Signalling mechanisms by which the aspartyl-tRNA synthetase IBI1 controls broad-spectrum induced resistance in *Arabidopsis thaliana*

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Thesis Summary

Plants possess an innate immune system that protects against the majority of attackers. Specialised pathogens, however, can de-regulate this system and cause disease. Treatment with β-aminobutyric acid (BABA) can render susceptible plants more resistant against virulent pathogens. This BABA-induced resistance (BABA-IR) is based on priming of defence, mediating a faster and/or stronger induction of basal defences after pathogen attack. Although BABA-IR provides protection in a wide range of plant species against a broad spectrum of pathogens, it is also associated with an undesirable plant stress response. The recent discovery that BABA is perceived by the aspartyl-tRNA synthetase (AspRS) IBI1 shed more light on the molecular perception mechanisms of BABA and the associated stress response. However, the mechanisms by which the BABA receptor IBI1 initiates downstream defence signalling remained unknown. This thesis describes results from various approaches to elucidate these pathways. Site-directed mutation of *IBI1* revealed that BABA perception relies on the aspartic acid-binding domain of IBI1, while domain-swapping between IBI1 and a close AspRS homologue showed that BABA-IR is not dependent on IBI1-specific protein domains, but rather a common function of AspRS enzymes that relies on sufficient levels of gene transcription. Yeast two-hybrid analysis identified numerous interactors of IBI1, which were confirmed by bimolecular fluorescence complementation analysis. Characterisation of a selection of these interactors revealed a role for VOZ transcription factors and photorespiration-derived reactive oxygen species in BABA-IR. Furthermore, genome-wide transcriptome analysis showed that IBI1-mediated BABA-IR coincides with global repression of abscisic acid-regulated genes. In support of this, metabolome analysis of the same samples in combination with targeted chlorophyll fluorescence analyses suggested a role for the xanthophyll cycle and non-photochemical quenching in IBI1-dependent BABA-IR. Based on these results, the roles of ABA and light signalling in BABA-IR are discussed in relation to the translational value of IBI1-dependent resistance in crops.

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Practical contributions:

The biological sample material that formed the basis of the microarray and metabolome analyses presented in Chapter 7 was prepared by Estrella Luna-Diez. She performed the pathogen colonisation analysis and *PR1* expression analysis presented in Figure 7.2. Estrella contributed to the statistical analysis of the micrarray data, and also designed the mutated IBI1 construct used in Chapter 3.

Joost Stassen performed the data preprocessing (RMA, normalisation, log transformation) and helped with the statistical analysis of the microarray data. He also performed the analysis underlying Figure 7.3b.

Pierre Pétriacq supervised the preparation of the metabolite extracts, ran samples on the MALDI-MS equipment and performed the data preprocessing of the MALDI-MS data. He also performed the OPLS-DA presented in Figure 7.5.

Chapter 1

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General Introduction

1.1 Sustainable pest and disease management is essential to safeguard food security

The Green Revolution that took place between the 1940s and 1960s is credited with saving billions of lives by increasing the world food production through promoting modern agricultural techniques together with new crop cultivars (Mann 1997; Macaray 2013). The challenge humanity faces today is of similar scope as the one Norman Borlaug and his colleagues had to overcome in the middle of the last century: By 2050, the global human population is estimated to rise by 2.2 billion people, an increase of ~30% from 2017 levels (United Nations Organisation 2017). Additionally, changes in dietary habits will require further growth in food production. At the same time, concerns about the destruction of our natural environment, human contribution to climate change and resource depletion mean that this will need to be done in a more sustainable way (The Royal Society 2009).

Pesticides are a critical component of post-Green Revolution agriculture. However, their indiscriminate use and persistent discharge in the environment has led to concerns about environmental and human health (Pimentel 2005; Skevas, Stefanou, and Lansink 2012). Legislation has limited pesticide release in the environment and led to the development of modern pesticides that are readily degraded outside plant tissues, thereby limiting their accumulation in the biosphere. As a consequence, modern pesticides require frequent applications to be effective, requiring heavy machinery, which leaves a bigger carbon foot print (Batey 2009; Woods et al. 2010). Furthermore, under the intense selection pressure of continuous pesticide use, many pests have evolved resistance against commonly used chemicals (Chandler et al. 2011). Future sustainable agriculture must therefore exploit alternative protection methods to manage pests and diseases. This includes selection of resistant cultivars, advanced monitoring that enables early interception of pests and diseases, more selective use of synthetic chemicals, and a combination of mechanical and biological control measures. This approach is commonly referred to as Integrated Pest Management (IPM) (Birch, Begg, and Squire 2011; Chandler et al. 2011; Savary et al. 2012). Interestingly, recent years have seen an increasing interest to exploit immune priming in IPM applications, which boosts quantitative disease resistance of plants (Beckers and Conrath 2007; Ahmad et al. 2010; Xu et al. 2017).

1.2 The plant innate immune system - inducible defence mechanisms

1.2.1: An evolutionary model of plant innate immunity - the "zig-zag" model

Plants have evolved a wide array of mechanisms to defend themselves against attack by pathogens and herbivores. Some mechanical barriers, like thorns, waxy cuticles or cell walls, are constitutively present, and are determined by the developmental programme of plants. In addition, plants store chemical compounds with antimicrobial properties, called phytoanticipins, which can rapidly provide chemical defence upon herbivore or pathogen attack (Van Etten et al. 1994; Bednarek et al. 2009, Ahmad et al. 2011;). Most defence mechanisms are only activated after perception of an attacker. Every plant cell has a sophisticated innate immune system that coordinates these inducible defences in response to attack by a herbivore or pathogen (Spoel and Dong 2012). Plant cells have the ability to recognise conserved structures that are characteristic for different classes of microbial pathogens. Detection of these pathogen-associated molecular patterns (PAMPs) elicits an immune response, termed PAMP-triggered immunity (PTI), which in most cases is sufficiently powerful to stop the invading pathogen. As a consequence, plants are resistant to the vast majority of potentially harmful microbes. This type of innate immunity is also known as non-host resistance (Heath 2000; Thordal-Christensen 2003). However, some pathogen strains have evolved "effector molecules", which allow the microbe to evade, overpower or de-regulate the immune system of their host plants (Jones and Dangl 2006; Deslandes and Rivas 2012). In most cases, effectors from (hemi)biotrophic pathogens are injected into the host cell by a type-3 secretion system (T3SS), where their action leads to effector-triggered susceptibility (ETS) (Alfano and Collmer 2004; Tang, Xiao, and Zhou 2006). Under the resulting selection pressure to counteract these effectors, plants have evolved a set of genes called disease resistance (R) genes. These genes usually encode cytoplasmic nucleotide binding - leucine rich repeat (NB-LRR) proteins, and any given R gene will confer resistance only against pathogen strains that have a "matching" effector-encoding avirulence (Avr) gene. This relationship between an R gene and a corresponding avirulence gene is traditionally referred to as gene-for-gene resistance or vertical resistance. In most cases this gene-for-gene relationship is not based on a direct physical interaction between the corresponding proteins, but involves an alternative perception mechanism, whereby R proteins survey the integrity of plant self-proteins against the activity of the avirulent effector protein. This mode of action is commonly known as the "guard hypothesis" (Jones and Dangl 2006). Irrespective of the exact molecular pathway, detection of an effector by an R protein often induces a multicellular cell death response, known as the hypersensitive response (HR). The resulting resistance is called effector-triggered immunity (ETI), which is sufficiently strong and fast to stop biotrophic pathogens before they can employ other effectors to induce ETS. Despite its effectiveness and relative ease to exploit by plant breeders, ETI increases the pressure on avirulent strains to evolve new Avr genes that are no longer recognised by existent R genes, breaking the resistance and reverting it to basal levels of resistance. This constant arms race between plants and their pathogens is described by the aptly named 'zig-zag model' (Fig. 1.1; Jones and Dangl, 2006).

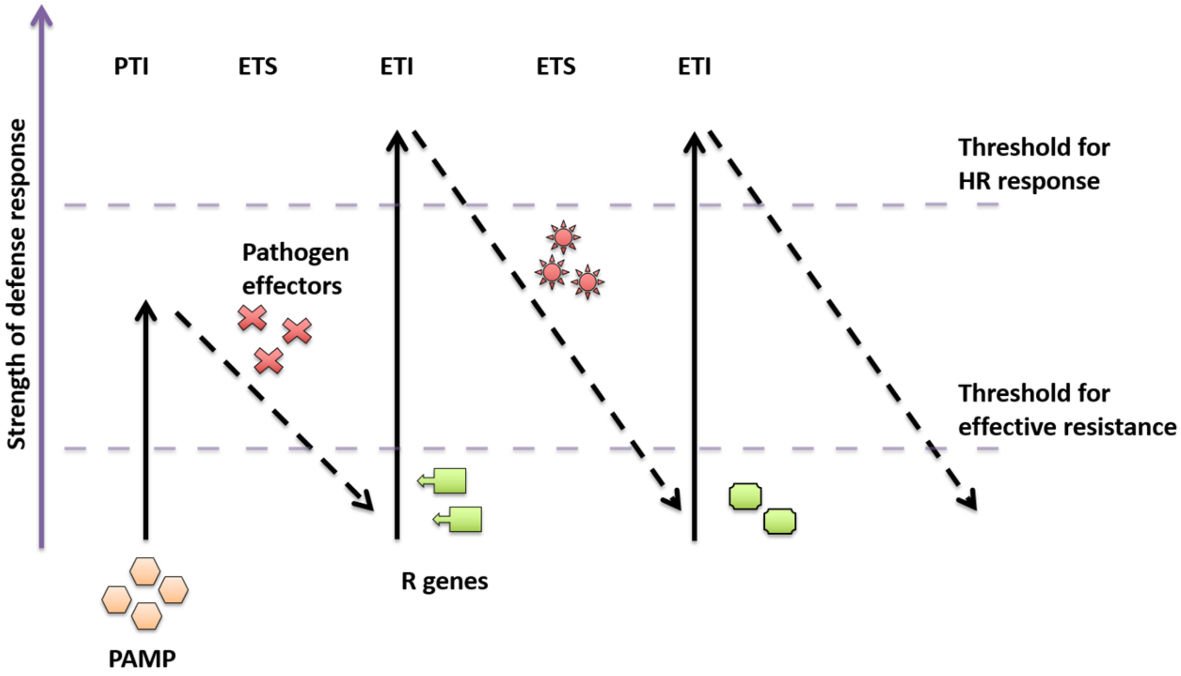

Figure 1.1: The 'zig-zag' model of co-evolution between plants and biotrophic pathogens. *Recognition of conserved microbial structures leads to PAMP-triggered immunity (PTI). Under the resulting selection pressure, pathogens have evolved effector molecules that suppress the plant immune response, causing effector-triggered susceptibility (ETS). Some plant varieties have acquired so-called resistance genes (R genes), which encode proteins that sense the presence of specific pathogen effectors and subsequently trigger an augmented defence response, called effector-triggered immunity (ETI). While R genes convey almost complete resistance against specific pathogens, microbes are constantly evolving novel effector molecules, which render their host once again susceptible. Figure recreated after Jones and Dangl (2006).*

1.2.2: A spatio-temporal model of plant innate immunity

Irrespective of the type of disease resistance (e.g. basal resistance, PTI or ETI), the resulting immune response is made up of different inducible defence layers that become active at different stages of pathogen infection (Ton, Flors, and Mauch-Mani 2009; Thomma, Nürnberger, and Joosten 2011).

Pre-invasive defence. For many bacteria, an intact epidermis is an insurmountable barrier, and can pose a very effective defence layer to prevent colonisation. However, physiological openings, such as stomata, can serve as gateways for bacterial invasion. To prevent entry via this route, plants rapidly close their stomata upon detection of PAMPs on their leaf surface, restoring the integrity of the leaf epidermis (Schulze-Lefert and Robatzek 2006). This abscisic acid (ABA)-dependent stomatal closure can stop bacteria even before they enter the plant tissue, but can be subverted by bacterial effectors such as coronatine (Melotto et al. 2006). An ABA-independent pathway for stomatal closure has been proposed, but the exact mechanisms are only being unravelled (Montillet et al. 2013; Guzel Deger et al. 2015; Su et al. 2017). However, many fungi, oomycetes and aphids can penetrate the epidermis directly (Pollard 1973; Kemen and Jones 2012), rendering stomatal immunity ineffective.

Early local post-invasive defence. Once a pathogen has successfully invaded the plant tissue, its presence activates the formation of callose-rich papillae, which strengthen the cell wall and form a matrix for other anti-microbial compounds, such as lignin, phenolic compounds and ROS intermediates, thus blocking the further spread of the pathogen (Conrath, Pieterse, and Mauch-Mani 2002; Luna et al. 2011). Recognition of PAMPs or effectors also triggers rapid production of nitric oxide (Wendehenne et al. 2001) and reactive oxygen species (ROS) (Wojtaszek 1997). This ROS burst proceeds in a biphasic manner, with a transient first increase in ROS followed by a stronger, more sustained accumulation (Torres, Jones, and Dangl 2006). While direct antimicrobial activities of ROS have been documented (Peng and Kuc 1992), the major contribution of ROS lies in their function as second messengers in the defence signalling network of the plant cell , leading to strengthening of the cell wall, synthesis of phytoalexins and regulation of salicylic acid (SA)-dependent pathways (Pastor et al. 2013). Another defence mechanism that is partially controlled by ROS and highly effective against biotrophic pathogens is the HR described above (Wojtaszek 1997; Jane Glazebrook 2005). The signals and responses taking place during expression of early post-invasive defence often lead to induction of later-acting post-invasive defences, explained below.

Late local post-invasive defence. If a pathogen manages to overcome the plant's early defence barriers, its continued presence leads to extensive transcriptional and metabolic reprogramming (van Loon, Rep, and Pieterse 2006). This defence layer depends on signalling mediated by plant hormones, such as salicylic acid (SA) or jasmonic acid (JA). The balance between these antagonistic hormones plays a pivotal role in shaping the outcome of the resulting defence response (De Vos et al. 2005; Leon-Reyes et al. 2010). For instance, SA signalling induces defences that are effective against biotrophic microbes, but can be detrimental against necrotrophic pathogens. JA-dependent defence mechanisms, on the other hand, provide protection against necrotrophic pathogens and insects, but can antagonise SA-dependent defences (Robert-Seilaniantz, Grant, and Jones 2011; Thaler, Humphrey, and Whiteman 2012). Other hormones, such as ethylene (ET) and abscisic acid (ABA), influence the plant defence response, and there is a growing appreciation of complex hormonal cross-talk underlying plant immunity (Robert-Seilaniantz, Grant, and Jones 2011). Depending on the nature of the perceived pathogen, SA- or JA-dependent genes are activated, leading to production of pathogenesis-related (PR) proteins and phytoalexins, amongst others. One class of transcription factors that play a prominent role in this large-scale transcriptional reprogramming are members of the WRKY family (Pandey and Somssich 2009), as well as the transcriptional regulator NPR1 (Cao et al. 1994; Dong 2004).

Metabolic reprogramming includes changes in both primary and secondary metabolism (Berger, Sinha, and Roitsch 2007). In some plant-pathogen interactions, the activity and expression of photosynthetic proteins is downregulated, whereas the expression of cell wall invertases and the rate of respiration are increased at the sites of infection, turning the infected tissue into a metabolite sink. The concomitant rise in hexose levels at the site of infection can induce defence gene expression (Berger, Sinha, and Roitsch 2007). While the association between sugars, infection and plant defence is the best-studied example of interplay between primary metabolism and defence, all aspects of primary metabolism are affected by pathogen or herbivore attack (Schwachtje and Baldwin 2008). For example, it has been shown that glutamate levels are reduced in sunflower upon infection with the fungus *B. cinerea*, even at sites distal from the attack, which was associated with enhanced levels of glutamate dehydrogenase (*GDH*) at the site of infection (Dulermo et al. 2009).

1.3 The plant acquired immune system - priming of defence

1.3.1: Systemic resistance responses: a combination of direct activation and priming of defence

In addition to the local innate immune responses described above, pathogen attack also generates long-distance systemic signals (Heil and Ton 2008), which prepares distal tissues against upcoming pathogen attack. Several types of systemic resistance responses have been identified, each partially differing in their triggering stimulus and underpinning signalling pathways. The best-known example is systemic acquired resistance (SAR), which is elicited by necrotising pathogens and is associated with activation of SA-dependent defence mechanisms (Durrant and Dong 2004). Plant beneficial microbes, on the other hand, promote a state of heightened immunity called induced systemic resistance (ISR), which is mediated by JA and ET signalling (Pieterse et al. 2014). A third form of whole-plant immunity is resistance that is induced in response to volatile organic compounds (VOC-IR) that are released upon wounding (Baldwin et al. 2006; Heil and Ton 2008). These long-distance signals can activate defence in undamaged organs and even in neighbouring plants.

Systemic defence is a powerful mechanism to restrain the spread of a disease or pest, because it allows a plant to prepare for attack before this attack occurs. Since the timing and initial strength of the immune response is a critical determinant of its effectiveness, systemic resistance shifts the odds in favour of the plant. Direct gene activation, however, does not fully account for the enhanced immunity associated with systemic resistance responses (Kohler, Schwindling, and Conrath 2002). It is now widely recognised that an important mechanism underpinning systemic resistance is the sensitisation of plant tissues for faster and/or stronger defence induction upon subsequent attack (Conrath, Pieterse, and Mauch-Mani 2002; Conrath et al. 2006; Pieterse et al. 2014). This phenomenon is known as priming.

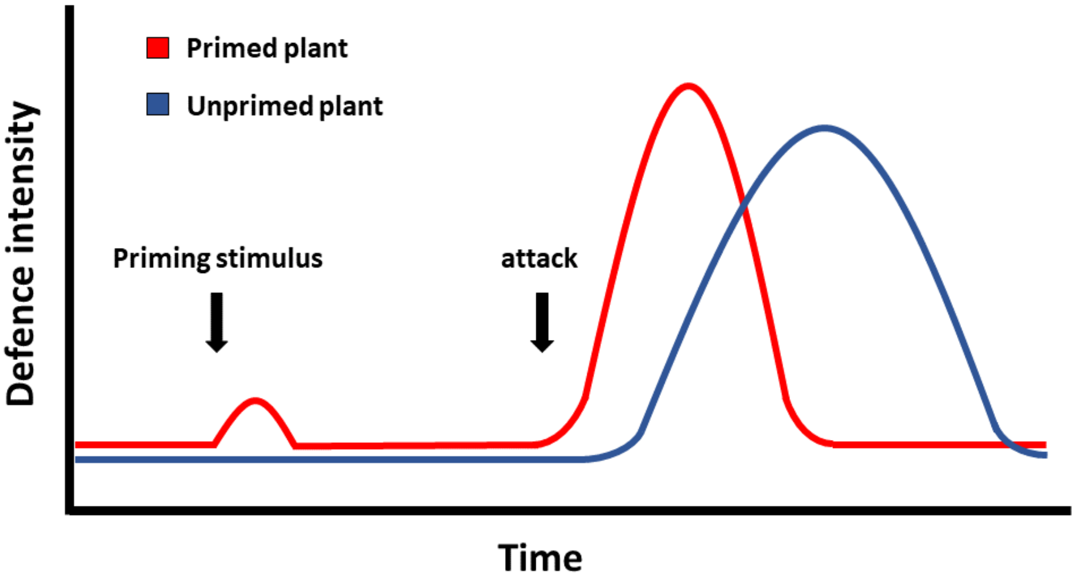

Figure 1.2: Conceptual model of the effect of priming on timing and amplitude of plant defence*.* *Treatment with a priming agent leads to a transient and relatively weak induction of defence mechanisms. However, upon subsequent attack, primed plants respond with a stronger and/or faster induction of defence compared to unprimed plants. Since the timing and strength of the initial immune response are important determinants of the outcome of infection, priming is a potent protective mechanism against various pathogens. Figure modified from Heil and Ton (2008).*

Priming phenomena have been described in different biological contexts, and can be defined as the implicit memory effect in which exposure to a stimulus influences the response to a subsequent stimulus (Gulan and Valerjev 2010). In the context of plant immunity, priming enables a plant to respond faster and/or stronger to subsequent attack compared to naïve un-primed plants. (Fig. 1.2) This immune sensitisation has been proposed as early as 1933 (Chester 1933). However, it was not until a few decades ago that plant scientists begun to unravel the importance and the physiological and molecular mechanisms underpinning priming (Kauss et al. 1992; Katz, Thulke, and Conrath 1998; Conrath, Pieterse, and Mauch-Mani 2002). Importantly, priming leads to augmented induction of PAMP- and effector-triggered immunity, affecting pathogens before they can fully mobilise their effectors (Ahmad et al. 2010). Depending on the strength of the augmented defence response, primed plants will either slow down or stop the spread of a pathogen entirely. Moreover, since priming involves augmentation of PTI, it provides protection against a broad range of biotic stresses (Jakab et al. 2001; Ton and Mauch-Mani 2004).

1.3.2: Costs and benefits of priming

From an evolutionary perspective, R gene-mediated resistance (vertical resistance or ETI) is a relatively unsustainable form of resistance that is constantly threatened by counter-evolution of new pathogen effector genes. Primed resistance, on the other hand, involves augmentation of multigenic basal resistance (quantitative resistance or horizontal resistance). Therefore, it is often assumed that resistance via priming is more difficult to evade or suppress by virulent microbes. Another benefit of defence priming is that it provides protection against a wide range of pathogens and herbivores (Conrath et al. 2006). Furthermore, some priming agents, such as β-aminobutyric acid (BABA), also increase the plant's capacity to resist abiotic stresses, such as drought or salt stress (Jakab et al. 2005). Intriguingly, an increasing body of evidence is emerging that priming can be inherited epigenetically to following generations, conferring enhanced resistance over several generations (Luna et al. 2012; Rasmann et al. 2012; Slaughter et al. 2012). Since primed plants do not mount a full immune response until needed, priming usually imposes a relatively small fitness cost compared to direct activation of defence (van Hulten et al. 2006). In relatively disease-free environments, however, primed plants show slightly reduced fitness compared to untreated counterparts (van Hulten et al. 2006).

The level of resistance delivered by priming depends on the speed and vigour of the augmented basal defence response, which is determined by a complex interplay between host- and pathogen-dependent factors (Truman, de Zabala, and Grant 2006). While some primed plants show full resistance against certain pathogens, the same priming stimulus only provides partial protection in other plant-microbe combinations (Ahmad et al. 2010). Furthermore, priming *per se* has only protective quality, but does not usually cure plants once a disease has established. To address this shortcoming, fungicides with both priming and curative effects have been developed (Beckers and Conrath 2007). Taking all these considerations into account, priming appears to be well-suited for agricultural exploitation in integrated pest and disease management schemes.

1.3.2: Molecular mechanisms of priming

Several molecular, biochemical and epigenetic mechanisms have been discovered that can have a contribution to priming in plants. This suggests that priming is determined by a multitude of different mechanisms, which can act redundantly and/or additively on the total level of resistance.

Accumulation of inactive defence compounds or defence signalling hormones. Many compounds with a range of different functions can be stored in plants in an inactive form, to be activated only when needed (Pastor et al. 2013). Phytoanticipins, for example, are constitutively present as glycosylated conjugates in the vacuole. Cellular disruption by chewing insects brings together the phytoanticipins with cytoplasmic glucosidases, thus releasing the toxic form of these defence compounds upon tissue damage (Pastor et al. 2013). Increased levels of the inactive conjugates upon priming treatment could mediate a stronger and/or faster release of the active compound upon attack. Interestingly, apart from their traditional role as herbivore deterrents, phytoanticipins also play a role in the regulation of cell wall-mediated defence against fungi: Benzoxazinoids are the most important phytoanticipins in cereals. Benzoxazinoid-deficient maize mutants show impaired callose deposition upon treatment with the fungal PAMP chitosan (Ahmad et al. 2011). A similar role has been demonstrated for glucosinolates in *Brassicaceae*, where early cell wall-mediated defence is associated with the hydrolytic activation of the indole glucosinolate 4-methoxyindol-3-ylmethylglucosinolate (4MI3G) by PEN2 (Clay et al. 2009; Luna et al. 2011). It is therefore conceivable that priming stimulates accumulation of inactive glycosylated phytoanticipins that can rapidly be mobilised upon pathogen or herbivore attack. Similarly, primed plants could harbour augmented pools of inactive hormone conjugates, such as ABA-glucose ester (ABA-GE) and SA-glucose (SAG).

Enhanced accumulation of inactive defence signalling proteins. Activation of an inducible defence reaction requires perception of extracellular alarm signal(s) and transduction into an intracellular defence response. These functions can be fulfilled by MAP kinase cascades, which are known to be involved in the signalling downstream of PAMP receptors (Asai et al. 2002; Chisholm et al. 2006). For example, activation of the cell surface receptor FLS2 by flagellin activates a signalling cascade consisting of MEKK1, MEK4/5 and MPK3/6 (Asai et al. 2002). The outcome of this signalling cascade is the activation of multiple genes that are involved in defence responses. It has been proposed that accumulation of signalling proteins in this pathway could enable cells to react more quickly to PAMP detection. This hypothesis was experimentally verified by Beckers et al. (2009), who demonstrated that priming treatment of *Arabidopsis thaliana* (Arabidopsis) by the SA analogue BTH or local pathogen attack is associated with systemic upregulation of *MPK3* and *MPK6* mRNA and protein (Beckers et al. 2009). Interestingly, both proteins remained in an inactive form until activated by a second challenge (*Pseudomonas syringae* pv *maculicola* dip-infection or water infiltration). Primed plants also showed enhanced expression of the defence genes *PR1* and *PAL1* upon challenge, which was reduced in *mpk3* and *mpk6* mutant plants, whereas direct activation of SA-inducible genes was not affected or even upregulated in both mutants. In support of these findings, overexpression of orthologues of Arabidopsis *MPK3* have previously been shown to induce resistance in other species (Lee et al. 2004; Shoresh et al. 2006). In addition to MAP kinases, this proposed mechanism may also apply to transcription factors. Van der Ent *et al.* (2009) showed that priming by BABA or *Pseudomonas fluorescens* (discussed below) is also accompanied by large-scale changes in the expression of transcription factors (Van der Ent et al. 2009). More specifically, 186 and 90 transcription factor genes showed more than 2-fold induction upon priming with BABA or *P. fluorescens* WCS417r, respectively. Conversely, 44 and 31 genes encoding TFs were downregulated by these treatments. Taken together, these results show that priming is, at least partly, mediated by enhanced accumulation of defence-regulatory MAP kinases and transcription factors that mediate an augmented defence reaction upon pathogen attack.

Epigenetic regulation of priming. While proteins are subject to relatively fast turn-overs, the heightened immune responsiveness of primed plants can persist throughout the life cycle of a plant, and even be transmitted to progeny over stress-free generations (Luna et al. 2012; Rasmann et al. 2012; Slaughter et al. 2012). The accumulation of precursors of antimicrobial compounds, inactive transcription factors and signalling proteins can therefore not account for these long-lasting aspects of priming. Epigenetic changes, on the other hand, are heritable long-term changes in gene expression that are independent of changes in the DNA sequence. Two main mechanisms of epigenetic control are histone modifications and DNA methylation, and both seem to play a role in priming phenomena (Bruce et al. 2007; Luna, López, et al. 2014; López Sánchez et al. 2016). For instance, the *drm1drm2cmt3* triple mutant, which is disrupted in non-CpG DNA methylation, shows constitutive priming of SA-dependent defences in the absence of priming treatment (Luna et al. 2012). Intriguingly, infection with *Pst*DC3000 has been reported to induce hypo-methylation in Arabidopsis (Pavet et al. 2006; Dowen et al. 2012), pointing to the possibility that priming of SA-dependent defence genes can be mediated by decreased methylation in these genes (Luna et al. 2012). This hypothesis was confirmed by further phenotypic analysis of the defence phenotypes of mutants affected in *NRPE1,* a component of the RNA-directed DNA methylation (RdDM) pathway, and *ROS1*, a DNA demethylase (López Sánchez et al. 2016). The hypo-methylated *nrpe1* mutant showed increased resistance against the biotrophic pathogen *Hyaloperonospora arabidopsidis* (*Hpa*), whereas the hyper-methylated *ros1* mutant was more susceptible and delayed in its activation of SA-dependent defence genes. Conversely, the two mutants showed opposing phenotypes against a necrotrophic pathogen, confirming the notion of negative crosstalk between SA- and JA-dependent defence. Intriguingly, while both mutants seemed unaffected in SAR against *Hpa,* neither *nrpe1* nor *ros1* developed trans-generational acquired resistance against this pathogen, highlighting the importance of DNA (de)methylation in transgenerational immune priming (López Sánchez et al. 2016).

The regulation of gene expression by histone modification is a complex phenomenon, and our understanding of this process is still rudimentary. However, it is widely accepted that acetylation of certain residues in histone tails facilitates access to DNA by transcription complexes, thus leading to an activated state of the associated gene (Kouzarides 2007; Li, Carey, and Workman 2007; Shahbazian and Grunstein 2007). Transgenerational SAR is associated with enhanced expression of SA-dependent genes in Arabidopsis (Luna et al. 2012). Interestingly, this type of priming correlated with acetylation of H3K9 in the promoter regions of SA-inducible genes *PR1*, *WRKY6* and *WRKY53* (Luna, López, et al. 2014). Conversely, primed plants showed triple methylation of H3K27 in the promoter region of the JA-inducible gene *PDF1.2*, a marker that is associated with gene repression (Zhang et al. 2007; Luna et al. 2012). These examples also suggest involvement of epigenetic mechanisms in the negative cross-talk between SA- and JA-/ET-inducible pathways (Robert-Seilaniantz, Grant, and Jones 2011). Thus, numerous studies have demonstrated that epigenetic changes play an important role in long-term adaptation of plants to stresses encountered in their environment, in particular to pathogen attack.

Changes in primary metabolism. While there is a growing appreciation that primary metabolism influences plants' defensive capacities beyond merely providing the energy for inducible defences (Schwachtje and Baldwin 2008), relatively little is known about its role in priming. However, it has been reported that antisense suppression of the plastid ATP/ADP antiporter AATP1 primed potato plants for an augmented oxidative burst and defence gene induction following attack by fungal and bacterial pathogens (Linke et al. 2002). The results of this study allowed speculation that a change in ATP/ADP transport over the plastid membrane is involved in the regulation of immunity. Although the underlying mechanisms behind these findings remained unknown, this report adds to the growing notion that interactions between primary and secondary metabolism can play a role in priming.

1.3.3: Types of priming inducing agents

The first systematic studies about the mechanisms underpinning defence priming were performed with cell suspension cultures in response to treatments with the defence signalling molecule salicylic acid and its synthetic analogues 2,6-dichloroisonicotinic acid (INA) and benzothiadiazole (BTH) (Kauss et al. 1992; Katz, Thulke, and Conrath 1998). Since then, a host of other priming agents have been described, including small chemical compounds, beneficial microbes, gene mutations and changes in DNA methylation. Although they all seem to induce resistance to a range of biotic and abiotic stresses, specific priming agents differ in their effectiveness against different stressors, and different combinations of defence signalling pathways can have a contribution to the primed resistance phenotype (Conrath et al. 2006).

As described above, systemic acquired resistance (SAR) is induced by localised pathogen attack and established by the accumulation of salicylic acid in distal plant parts (Durrant and Dong 2004). SAR requires the intact defence regulatory protein NPR1 (Durrant and Dong 2004), which in recent years has been proposed to act as a receptor of nuclear SA (Fu et al. 2012; Wu et al. 2012; Kuai, MacLeod, and Després 2015). In addition to infection by necrotising pathogens, SAR can also be induced by exogenous application of SA or its functional analogues INA and BTH (Conrath 2006). In the 1990s, it was found that pre-treatment of suspension-cultured parsley cells with the SAR inducers SA, INA or BTH prompted them to respond with robust induction of various defence mechanisms to otherwise non-inducing concentrations of a PAMP from the oomycete *Phytophthora megasperma* (Kauss et al. 1992; Katz, Thulke, and Conrath 1998). Among the augmented responses were the early oxidative burst, cell-wall deposition of phenolic and lignin-like compounds and the production of phytoalexins. Strikingly, treatment with either elicitor alone did not trigger a full immune response. Subsequent research revealed that treatment with SAR inducers directly activates some defence genes, but merely facilitates expression of others (e.g. *PAL1*), resulting in augmented transcriptional induction following a second stress stimulus (Katz, Thulke, and Conrath 1998). Similar to the results in parsley cell cultures, it was found that BTH can directly activate *PR1* expression in *Arabidopsis thaliana*, whereas *PAL1* expression is only augmented following infection by virulent *Pseudomonas syringae tomato* (*Pst*), mechanical wounding or water infiltration of the leaves (Kohler, Schwindling, and Conrath 2002). The priming effects of chemically-induced SAR can be mimicked by infection with avirulent *Pst* (Kohler, Schwindling, and Conrath 2002). It is now thought that the protective effect of SAR mainly involves potentiation of SA-dependent and SA-independent defences upon subsequent attack, rather than direct activation of defence genes (Goellner and Conrath 2008; Bernsdorff et al. 2015).

The discovery of several Arabidopsis mutants altered in the speed and intensity by which they activate inducible defences led to further insight into the mechanisms of priming (Frye and Innes 1998; Ton et al. 2005). For example, mutations in *EDR1* lead to a constitutively primed phenotype in that these mutant plants show augmented induction of callose deposition and HR upon challenge in the absence of a priming treatment, yet do not express constitutively higher levels of *PR1* and *BGL2* prior to infection (Frye and Innes 1998). *EDR1* encodes a MAP3K, confirming the notion that MAP kinase pathways are involved in the regulation of priming. Other mutations that are affected in priming include *npr1*. While NPR1 also plays a critical role in PTI and ETI (Shah, Tsui, and Klessig 1997), it is fully blocked in SAR (Cao et al. 1994) and lacks the ability to develop BTH-induced priming of *PAL* gene expression and callose deposition (Kohler, Schwindling, and Conrath 2002). Further mutations that have been shown to affect priming in Arabidopsis, either positively or negatively, are listed in Table 1.1.

Table 1.1: Mutations affecting priming in Arabidopsis

Mutant	Primed response	Effect	Encodes	References
ald1	pathogen-induced SAR	absent	aminotransferase AGD2-LIKE DEFENSE RESPONSE PROTEIN1	(Návarová et al. 2012)
drm1drm2 cmt3 (ddc)	transgenerational SAR	constitutively expressed	enzymes required for non-CpG DNA methylation	(Luna et al. 2012)
edr1-1	SA-inducible genes, callose deposition, HR	enhanced	MAPKKK	(Frye and Innes 1998; Frye, Tang, and Innes 2001; van Hulten et al. 2006)
ibi1-1	SA-dependent and independent BABA-IR	impaired	Asp-tRNA synthetase IMPAIRED IN BABA-INDUCED IMMUNITY1	(Luna et al., 2013, *under revision*)
ibs1	BABA-induced SA-dependent genes, callose deposition	absent	Cyclin-dependent kinase-like protein	(Ton et al. 2005)
ibs2	BABA-induced ABA-dependent salt resistance	impaired	AtSAC1b, polyphosphoinositide phosphatase	(Ton et al. 2005)
ibs3	BABA-induced ABA-dependent salt resistance	impaired	zeaxanthin epoxidase ABA1	(Ton et al. 2005)
jar1	JA-dependent genes, ISR	impaired or absent	jasmonic acid-amido synthetase JASMONIC ACID RESPONSE LOCUS1	(Ahn, Lee, and Suh 2007; Kravchuk et al. 2011)
lecRK-VI.2-1	PTI-marker genes, callose deposition, stomatal closure	impaired	LECTIN RECEPTOR KINASE VI.2	(Singh et al. 2012)
npr1	BTH-induced PAL expression,	absent	transcriptional coactivator NON-EXPRESSOR OF PR1	(Kohler, Schwindling, and Conrath 2002)
ocp1	SA- and JA-inducible genes	SA: impaired JA: enhanced	RNA Pol IV/V subunit NRPD2	(López et al. 2011)
ocp3	ABA-dependent callose deposition	constitutively primed	transcription factor OVEREXPRESSOR OF CATIONIC PEROXIDASE 3	(García-Andrade et al. 2011)

A second form of systemic resistance, termed induced systemic resistance (ISR), is characterised by activation of jasmonic acid (JA)- and ethylene (ET)- dependent signalling pathways (Pieterse et al. 1998). Unlike SAR, ISR is not induced by pathogen attack, but by root colonisation of plant-beneficial rhizobacteria (PGPR) or fungi (PGPF) (Pieterse et al. 1998; Conrath et al. 2006; Van der Ent et al. 2009). ISR is not dependent on endogenous production of SA, nor is it marked by systemic activation and/or priming of *PR* genes (Pieterse et al. 1998). Instead, ISR is mediated by sensitisation of the tissue to the plant hormones JA and ET (Wees et al. 1999). Like SAR, the enhanced immunity during ISR is at least partly conveyed through priming (Pieterse et al. 2002; Van der Ent et al. 2009). *P. fluorescens* WCS417 is widely used as an ISR-inducing agent. Using this bacterium, it was found that ISR in carnation (*Dianthus caryophyllus*) is associated with priming for the incorporation of callose and phenolic compounds into the cell wall, thus increasing resistance against *Fusarium oxysporum* (Peer 1991). In Arabidopsis, genes that are primed during *P. fluorescens* WCS417-mediated ISR include *AtVSP2*, *PDF1.2* and *HEL* (Wees et al. 1999; Hase et al. 2003). In Arabidopsis, ISR establishment leads to a direct upregulation of the JA-regulatory transcription factor *MYC2* in the shoot (Pozo et al. 2008), which can facilitate a faster and stronger transcriptional induction of JA-dependent target genes. Additionally, ISR in Arabidopsis enhances resistance through ABA-dependent, JA-independent priming of callose deposition (Van der Ent et al. 2009).

When the integrity of green plant tissues is compromised by herbivore damage, volatile organic compounds are released from the damaged plant (Kessler et al. 2006; Ton et al. 2007). These volatiles can evoke resistance in systemic plant parts as well as in neighbouring plants (VOC-IR; Heil and Ton, 2008). Interestingly, the priming aspect of VOC-IR is mediated only by a subset of JA-inducible genes (Ton et al. 2007). Several candidates have been suggested to mediate airborne long-distance signalling (Heil and Ton 2008): Upon methylation, the plant hormones SA and JA become volatile, and both MeSA and MeJA can induce a defence response (Farmer and Ryan 1990; Shulaev, Silverman, and Raskin 1997). C6-compounds that are rapidly released upon tissue damage, collectively called green-leaf volatiles (GLV), are also effective at eliciting direct and indirect defence responses, both in the context of herbivore and pathogen attack (Arimura, Ozawa et al. 2000; Kessler and Baldwin 2001; Shiojiri et al. 2006). The gaseous plant hormone ethylene, on the other hand, seems to be important only locally at the site of pathogen infection (Verberne et al. 2003), where it augments GLV emission (Ruther and Kleier 2005) but does not act as a long-distance signal (Verberne et al. 2003; Heil and Ton 2008). Besides their relatively well-known function in attracting natural predators of herbivores (Arimura, Ozawa et al. 2000; Ton et al. 2007), volatiles can also regulate transcription of defence genes (Arimura, Tashiro et al. 2000) or increase the production of secondary metabolites such as phenolic compounds (Tscharntke et al. 2001) and defensive enzymes like proteinase inhibitors (Farmer and Ryan 1990).

In addition to SAR-inducing pathogens, ISR-inducing rhizomicrobes and herbivore-induced volatiles, a wide range of chemical priming agents has been described (Table 1.2). It is not always straightforward to reconcile the host of findings reported in these studies. Indeed, it seems that some mechanisms are shared by multiple priming phenotypes, whereas others are specific to certain plants, priming agents or pathogens (Conrath et al. 2006). For example, several stimuli, including pipecolic acid (Návarová et al. 2012), probenazole (Yoshioka et al. 2001) and azelaic acid (Jung et al. 2009) can elicit a SAR-like phenotype. At the moment, it is unclear at what stage the pathways induced by these chemicals converge, or which act as a systemic messenger molecule to mediate whole-plant priming. In addition, thiamine (Vitamin B1) and quercetin (a plant flavonoid) can prime both SA-inducible gene expression as well as SA-independent mechanisms like ROS accumulation and callose deposition in rice and Arabidopsis, respectively (Ahn, Kim, and Lee 2005; Jia et al. 2010). Both compounds are effective in inducing resistance to *P. syringae*.

Interestingly, several more recent publications identified members of the plant growth hormone family cytokinins as priming agents, but reported differing mechanisms for different species (Choi et al. 2010; Dervinis et al. 2010). For example, in Arabidopsis, cytokinin-induced resistance against *Pst DC*3000 is associated with priming of SA-inducible genes (Choi et al. 2010), whereas in poplar (*Populus spp.)*, cytokinin treatment confers increased resistance against herbivorous insects mediated by priming of JA-inducible genes (Dervinis et al. 2010). Moreover, in tobacco, the resistance-enhancing effect of the cytokinin kinetin relies on direct induction of increased production of the phytoalexins scopoletin and capsidiol, rather than priming (Großkinsky et al. 2011).

Examples of priming in crops include the above-mentioned thiamine-induced potentiation of SA-inducible genes (*PR1a, PR10, PR11*) following infection with *Xanthomonas oryzae* or *Magnaporthae grisea* in rice (Ahn, Kim, and Lee 2005), as well as priming of barley by the PGPF *Piriformospora indica*, rendering the plant more resistant against infection and salt stress (Waller et al. 2005). Resistance against downy mildew can also be induced in barley by saccharin, and is associated with priming of cinnamyl alcohol dehydrogenase activity (Walters et al. 2008). Finally, several commercial fungicides, such as Brotomax™ and pyraclostrobin, act not only by directly inhibiting fungal growth, but also prime plants for induction of multiple defence pathways after stress. The aluminium lignosulphonate-containing formulation Brotomax™ has been reported to prime *Citrus* fruits for augmented synthesis of the phytoalexins scoparone and flavanone, resulting in increased resistance to *Phytophthora* *parasitica* (Ortuño et al. 1997). Pyraclostrobin, a strobilurin fungicide, enhances resistance to the bacterial pathogen *P. syringae* pv. *tabaci* and to tobacco mosaic virus (TMV) (Herms et al. 2002). This is associated with more rapid accumulation of PR1 in infected, but not in uninfected leaves. Pyraclostrobin induces priming either downstream or independent of SA accumulation, because enhanced resistance was also expressed in *NahG* tobacco plants (Herms et al. 2002). Interestingly, this study shows that the priming effects by fungicides is not restricted to enhanced resistance against fungi, but can also be effective against bacteria and viruses, showing the potential of primed immunity for agricultural application.

Table 1.2: Chemical and biological treatments known to induce priming

Priming agent	Augmented response	Protection against	Plant species	References
Aluminium ligno-sulphonate	Phytoalexins (scoparone, flavonone)	Fungal pathogens	*Citrus*	(Ortuño et al. 1997)
Azelaic acid	SA-inducible genes	*Pseudomonas syringae*	Arabidopsis	(Jung et al. 2009)
Benzothiadiazole (BTH)	PAL expression, callose deposition	*Pseudomonas syringae*, wounding, water infiltration	Arabidopsis	(Kohler, Schwindling, and Conrath 2002)
2,6-dichloro-isonicotinic acid (INA)	PAL expression, coumarin secretion	*Pseudomonas syringae*	Parsley (Petroselinum crispum) cell culture	(Katz, Thulke, and Conrath 1998)
β-aminobutyric acid (BABA)	SA-inducible genes, callose deposition	*Peronospora parasitica, Pseudomonas syringae, H. arabidopsidis*	Arabidopsis	(Zimmerli et al. 2000)
β-aminobutyric acid (BABA)	SA- and ABA-dependent genes	Salt and drought stress	Arabidopsis	(Jakab et al. 2005)
β-aminobutyric acid (BABA)	Callose deposition, ROS	Oomycetes and necrotrophic fungi	Arabidopsis	(Ton and Mauch-Mani 2004; Victoria Pastor et al. 2013)
β-aminobutyric acid (BABA)	??	Nematodes, aphids, microbial pathogens	Tomato, legumes, tobacco, grapevine, potato	(Cohen 1994, 2002; Cohen, Reuveni, and Baider 1999; Hodge, Thompson, and Powell 2005; Oka, Cohen, and Spiegel 1999)
Cytokinins	SA-inducible genes	*Pseudomonas syringae*	Arabidopsis	(Choi et al. 2011)
Cytokinins	JA-inducible genes	Insects	Poplar	(Dervinis et al. 2010)

Cytokinins	Phytoalexins (scopoletin capsidiol)	*Pseudomonas syringae*	Tobacco	(Großkinsky et al. 2011)
Pipecolic acid	SA-inducible genes, camalexin	*Pseudomonas syringae*	Arabidopsis	(Návarová et al. 2012)
PGPR (*Pseudomonas fluorescens*, *Bacillus pumilus*)	Induced systemic resistance (ISR)	*Fusarium oxysporum, H. arabidopsidis, Magnaporhtae oryzae*	Arabidopsis, rice, bean, carnation (*Dianthus caryophyllus)*	(Peer 1991; Benhamou et al. 1996; Pieterse et al. 1998; Pieterse et al. 2002; Vleesschauwer et al. 2008)
Quercetin	PR1, callose deposition, ROS	*Pseudomonas syringae*	Arabidopsis	(Jia et al. 2010)
Saccharin	Peroxidase activity	Downy mildew (*Blumeria graminis*)	Barley	(Boyle and Walters 2006)
Strobilurin	PR1 expression	*Pseudomonas syringae,* tobacco mosaic virus (TMV)	Tobacco	(Herms et al. 2002)
Thiamine (Vit B1)	SA-inducible genes, callose deposition, ROS	Bacterial, fungal and viral infection	Arabidopsis, rice, crop vegetables	(Ahn, Kim, and Lee 2005)
Volatile organic compounds	JA- and SA-inducible genes	Insects, microbial pathogens	Alder, Arabidopsis, *Artemisia tridentata*, maize, tobacco	(Farmer and Ryan 1990; Arimura, Ozawa et al. 2000; Arimura, Tashiro et al. 2000; Kessler et al. 2006; Shiojiri et al. 2006; Ton et al. 2007; Heil and Ton 2008)

1.4 Priming by ß-aminobutyric acid

ß-aminobutyric acid (BABA) was first described as a plant defence elicitor in 1963, but more targeted and systematic investigations into the resistance-inducing properties of BABA started in the 1990s (Papavizas and Davey 1963; Cohen et al. 1994; Cohen, Vaknin, and Mauch-Mani 2016). Interest in BABA-induced resistance (BABA-IR) is partly driven by the remarkably wide range of stresses to which it provides protection (Zimmerli et al. 2000; Jakab et al. 2001; Ton and Mauch-Mani 2004; Jakab et al. 2005; Conrath et al. 2006; Olivieri 2012; Slaughter et al. 2012), including diseases that are difficult to control by conventional strategies. To date, BABA-IR has been shown to be effective against more than 80 pathogens and pests, including pathogenic bacteria, fungi, insects, nematodes and viruses, as well as against abiotic stresses like drought, heat, cold and high salt concentrations (Cohen et al. 1994; Cohen, Reuveni, and Baider 1999; Cohen 2002; Conrath, Pieterse, and Mauch-Mani 2002; Conrath et al. 2006; Beckers and Conrath 2007; Van der Ent et al. 2009; Liljeroth et al. 2010; Conrath 2011; Cohen, Vaknin, and Mauch-Mani 2016). This wide-range efficacy of BABA is achieved by simultaneously priming multiple defence pathways (Zimmerli et al. 2000; Ton et al. 2005). For example, BABA-IR against the biotrophic bacterium *P. syringae tomato* DC3000 (*Pst* DC3000) and the necrotrophic fungus *B. cinerea* is dependent on SA accumulation and a functional NPR1 protein (Zimmerli et al. 2000; Zimmerli, Metraux, and Mauch-Mani 2001). Like BABA-IR against the oomycete *Hpa*, BABA-IR against the necrotrophic fungi *Alternaria brassicicola* and *Plectosphaerella cucumerina* is not affected in mutants impaired in SA-, JA- or ET-signalling or camalexin production, but relies on augmented deposition of callose that involves regulation by an abscisic acid (ABA)-dependent signalling pathway (Ton and Mauch-Mani 2004). Consequently, inhibition of callose formation by 2-deoxy-D-glucose diminished BABA-mediated protection against *A. brassicicola* (Ton and Mauch-Mani 2004). Similarly, BABA did not induce enhanced resistance against *P. cucumerina* in the callose-deficient mutant *pmr4-1* (Ton and Mauch-Mani 2004). BABA-induced priming of callose defence against necrotrophic fungi appears to involve regulation by ABA, because exogenous application of ABA mimics BABA-IR against these fungi, and the ABA-deficient *aba1-5* and ABA-insensitive *abi4-1* mutants were found to be impaired in BABA-IR against these fungi (Ton and Mauch-Mani 2004; also see Chapter 8). Surprisingly, ABA has also been reported to suppress callose deposition in response to the bacterial elicitor flg22 (Clay et al. 2009). Further systematic investigation into the role of ABA in callose deposition revealed that the abiotic growth conditions, such as light intensity, influence the regulatory function of ABA on PAMP-induced callose deposition (Luna et al., 2011). However, the exact role of ABA in callose priming by BABA remains open for further investigation.

The potential of BABA is underpinned by a series of studies that have found effective protection by this chemical in a number of crop plants, such as tomato (Cohen et al. 1994; Jeun, Siegrist, and Buchenauer 2000; Cohen 2002; Wilkinson et al. 2017), potato (Cohen 2002; Olivieri 2012) and grapevine (Cohen, Reuveni, and Baider 1999; Cohen 2002; Hamiduzzaman et al. 2005). Some of these studies were performed under field conditions (Walters et al. 2011). Overall, BABA-IR has been described to be effective in more than 40 species (Cohen, Vaknin, and Mauch-Mani 2016). However, at the moment, BABA is not used at a large scale in commercial agriculture. One of the factors preventing the use of BABA in crop protection is the fact that it leads to a reduction in plant growth, particularly when applied in higher doses. In Arabidopsis, BABA results in a phytotoxic response that has been referred to as a stress-induced morphogenic response (SIMR), which is characterised by growth inhibition and increased accumulation of anthocyanins (Wu et al. 2010). While this growth penalty is outweighed by the positive effect of enhanced resistance in environments with disease pressure, it can be quite severe under disease-free conditions (van Hulten et al. 2006). However, it has been demonstrated that BABA-induced resistance and growth repression are controlled by separate pathways (Luna et al. 2014; Chapter 1.5.4)

1.5 IMPAIRED IN BABA-INDUCED IMMUNITY 1 (IBI1) - the receptor of BABA

1.5.1: Identification of IBI1

Despite the wealth of literature describing the effectiveness of BABA treatment in various host-pathogen combinations, the biological relevance, as well as the molecular pathways underpinning BABA-IR, remained poorly understood for decades. Only more than 20 years after the first systematic research on the resistance-inducing properties of BABA, a receptor protein for this amino acid was identified in Arabidopsis (Luna et al. 2014). This discovery was based on the identification of an EMS-induced mutant of Arabidopsis that failed to develop BABA-IR against downy mildew (*Hpa*) after soil-drenching treatment with relatively low concentrations of BABA. Through a genetic mapping approach, it was revealed that independent mutant lines in the same gene, At4g31180, resulted in the same loss of BABA-IR. Consequently, this gene was named *IMPAIRED IN BABA-INDUCED IMMUNITY 1 (IBI1)*. Both the SA-dependent and SA-independent components of BABA-IR were nearly completely abolished in *ibi1* mutants, indicating that IBI1 is indispensable for BABA-IR (Luna et al. 2014). Interestingly, expression of *IBI1* is induced by pathogen inoculation, and overexpression of *IBI1* enhanced resistance against *Hpa* in the absence of BABA, indicating that the protein also plays a role in basal defence.

IBI1 encodes an aspartyl tRNA synthetase (AspRS), which belong to the class of aminoacyl tRNA synthetases (aaRSs). These enzymes are essential in all cells as they mediate ATP-dependent ligation of free amino acids to their corresponding tRNA molecules (Guo, Yang, and Schimmel 2010). The resulting charged aminoacyl-tRNAs are then used by ribosomes for the synthesis of protein (Guo and Schimmel 2013). While at first sight it may seem surprising that such a basal and ubiquitous enzyme is responsible for induction of primed defence following treatment with BABA, non-canonical functions of aaRSs are well described in other organisms ranging from *Drosophila* to mammals (Lee et al. 2004; Sampath et al. 2004; Kim, You, and Hwang 2011; Guo and Schimmel 2013). In fact, the acquisition of new functions via addition of novel domains to aaRSs has been suggested to be an important evolutionary mechanism implicated in the emergence of new phenotypic traits, including functions in the adaptive immune system of vertebrates (Guo, Yang, and Schimmel 2010). For example, GlnRS can inhibit the MAP3K APOPTOSIS SIGNAL-REGULATING KINASE1 (ASK1). Intriguingly, in Arabidopsis, MAP triple kinases such as EDR1 and MEKK1 play an important role in regulating plant immunity (Christiansen et al. 2011; Frye, Tang, and Innes 2001). Moreover, *edr1* mutants show a constitutively primed phenotype (van Hulten et al. 2006). It is, therefore, tempting to speculate that IBI1 could interact with a MAP3K, thereby inhibiting its immuno-suppressive activity.

1.5.2: The ‘four-stage model’ of IBI1-dependent regulation of BABA-IR

Based on confocal microscopy of Arabidopsis plants overexpressing YFP-tagged IBI1, a 4-stage model of IBI1-mediated BABA-induced resistance has been developed (Fig. 1.3). In unstressed cells, IBI1 localises to the endoplasmic reticulum (ER) and/or cytoplasmic strands, where it performs its canonical function as an aspartyl tRNA synthetase (stage I). While BABA treatment alone does not change the subcellular localisation of IBI1, it is postulated that binding of R-BABA to the enzyme weakens the attachment of IBI1 to the ER, thus priming its cytoplasmic translocation following pathogen attack (stage II). Upon perception of pathogen attack, a fraction of IBI1 translocates to the cytoplasm, where it contributes to basal resistance, presumably by interacting with additional regulatory proteins (stage III). In BABA-primed plants, where the connection of IBI1 to the ER has been loosened, the perception of pathogen attack leads to translocation of a greater fraction of IBI1 to the cytoplasm, resulting in faster and/or stronger defence activation (stage IV). This model postulates that the defence function of IBI1 is activated when the protein translocates to the cytoplasm, which brings the enzyme into a new protein environment that enables it to interact with specific defence regulatory protein partners.

1.5.3: R-BABA presumably binds to the L-aspartic acid binding site of IBI1

A possible explanation of how R-BABA interacts with IBI1 lies in the structural similarity between the canonical substrate of IBI1, L-aspartic acid, and the R-enantiomer of BABA. (Fig. 1.4). Using information from the co-crystallised structure of a bacterial AspRS and L-Asp, molecular interaction models predicted that R-BABA binds with high affinity to the L-Asp-binding domain of AspRS enzymes, whereas S-BABA showed binding in a different orientation and with lower affinity (Luna et al. 2014). In agreement with these *in silico* modelling results, treatment with R-BABA induced full resistance against *Hpa* in Arabidopsis, whereas S-BABA had only a modest effect (Luna et al. 2014). This finding supported previous findings that only the R-enantiomer of BABA induces resistance in tobacco, grapevine and lettuce (Cohen 1994; Cohen, Reuveni, and Baider 1999; Cohen, Rubin, and Vaknin 2011). Importantly, the study by Luna et al. (2014) also demonstrated *in planta* binding between active R-BABA and IBI1, using transgenic plants carrying a 35S:*IBI1-YFP* construct. Expression of this chimeric gene restores BABA-IR in *ibi1-1* plants, demonstrating full functionality of this fusion protein. Using an antibody against YFP, immunoprecipitation (IP) from extracts of water-, R-BABA- and S-BABA-treated plants only revealed the presence of R-BABA in the IP from R-BABA-treated 35S:*IBI1-YFP* plants. No BABA could be detected in the IP fraction of R-BABA-transformed wild-type plants or the IP fraction from water- and S-BABA-treated 35S:*IBI1-YFP* plants, providing direct evidence for the

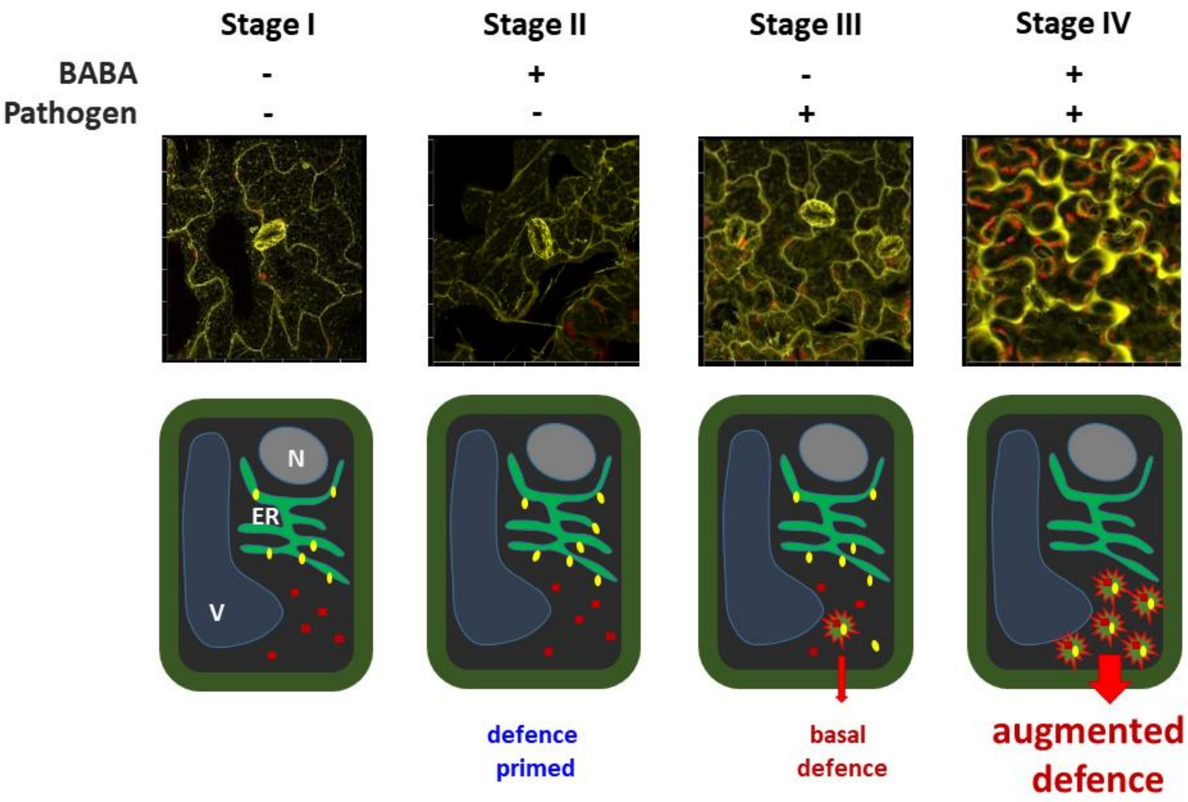

Figure 1.3: The four-stage model of IBI1-dependent BABA-IR. Based on confocal microscopy analysis of YFP-tagged IBI1, this model links the defence function of IBI1 in BABA-IR with the enzyme's subcellular localisation. The figure is adapted from Luna et al. (2014). The lower cartoons depict hypothesised cellular interactions, partially inferred from the microscopy analysis; see text for details. Yellow dots: IBI1. Red dots: cytoplasmic IBI1-interacting proteins with defence regulatory activity. ER: endoplasmic reticulum. V: vacuole. N: nucleus.

enantiomer-specific binding of BABA to IBI1 (Luna et al. 2014). However, experimental evidence that R-BABA specifically binds to the L-Asp-binding domain of IBI1 is still lacking. This would require analysing the activity and/or binding of R-BABA in transgenic *ibi1* mutant plants overexpressing IBI1 protein carrying a mutation in the L-Asp-binding domain.

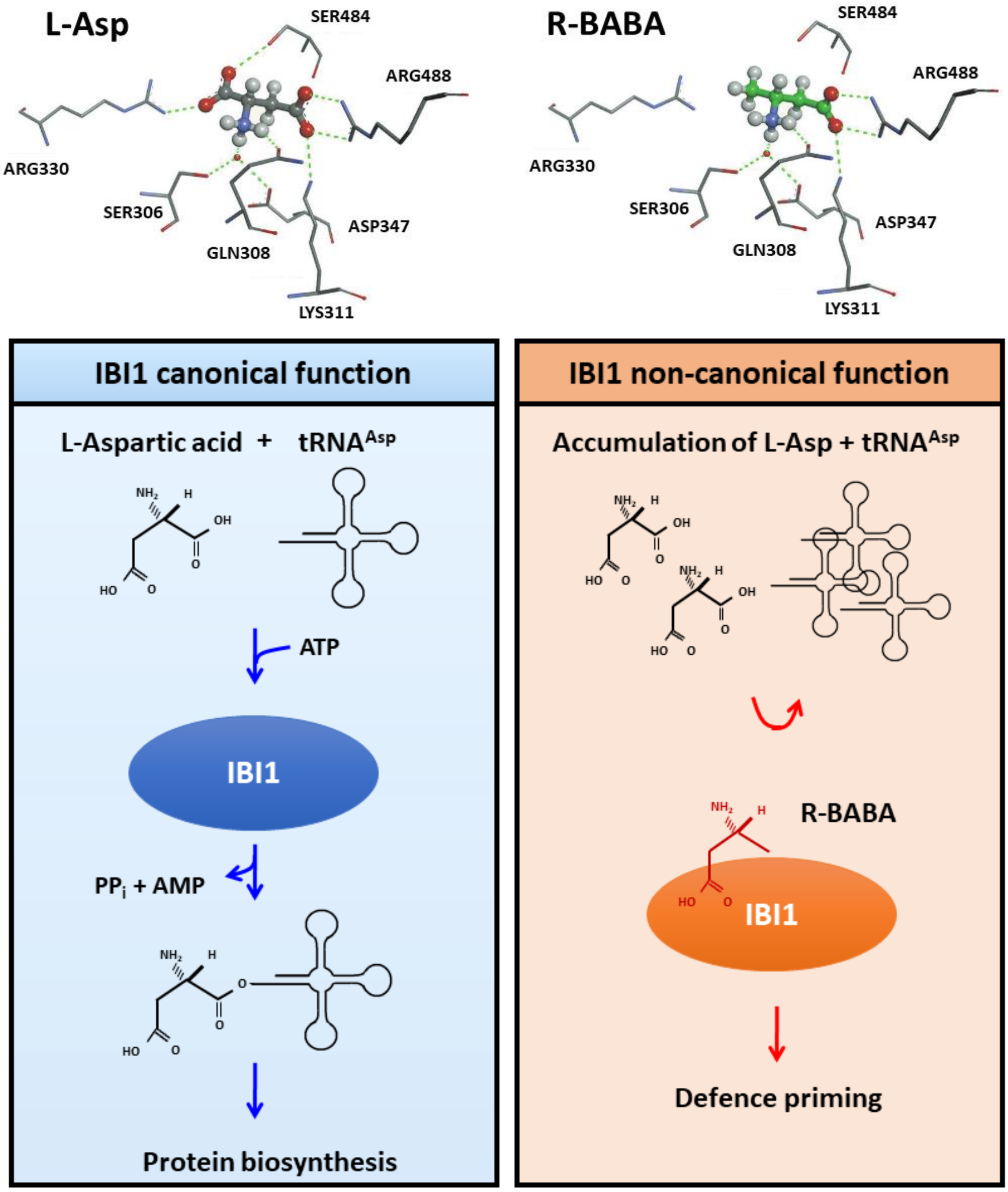

Figure 1.4: Model of the interaction between R-BABA and IBI1. Upper panel: *3D-interaction models showing key hydrogen bonds between IBI1 and L-Asp (left) or IBI1 and R-BABA (right). Models were taken from Luna et al. (2014).* ***Lower panel:*** *model of the canonical and non-canonical function of IBI1. In cells of un-treated plants (left), IBI1 acts as an Asp-tRNA synthetase (AspRS), catalysing the ATP-consuming synthesis of L-Asp-tRNA*^{Asp}*. In cells of BABA-treated plants (right), R-BABA binds to the L-Asp-binding site of IBI1, priming the enzyme for its non-canonical defence function upon pathogen attack. As a side effect, the binding of R-BABA to IBI1 leads to enhanced accumulation of free aspartic acid and uncharged tRNA*^{Asp} *in the cell. Consequently, high concentrations of R-BABA repress canonical AspRS activity in the cell, leading to a specific stress response.*

1.5.4: BABA-induced resistance and BABA-induced stress are controlled by separate pathways

The model emerging from the above findings is that binding of R-BABA to IBI1 interferes with L-Asp binding, loosening the attachment of IBI1 to the ER and priming the enzyme for pathogen-induced translocation into the cytoplasm, where it assumes its non-canonical function in defence (Fig. 1.4). This model predicts that R-BABA – IBI1 interaction leads to enhanced accumulation of free L-Asp and uncharged $tRNA^{Asp}$. Indeed, mass spectrometry-based quantification of L-Asp confirmed increased levels of this amino acid in R-BABA-treated plants, but not in S-BABA-treated plants (Luna et al. 2014). While direct quantification of uncharged $tRNA^{Asp}$ is more difficult, it is known that its accumulation activates the protein kinase GCN2, which results in phosphorylation of eIF2α (Dever and Hinnebusch 2005). Using phosphorylated eIF2-α as a proxy of uncharged tRNA accumulation, Western blot analysis confirmed that BABA treatment greatly induces tRNA-dependent GCN2 activity (Luna et al. 2014). Phosphorylation of eIF2α leads to large-scale remodelling of cellular metabolism and inhibition of protein synthesis (Dever and Hinnebusch 2005). Competition between L-Asp and R-BABA for binding to IBI1 thus provides a possible explanation for the BABA-induced stress phenotype. Corroborating the hypothesis that BABA-induced GCN2 activity is responsible for BABA-induced growth repression, *gcn2-1* mutant plants showed little growth inhibition upon treatment with BABA (Luna et al. 2014). Strikingly, the *gcn2-1* mutant was unaffected in BABA-IR against *Hpa*. Conversely, while the *ibi1-1* mutant failed to show BABA-IR, it was hyper-sensitive to BABA-induced stress, presumably due to its reduced cellular AspRS capacity (Luna et al., 2014). Thus, IBI1 controls BABA-IR and BABA-induced stress via two independent pathways.

1.5.5: The Arabidopsis genome harbours several aspartyl-tRNA synthetases

Since the *ibi1-1* mutant does not produce functional IBI1 due to a premature STOP codon in the first exon of the *IBI1* gene, the fact that this mutation is not lethal suggests gene redundancy for the canonical AspRS activity of IBI1. Arabidopsis harbours three isoforms of Asp-tRNA synthetase, encoded by *At4g31180* (*IBI1*), *At4g26870* and *At4g33760*. While *At4g33760* encodes a prokaryotic-like AspRS enzyme that is likely active in chloroplasts, *IBI1* and *At4g26780* encode eukaryotic-like AspRS isoforms with 74.2% amino acid identity (Duchêne et al. 2005; Luna et al. 2014). Since BABA-IR is completely abolished in the *ibi1-1* mutant, it has been suggested that its homologue can substitute the canonical AspRS function of IBI1, but not its non-canonical defence function in BABA-IR (Luna et al., 2014). Interestingly, IBI1 contains several unique sequences that are absent in the homologue. The addition of novel domains to aminoacyl tRNA synthetases has been suggested to confer new functions to these proteins (Guo, Yang, and Schimmel 2010).

1.6 Scope of the PhD thesis

Various mechanisms and pathways contributing to BABA-IR have been suggested, including phytohormone-dependent signalling and modulation of primary metabolism (Zimmerli et al. 2000; Ton and Mauch-Mani 2004; Victoria Pastor et al. 2014). Most of these studies revealed partial involvements of major defence signalling components, pointing to a mechanism by which BABA-induced augmentation of these basal defence mechanisms contributes to BABA-IR. However, little is still known about the early signalling steps from the molecular perception of BABA via its receptor IBI1 to the expression of the augmented defence response during BABA-IR. Furthermore, the effects of BABA-induced stress could have confounded previous reports about the signalling defence pathways contributing to BABA-IR. The overarching aim of this PhD study was to dissect the molecular pathways controlling IBI1-dependent BABA-IR, and to separate these from the responses that are caused by BABA-induced stress. To this end, two complementary approaches were used. Firstly, a bottom-up approach was followed, studying the roles of the primary amino acid sequence of IBI1, and of IBI1-interacting proteins in BABA-IR. Secondly, a top-down approach was used, in which global transcriptomic and metabolomic impacts of IBI1-dependent BABA-IR were studied.

As described above, while molecular docking models of R-BABA and AspRS enzymes suggest a critical role for the L-Asp-binding domain of IBI1 in plant responses to BABA, experimental evidence for this hypothesis is lacking. Furthermore, the lack of gene redundancy for the non-canonical defence function of IBI1 in BABA-IR suggests that IBI1-specific amino acid sequences that are absent in a close AspRS homologue of IBI1 (At4g26870; hereafter referred to as HM1) are critical for the role of IBI1 in plant immunity. To address the importance of these protein domains, Chapter 3 describes a mutational approach of the IBI1 protein. Site-directed mutagenesis of the L-Asp-binding domain of IBI1 revealed that this domain is critical for both BABA-IR and tolerance to BABA-induced stress. To identify the role of IBI1-specific amino acid sequences, chimeric gene constructs were generated between *IBI1* and its close gene homologue *HM1* and tested for complementation of BABA-IR and BABA tolerance in the *ibi1-1* mutant. While some chimeric gene constructs failed to complement the *ibi1-1* mutant phenotype, this Chapter provides evidence that the ability of IBI1 to mediate BABA-IR and BABA tolerance is a general property of AspRS enzymes that is determined by the level of gene expression, rather than IBI1-specific amino acid sequences.

Defence signalling cascades in plants crucially rely on protein interactions (Asai et al. 2002). Chapter 4 describes the results of a yeast two-hybrid screen to identify interaction partners of IBI1. Overall, the screen identified 25 putative partners. Proteins with robust expression levels in leaves (the tissues where IBI1-dependent BABA-IR is assayed) and predicted roles in plant immunity were confirmed as IBI1 interaction partners using bimolecular fluorescence complementation (BiFC) assays. Chapters 5 and 6 follow leads from the most promising IBI1-interacting proteins identified in Chapter 4. Chapter 5 describes the role of the IBI1-interacting VOZ transcription factors in BABA-IR, based on phenotypic characterisation of (double) mutants and overexpression lines of the corresponding *VOZ* genes. This analysis revealed a role for the VOZs in SA-independent BABA-IR against *Hpa*, mediated by increased efficiency of callose deposition. Chapter 6 describes the involvement of photorespiration, based on the finding that the plastidic NAD-dependent malate dehydrogenase plNAD-MDH was identified as an interactor of IBI1. While genetic manipulation of the *MDH* gene was avoided to prevent severe developmental phenotypes that would complicate reliable interpretation of *Hpa* patho-assays, Chapter 6 provides evidence that interference with photorespiration by knock-down mutations in glycolate oxidase genes affects BABA-IR against *Hpa*.

Chapter 7 describes the results of a combined transcriptome and metabolome analysis aimed at identifying pathways which contribute to BABA-induced resistance. Previous studies on the transcriptomic and metabolomic impact of BABA did not distinguish between the immune-priming and stress-inducing effects of BABA (Van der Ent et al. 2009; Tsai et al. 2011; Pastor et al. 2014). By taking advantage of the fact that BABA-treated *ibi1-1* mutant plants do not respond with augmented defence to pathogen attack, but show a hyper-susceptible stress response to BABA, the effects of these independent responses could be separated. While the exaggerated stress response of the *ibi1-1* mutant dominated the transcriptional differences between the wild-type and the mutant during BABA-IR against *Hpa*, the global transcriptomic pattern revealed that the expression of IBI1-dependent BABA-IR is associated with a genome-wide repression of ABA/abiotic stress-responsive genes. Furthermore, mass-spectrometry analysis of metabolic profiles during expression of BABA-IR against *Hpa* suggested IBI1-dependent activity of pathways that converge at the xanthophyll cycle. This cyclic pathway is essential for the safe release of excessive excitation energy via non-photochemical quenching (NPQ). Direct chlorophyll fluorescence measurements showed different levels of NPQ in Col-0 wild-type and *ibi1-1* mutant plants during expression of BABA-IR, thus supporting the results obtained in the mass spectrometry analysis. Interestingly, the compounds involved in the xanthophyll cycle are direct precursors of abscisic acid. Thus, both the transcriptome and metabolome analysis in this Chapter point to a complex role of regulation of ABA-mediated signalling in BABA-IR. This role is further discussed in Chapter 8, the General Discussion, which places the main findings of this thesis in a wider context of environmental signalling in plants.

Chapter 2

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Materials and methods

2.1 Plant material and growth conditions

Unless stated otherwise, *Arabidopsis thaliana* (Arabidopsis) for bioassays and gene expression assays were sown in 60 mL pots containing a mixture of Levington M3 soil and sand (2:1 v/v). The soil was saturated with tap water, and seeds were stratified at 4°C in the dark for 2 to 3 days. Plants were subsequently cultivated at 8.5 h light (115-140 µmol/m^2/s, 21°C) and 15.5 h darkness (19°C) at 70-80% relative humidity (RH) in controlled growth chambers. Long day conditions to promote flowering were at 15.5 h light (115-140 µmol/m^2/s, 21°C) and 8.5 h darkness (19°C) at 70-80% RH. *Nicotiana benthamiana* seeds were sown on wetted paper tissue in Petri dishes, and kept at 15.5 h light (115-140 µmol/m^2/s, 21°C) and 8.5 h darkness (19°C) in controlled growth chambers. Seven to ten day-old *N. benthamina* seedlings were transplanted individually into 60 mL pots containing a mixture of Levington M3 soil and sand (2:1 v/v). Leaf infiltration assays were performed on 4-6 week old plants (i.e. before plants developed flowers).

2.2 Cloning of genes in expression vectors

Expression vectors in the pEarleygate101-YFP (pEG101, Earley et al. 2006) plasmid were created as follows: coding sequences (CDSs) of the respective genes were cloned from Arabidopsis Col-0 cDNA, using gene specific primers (see Appendix 5 for a full list of all primers). The sequence 'CACC' was added at the 5'-end of the forward primer to allow subsequent topoisomerase-mediated ligation into the entry plasmid pENTR. Reverse primers were designed to omit the STOP codon and to allow the C-terminal addition of YFP tags (see Appendix 5 for primer sequences). Phusion® High-Fidelity DNA Polymerase (New England Biolabs, M0530L) was used to amplify the CDSs from cDNA. Phusion® High-Fidelity DNA Polymerase is a novel Pyrococcus-like DNA polymerase, which has a 50-fold lower error rate than *Thermus aquaticus* (*Taq*) DNA polymerase, was used according to the manufacturer (https://www.neb.com/products/m0530-phusion-high-fidelity-dna-polymerase, accessed on 21/08/2017). This DNA polymerase possesses 3'-5' exonuclease ('proof-reading') activity, and generates blunt-ended products. Volumes of 20 µl were set up for polymerase chain reactions (PCR), according to manufacturer's recommendations (without DMSO), using following PCR program used:

Initial denaturation	98°C	30 seconds
30 cycles	98°C 57-64°C (depending on specific primer T_m) 72°C	10 seconds 20 seconds 30 seconds
Final extension	72°C	10 minutes
Final hold	4°C	

The PCR product was subsequently cloned into a Gateway®-compatible pENTR vector, using a pENTR™/D-TOPO® Cloning kit (Invitrogen, K243520). Typically, a yield of ~7 ng/µl was achieved by the Phusion PCR. Two µl of the PCR reaction were loaded and separated by electrophoresis on a 1.5% agarose gel containing 0.1 µg/mL ethidium bromide, after which the PCR product was extracted from the gel and purified for a TOPO® cloning reaction using a QIAquick gel extraction kit (QIAGEN, Cat No. 28704) , according to the manufacturer's protocol. Two µl of the cloning reaction was transformed into Mach1™-T1^R chemically competent *E. coli* cells via heat-shock. Bacterial cells were incubated on ice for 20-30 minutes in the presence of the cloning reaction product, heat-shocked at 42°C for 30 seconds, and transferred back onto ice. Cells were re-suspended in 250 µl room-temperature S.O.C. medium, incubated at 37°C for 1 hour at a shaking platform (220 rpm), after which 20 and 100 µl of the transformation suspension was plated onto selective agar (LB + kanamycin, 50 µg/mL). Plates were incubated at 37°C overnight. Individual colonies were tested the following day for presence of the recombined pENTR vector via colony PCR, using a gene-specific Forward primer, and an M13 Reverse primer, which binds to the pENTR backbone (see Appendix 5 for primer sequences). The following settings were used for colony PCRs:

Initial denaturation	95°C	12 minutes
35 cycles	92°C 57°C 72°C	30 seconds 30 seconds 1 minute
Final extension	72°C	10 minutes
Final hold	10°C	

PCR products were separated and visualised by agarose gel electrophoresis on 1-1.5% agarose gels, containing 0.1µg/mL ethidium bromide. Successfully transformed colonies were resuspended in 10 or 50 mL liquid LB medium, containing 50 µg/mL kanamycin, and incubated at 37°C overnight. Glycerol stocks (15% v/v/) were prepared from bacterial overnight cultures and stored at -80°C. Recombined pENTR plasmids were isolated from 5 mL of the bacterial overnight cultures, using a GeneJET Plasmid Miniprep kit (Thermo Scientific, K0503). Sequences of CDS inserts were verified by Sanger sequencing, using Eurofins' tube sequencing service (Eurofins Genomics, Ebersberg, Germany). After sequence verification, gene inserts were cloned into pEarleygate101 (pEG101), using Gateway® cloning. However, since pENTR and pEarleygate101 both use kanamycin resistance as selection marker, selection of *E. coli* cells carrying transformed pEarlygate101 vectors was impossible. Therefore, the CDS plus flanking attL1/attR1 sites of the pENTR vector was first amplified by PCR, using M13 Fwd + Rev primers (see Appendix 5 for primer sequences) and Phusion High Fidelity DNA polymerase. The resulting PCR product was then recombined into pEG101 using the Gateway® LR Clonase® II Enzyme mix (Invitrogen, 11791100) according to manufacturer's protocol. The recombinant pEG101 was transformed into Mach1™-T1^R chemically competent *E. coli* via heat-shock transformation, as described above for the pENTR vectors, after which 50-200 µl of the transformation was plated onto selective agar (LB + 50 µg/mL kanamycin). Plates were incubated at 37°C overnight. Individual colonies were tested the following day for the presence of the recombined pEG101 vector via colony PCR, using a gene-specific Forward primer, and Reverse primer Gen007 (see Appendix 5), which binds to the YFP coding sequence of pEG101. As described above for pENTR clones, positive colonies were grown overnight to prepare glycerol stocks. Recombinant pEG101 plasmids containing the inserted CDSs were isolated by miniprep (GeneJET Plasmid Miniprep kit, Thermo Scientific, K0503).

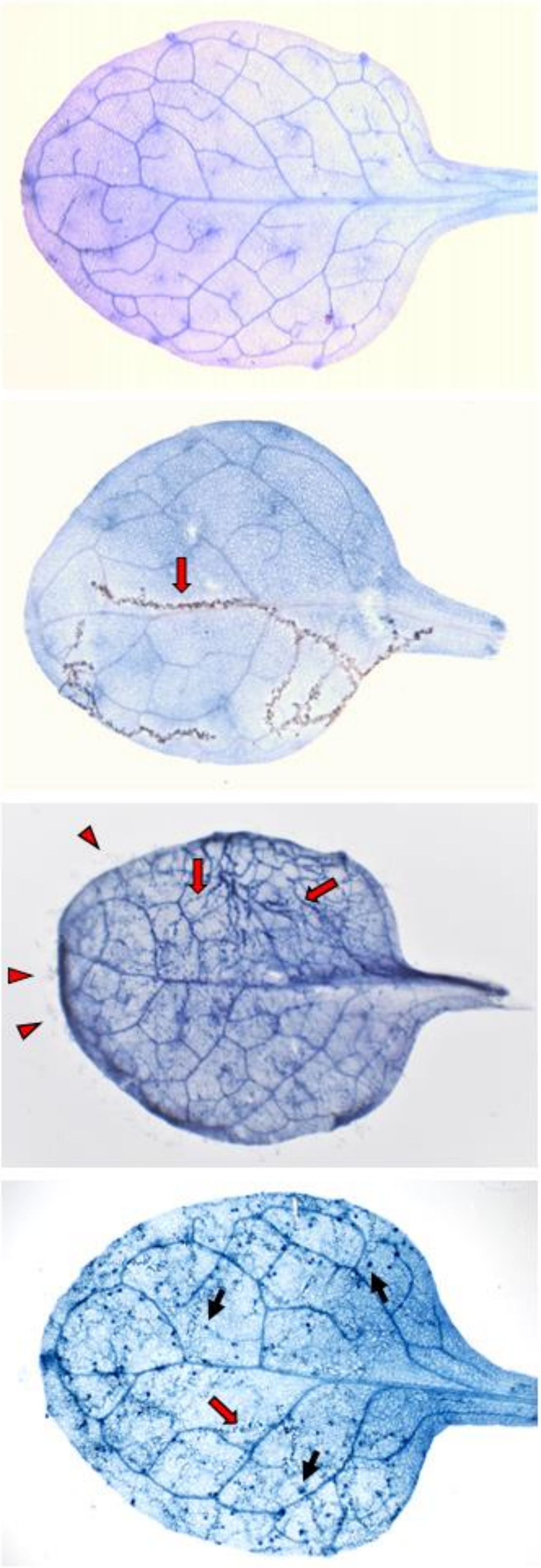
2.3 Generation of transgenic Arabidopsis lines by floral dip transformation

Transgenic Arabidopsis lines were generated by the floral dip transformation method, described by Clough and Bent (Clough and Bent 1998). *Agrobacterium tumefaciens* strain GV3101::pMP90 was transformed with expression plasmids via electroporation. To this end, 5 to 20 µl of the transformation reaction was plated onto selective agar (LB + 50 µg/mL kanamycin + 50 µg/mL rifampicin + 50 µg/mL gentamicin, and the plates were incubated at 28°C for 2 days. The resulting colonies were tested for presence of the recombined pEG101 vector via colony PCR, using a gene-specific Forward primer, and Reverse primer Gen007, as described above for *E. coli.* Overnight cultures of successfully transformed cultures were incubated in liquid LB medium, supplemented with kanamycin, rifampicin and gentamicin, at 28°C, and 15% glycerol stocks were prepared from these cultures. Of these overnight cultures, 50 mL was centrifuged for 15 minutes at ~1,400 x *g*. The pellet was then re-suspended in transformation solution (2.5 g sucrose in 50 mL tap water, pH~7.0, plus 10 µl Silwet L-77 [‘Vac-in-Stuff’, Lehle Seeds, Cat. No VIS-30]). Inflorescences of approx. 5- to 6-week old *ibi1-1* Arabidopsis were immersed in the transformation solution for 2 minutes. After transformation, plants were placed horizontally on wetted paper towels, and kept at 100% relative humidity (RH) in darkness. The next day, plants were put upright, and humidity was reduced to ~60% RH. Plants were kept under long-day conditions (15.5 h light per day) until siliques were mature. Siliques were dried at 28°C before seeds were harvested. T1 seeds were mass-sown on soil, stratified and grown under short-day conditions, as described above. Successfully transformed seeds were selected by repeated spraying with 125 mg/L glufosinate-ammonium (Pearl, SKU:1345, Agrigem Ltd., UK). Glufosinate-resistant seedlings were transplanted into individual pots and kept under long-day conditions (15.5 h per day) to induce flowering. Seeds were harvested from individual glufosinate-resistant plants and individual T2 lines were tested for complementation of germination on β-aminobutyric acid (BABA)-supplemented agar plates and BABA-induced resistance, as described below.

2.4 BABA-induced resistance assays.

To quantify basal and BABA-induced resistance (BABA-IR), 2 week-old seedlings grown in ~60 mL soil were soil-drenched by injecting 6 mL of 10x concentrated BABA solutions (racemic mixture of R-/S-BABA; Sigma-Aldrich A44207) or an equal volume of water (control). Figure legends and texts refer to the estimated final BABA concentrations in the soil, ranging from 2.5 to 10 mg/L. At 2 days after soil-drench treatment, seedlings were inoculated by spraying a suspension of $5x10^4$ - 10^5 conidiospores of *Hyaloperonospora arabidopsidis* (*Hpa*) pathovar WACO9 onto the shoots. The pathogen was maintained on hyper-susceptible WS *NahG* seedlings and transferred to fresh host plants on a weekly basis. Conidiospores were collected from hyper-susceptible WS *NahG* plants by washing them off in distilled water and filtering through miracloth (Merck Millipore). Spore density was determined in a hemocytometer (Beckman Coulter). Spore density was adjusted to $5x10^4$ - 10^5 conidiospores/mL and plants were sprayed with the spore suspension until run-off. Since lack of oxygen can inhibit sporulation of conidiospores suspended in water, inoculated plants were air-dried by keeping the lid off the tray for 5-30 minutes after spraying. Trays were then sealed with parafilm to maintain at 100% RH, which facilitates *Hpa* germination.

Disease resistance was determined by measuring *Hpa* colonisation in seedlings at 5 or 6 days post inoculation (dpi). To this end, 20 - 50 seedlings were stained with lactophenol trypan blue, as described previously (Koch and Slusarenko 1990). This method stains fungal cell walls, dead plant cells and, to a lesser extent, leaf vasculature and can distinguish between various degrees of *Hpa* pathogenesis and host resistance. To quantify the extent of *Hpa* colonisation, approximately 100 individual leaves per condition were assigned to one of four different colonisation classes (Fig. 2.1): I = healthy leaf, no sporulation; II = hyphal growth, <8 conidiophores; III = hyphal growth, and more than 8 conidiophores; IV = extensive hyphal growth, conidiophores and oospores present. Differences in class distribution between samples were tested for statistical significance by Fisher's exact test, using SPSS software (IBM SPSS statistics 22).



☐ **Class I**

healthy leaf, no sporulation

🟦 **Class II**

hyphal growth (arrow)

<8 conidiophores

🟦 **Class III**

hyphal growth (red arrows)

more than 8 conidiophores (arrowheads)

■ **Class IV**

extensive hyphal growth (red arrows)

conidiophores and oospores (black arrows)) present

Figure 2.1: Classification of trypan blue-stained leaves according to H. arabidopsidis *colonisation.*

Defence effectiveness of callose deposition was analysed at 2 or 3 dpi, as described by Ton et al., 2005. Seedlings were harvested and stored in 96% ethanol for at least 24 hours to clear leaves. Once samples were completely transparent, they were incubated in 0.07 M phosphate buffer (pH = 9.0) for 30 minutes. Samples were then incubated for 15 minutes in a 4:1 mixture of 0.05% aniline blue (w/v) and 0.005% calcofluor (w/v) in phosphate buffer, followed by overnight incubation in phosphate buffer containing 0.05% aniline blue (w/v), in order to wash off excess calcofluor. Samples were mounted on microscope slides in a matrix of phosphate buffer containing 0.05% (w/v) aniline blue and analysed on an Olympus BX51fluorescence microscope (excitation filter 330 nm wide band, emission filter 400 nm LP, dichromatic filter 400 nm). Effectiveness of callose deposition was determined by classifying conidiospores on 8-10 leaves per treatment/genotype combination into one of the following 5 categories: 1 = spore did not germinate and was not associated with callose; 2 = spore germinated with the emerging germ tube/hyphae not associated with callose deposition, indicating lack of callose-mediated defence; 3 = spore germinated with emerging germ tube/hyphae trailed by callose, suggesting that the callose deposits were ineffective in arresting colonisation; 4 = spore germinated with the proximal end of the emerging germ tube fully encapsulated in callose, indicating effective arrestment of colonisation; 5 = spore without visible germ tube, but fully encapsulated by callose, indicating early effective arrestment of colonisation. Callose effectiveness is expressed as the average percentage of germinated conidiospores that were arrested by callose deposition: [number of spores in classes 4+5]/ [number of spores in classes 2+3+4+5]. Differences in class distribution between samples were tested for statistical significance by Fisher’s exact test, using SPSS software (IBM SPSS statistics 22).

2.5 Germination assays to assess BABA tolerance.

Seeds were stratified in Eppendorf tubes filled with distilled water and kept for 2 days at 4°C prior to sowing on ½ MS plates with and without 50 mg/L BABA (racemic mixture of R-/S-BABA; Sigma-Aldrich A44207). Seedlings were allowed to grow for one to two weeks before germination rates were calculated. Where appropriate, transgene expression in seedlings was confirmed by assessing YFP fluorescence under a Leica stereomicroscope (Leica MZ FLIII fluorescence stereomicroscope, excitation filter BP 470/40 nm, barrier filter BP 525/50 nm).

2.6 Gene expression assays.

For gene expression analysis, the above-ground parts of 4 - 10 seedlings were snap-frozen in liquid nitrogen, homogenised in 2 mL tubes containing 3 metal beads, and RNA extracted using the Trizol extraction method as described by López Sánchez et al., 2016. In brief, samples were collected into a 2-mL Eppendorf tube with 3 stainless steel balls in liquid nitrogen. Samples were homogenised by shaking 2 x 45 seconds in an 8000M mixer/mill (Glen Creston Ltd.). Homogenised samples were re-suspended in 1 mL trizol by vortexing, and incubated at room temperature for 5 - 20 minutes. All subsequent steps were performed on ice/in cooled centrifuges to minimise RNA degradation. Samples were centrifuged at 17,000 x *g*, 5 minutes, 4°C. Supernatant was transferred to new Eppendorf tubes containing 220 µl chloroform, and vortexed for 15 seconds. After centrifugation (17,000 x *g*, 5 minutes, 4°C), the aqueous upper phase was transferred to new Eppendorf tube containing 350 µl of isopropanol and 350 µl of 0.8 M citrate/1.2 mM NaCl. Samples were mixed by inverting several times, and kept at room temperature for 10 minutes. RNA was precipitated by centrifugation (17,000 x *g*, 5 minutes, 4°C), washed twice with 1 mL of 70% ethanol, and re-suspended in 50 µl nuclease-free water. The quality and concentration of the extracted RNA were assessed on a NanoDrop™ spectrophotometer. A 260/280 nm absorption ratio of 1.5 was considered to be an acceptable minimum purity of RNA to be used for reverse transcription quantitative PCR (RT-qPCR). RNA concentrations were standardised by diluting to 100 ng/µl and treated with DNase (RQ-RNase free DNase, M6101, Promega) to degrade contaminating genomic DNA. The cDNA synthesis was performed with 800 ng RNA, using oligo(dT) primers and SuperScript III reverse transcriptase (Invitrogen, 18080085) according to the manufacturer’s protocol. The obtained solution of cDNA was diluted 1:10 before use in qPCR reactions.

RT-qPCR reactions were performed with a RotorGene Q real-time PCR cycler (Qiagen; Q-Rex Software v1.0), using the Rotor-Gene SYBR® Green Kit (Qiagen, Cat No. 204074), and gene specific primer pairs at a final concentration of 250 nM (see Appendix 5 for primer sequences). The following 2-step PCR settings were used for all qPCRs:

Initial denaturation	95°C	10 seconds
40 cycles	90°C 60°C	10 seconds 45 seconds

The specificity of all primers used was verified by assessing melting curves of PCR products. Comparative quantitation of gene transcripts was calculated using PCR efficiency correction and normalisation to two or three established housekeeping genes (see below). Briefly, for each primer combination, fold-changes in comparison to an arbitrary calibrator sample were calculated as [PCR efficiency]$^{Ct1\text{-}Ct2}$, where Ct (take-off cycle) was defined as the cycle in which the increase in fluorescence is 20% of the peak increase in fluorescence (QIAGEN 2012). PCR efficiencies (1 + E) were estimated for each PCR reaction separately ('per tube' estimation). Relative transcript values of samples were normalised to the mean relative transcript value of control samples (usually from water-treated, mock-inoculated wild-type plants), as indicated in figure legends. *GAPDH (*At1g13440*), UBC21 (*At5g25760*)* and *SAND family protein (*At2g28390*)* were used as housekeeping genes (Czechowski et al. 2005). Primer sequences are listed in Appendix 5.

2.7 Yeast Two-Hybrid Analysis.

To identify protein interaction partners of IBI1, a yeast two-hybrid (Y2H) approach was used. Due to the technical difficulties and time requirements involved in setting up large-scale Y2H screens, the screening itself was carried out by Hybrigenics Services, S.A.S., Paris, France (http://www.hybrigenics-services.com). The coding sequence for Arabidopsis - IBI1 (aa 1-558) (NCBI reference NM_119268.4) without STOP codon was PCR-amplified from pENTR-IBI1 and cloned into pB29 as an N-terminal fusion to LexA DNA-binding domain (IBI1-LexA; first screen) and into pB43 as an N-terminal fusion to the Gal4 DNA-binding domain (IBI1-Gal4; second screen). Before screening, constructs were verified by sequencing. The LexA construct was used as a bait to screen a random-primed Arabidopsis seedlings library, whereas the Gal4 construct was used as a bait to screen a dT primed Universal Arabidopsis Normalized cDNA library from Clonetech. This latter library consists of cDNAs from poly-A^+ RNAs isolated from a range of Arabidopsis Col-0 tissues (intact seedlings with roots, taken every 4hr over a 24h period and again at 10 days, from long and short day grown seedlings; etiolated seedlings [grown for 5 days in the dark]; open flowers; closed flower buds; pollen; siliques at various developmental stages; leaves from plants grown in soil, before and after bolting; stems). Importantly, the mRNA used for the Clontech library was normalised prior to library construction by a combination of duplex-specific nuclease and SMART technology, resulting in less bias towards highly expressed transcripts and more reliable detection of low-abundance interaction partners. The normalised cDNA was cloned into pGADT7-RecAB, resulting in a collection of Y2H prey plasmids encoding the fusion protein N – NLS_{SV40} - GAL4-AD – HA tag - prey – C. pB29, pB43 and pP6 derive from the original pBTM116 (Vojtek and Hollenberg 1995; Béranger et al. 1997), pAS2ΔΔ (Fromont-Racine, Rain, and Legrain 1997) and pGAD.GH (Bartel and Fields 1995) plasmids, respectively. An initial series of test runs without 3-aminotriazol in the selection medium was carried out to verify that the IBI1 - GAL4-DBD fusion protein is toxic nor auto-activating. For the LexA bait construct, 72 million clones (7-fold the complexity of the library) were screened, using a mating approach with YHGX13 (Y187 ade2-101::loxP-kanMX-loxP, matα) and L40ΔGal4 (mata) yeast strains, as described previously (Fromont-Racine, Rain, and Legrain 1997). Thirty His+ colonies were selected on a medium lacking tryptophan, leucine and histidine. For the Gal4 construct, 79.8 million (7-fold the complexity of the library) clones were screened, using a mating approach with YHGX13 (Y187 ade2-101::loxP-kanMX-loxP, matα) and CG1945 (mata) yeast strains, as described described (Fromont-Racine, Rain, and Legrain 1997). Two hundred and thirty three His+ colonies were selected on a medium lacking tryptophan, leucine and histidine. The prey fragments of the positive clones were amplified by PCR and sequenced at their 5' and 3' junctions. The resulting sequences were used to identify the corresponding interacting proteins in the GenBank database (NCBI), using a fully automated procedure. A confidence score (PBS, for Predicted Biological Score) was attributed to each interaction as previously described (Formstecher et al. 2005). This PBS score is determined by two different parameters. Firstly, a local score takes into account the redundancy and independency of prey fragments, as well as the distribution of reading frames and stop codons in overlapping fragments. Secondly, a global score takes into account the interactions found in all screens performed at Hybrigenics, using the same library. This global score represents the probability of an interaction being nonspecific. For practical use, the scores were divided into four categories, from A (highest confidence) to D (lowest confidence). A fifth category (E) specifically flags interactions involving highly connected prey domains previously found several times in screens performed on libraries derived from the same organism. Finally, several highly connected protein domains have been confirmed as false-positives of the technique, and have therefore been assigned to class F. The PBS scores have been shown to correlate positively with biological significance of interactions (Rain et al. 2001; Wojcik, Boneca, and Legrain 2002).

2.8 Bimolecular Fluorescence Complementation (BiFC) analysis.

Putative protein interactions detected in the yeast two-hybrid screen described above were confirmed by Bimolecular Fluorescence Complementation (BiFC) assays in *Nicotiana benthamiana*, as follows. Coding sequences (CDSs; without STOP codon) of the putative interaction partners were cloned into pENTR, transformed into *E. coli* and sequenced, as described above (section 2.2). CDSs plus flanking attL1/attR1 sites were amplified by PCR from the pENTR, using M13 Fwd/Rev primers (Appendix 5). The resulting PCR products were recombined into the Gateway-compatible BiFC vectors pEarleygate201-YN (pEG201-YN) and pEarleygate202-YC (pEG202-YC). After amplification of the recombinant BiFC plasmids in *E. coli* Mach1™-T1^R cells, *A. tumefaciens* strain GV3101::pMP90 was transformed with these plasmids via electrotransformation. Transformed Agrobacteria were selected on LB agar plates, supplemented with kanamycin, rifampicin and gentamicin, as described above (section 2.3). The presence of the plasmids was confirmed via colony PCR, using a gene-specific Forward primer, and pEG201-YN Rev (for pEarleygate201-YN) or Gen007 (for pEarleygate202-YC) Reverse primer (Appendix 5).

A. tumefaciens cell suspensions carrying the appropriate recombinant plasmids were infiltrated into leaves of 4-6 week old *N. benthamiana*. To this end, frozen glycerol stocks of *A. tumefiaciens* strains were inoculated in 7 mL of LB medium (supplemented with 50 µg/mL kanamycin, 50 µg/mL rifampicin and 50 µg/mL gentamicin) and grown for 2 days at 28°C at a shaking platform (200-220 rpm). Optical densities at 600 nm (OD_{600}) of cultures were measured with a Jenway 7200 spectrophotometer (Cole-Parmer, Part Code 720001). Appropriate volumes of bacterial cultures were precipitated by centrifugation at 3,000 *g* for 10 minutes, washed once in resuspension solution, and centrifuged again at 3,000 *g* for 10 minutes. The resulting pellets were re-suspended in appropriate volumes of resuspension solution to adjust the OD_{600} of each culture to 1.0. Subsequently, cultures containing complementary pEG201-YN/pEG202-YC plasmids were mixed as follows:

- 400 µl of Agrobacterium containing gene of interest in pEG201-YN backbone
- 400 µl of Agrobacterium containing gene of interest in pEG202-YC backbone
- 200 µl of Agrobacterium containing pBin-p19

The pBin-p19 plasmid is a binary vector containing the sequence of tomato bushy stunt virus (BSV) p19, which suppresses silencing of the transgene after infiltration. Bacterial mixtures were incubated at room temperature with gentle shaking (~50 rpm) on an orbital shaker (Stuart Scientific Gyro rocker

STR9, Z316520 ALDRICH, Sigma-Aldrich) for 2-3 hours. Leaves of 4-6 week-old *N. benthamiana* plants were infiltrated with approximately 250 µl of the *A. tumefaciens* mixtures. Each BiFC pair was infiltrated in at least two individual leaves. Two or three days after infiltration, interactions were assessed by mounting three leaf discs (6 mm diameter) from at least two individually infiltrated leaves on microscope slides in a matrix of water. Fluorescence complementation was assessed using an Olympus BX51 fluorescence microscope (light source: CoolLED pE-2 at 470 nm; filter: U-MWIBA2 [excitation 460-490 nm, emission 510-550 nm]).

2.9 Site-directed mutagenesis of IBI1.

A point mutation converting the glutamine residue (Gln308) in the L-Asp-binding site of IBI1 into an apolar alanine residue (Gln308Ala) was introduced in IBI1, using the Phusion Site-Directed Mutagenesis kit of ThermoScientific (UK; #F-541). PCR was carried out on a pENTR plasmid (Invitrogen, UK; # K243520), containing a 1674 bp wild-type coding sequence of IBI1 without stop (Luna et al. 2014), using the following 5'-phosphorylated primers:

Reverse wild-type primer: 5'- ACTGAGCTAGACAAGCAGGTTG -3'

Forward mutant primer: 5'-CTCCTGCTCTCCACAAGCAAATGGC -3'

After ligation following the manufacturer's protocol, the pENTR plasmid was transformed into *E. coli* (One-shot Mach1TM-T1R Chemically Competent *E. coli*, Invitrogen, UK; #C8620-03) to confirm the mutation in the *IBI1* cDNA. A PCR-amplified fragment (M13 Fwd / M13 Rev) from the plasmid with boarding attL1 and attL2 sequences was used for used for recombination into pEarleyGate-101, as described above in section 2.3. Transformation of *Agrobacterium tumefaciens* GV3101::pMP90 and Arabidopsis *ibi1-1* was carried out as described in section 2.3. Selected T2 progeny were verified for monogenic segregation of glufosinate resistance and YFP fluorescence (Leica MZ FLIII fluorescence stereomicroscope, excitation filter BP 470/40 nm, barrier filter BP 525/50 nm). BABA tolerance of segregating T2 progeny was analysed at 11 days after planting by comparing growth phenotypes of YFP-fluorescent plants on water- and BABA-containing ½ MS agar plates, as described above (section 2.5).

2.10 Microarray analysis.

Col-0 and *ibi1-1* plants were grown as described above. Ten-day old seedlings were soil-drenched with water or 10 mg/L BABA, and infected with *H. arabidopsidis* or mock solution by spray-inoculation, as described above. Three biological replicates (each consisting of pooled leaves from ~20 plants from 1 pot) were collected at 2 days after inoculation (dpi) and snap-frozen in liquid nitrogen for RNA extraction. Successful induction of defence by BABA in Col-0 was confirmed via callose staining at 2 dpi, RT-qPCR of *PR1*, and trypan blue staining 5 dpi. The callose defence and induced resistance phenotypes were confirmed to be absent in BABA-treated *ibi1-1* plants. RNA hybridisation to Arabidopsis Gene ST 1.1 arrays (median 22 probes/gene, Affymetrix, 901913) was performed at the Nottingham Arabidopsis Stock Centre (NASC). Microarray data were extracted, quality-checked and normalised (via Robust Multi-array Average, RMA) using the R Bioconductor software package (version 3.2; Huber et al. 2015) as follows: an image of each microarray was produced with the R Bioncoductor package 'affy' (version 1.48.0; Gautier et al. 2004), and checked for bubbles and smears. Normalisation was performed using the Robust Multiarray Average (RMA) algorithm, as implemented in the R Bioconductor package 'oligo' (version 1.34.2; Carvalho and Irizarry 2010). RMA consists of background correction, log2 transformation and quantile normalisation of the raw intensity values, to confer identical statistical properties on the distribution of intensity values on each microarray. A linear model is then fit to the normalised data to obtain an expression value for each probe set on each microarray (Irizarry et al. 2003). Annotations of probe sets and transcripts were retrieved from the NetAffx database (G. Liu et al. 2003) using the 'oligo' package. A PCA at the transcript level across all 24 microarrays was performed using the R Bioconductor package 'arrayQualityMetrics' (Kauffmann, Gentleman, and Huber 2009). Pairwise comparisons between matched samples were performed using Welch's *t*-test (unequal variances *t*-test), to identify genes whose expression differs significantly (p-value =<0.05, fold-change => 2) between Col-0 and *ibi1-1.* The RMA-processed log2-transformed expression values of these genes were normalised by row (Value = [Value-RowMean]/RowStandardDeviation] and column (Value = [Value-ColumnMean]/ColumnStandardDeviation], and hierarchical clustering of differentially expressed genes by Pearson correlation was performed after using the software MeV, version 4.9.0 (Saeed et al. 2006). The web tool Gorilla (Eden et al. 2009; http://cbl-gorilla.cs.technion.ac.il/; accessed 11/02/2015) was used for GO term analysis of differentially expressed genes. Differentially expressed genes were mapped to cellular pathways using MapMan software (Thimm et al. 2004).

2.11 Metabolic profiling by MALDI-MS – data acquisition.

Arabidopsis leaf metabolome was analysed by metabolic fingerprinting using Matrix Associated Laser Desorption Ionisation (MALDI) Mass Spectrometry (MS), as described previously (Pétriacq, Stassen, and Ton 2016). Samples were obtained from the same experiment that was used for the microarray analysis. Seedlings were freeze-dried under a vacuum for 48 h, yielding ~10 mg dry weight per sample. The exact weights were recorded to allow normalisation of detected compounds by dry weight of the sample. Freeze-dried leaf tissues were subsequently ground in a ball mixer/mill for 2 min at 30 Hz. Metabolites from the resulting powder were extracted in a ball mixer/mill (2 min, 30 Hz) using 1 mL of MeOH:H_2O:formic acid (95:4.9:0.1; v/v/v) to obtain both polar and non-polar compounds. After centrifugation (15 minutes, 17,000 *g*, 4 °C), supernatants (~800 µL) were transferred into fresh 2-mL tubes, and the remaining pellet was subjected to re-extraction in 500 µl extraction buffer by grinding in a ball mixer/mill (2 min, 30 Hz). After centrifugation (15 min, 17,000 *g*, 4 °C), the supernatants (ca. 400 µL) was pooled with the first supernatant, vortexed, then centrifuged again to pellet any remaining rough particles (15 min, 17,000 *g*, 4 °C). The pooled and centrifuged supernatant was then split into 3 aliquots (ca. 300 µL each) and dried overnight in a speed-vacuum at 4 °C. Concentrated extracts were re-suspended in 100 µl MeOH:H_2O:formic acid (50:49.9:0.1; v/v/v) by sonication for 10 minutes, vortexed and centrifuged (15 min, 17,000 *g*, 4 °C) before MS analysis. Samples were analysed using a Synapt G2 HDMS quadruple time-of-flight mass spectrometer (Waters, Manchester, UK) interfaced with a MALDI head, as reported before (Luna et al. 2014; Pétriacq, Stassen, and Ton 2016). The metabolites were detected in positive and negative ionisation mode, using two different matrices to increase the number of ionised analytes. For positive ionisation, α-Cyano-4-hydroxycinnamic acid (CHCA; Sigma-Aldrich) solution was prepared at 5 mg/mL in methanol (MeOH)/trifluoroacetic acid (TFA) (99.9/0.1, v/v) and the following MS settings were adopted according to existing laboratory protocols (Pétriacq, Stassen, and Ton 2016): Sample Plate 2V, Extraction Grid 3V, Hexapole 1V and Aperture 5V. For negative ionisation, 9-aminoacridine (9AA; Sigma-Aldrich) has been used and prepared at 10 mg/mL in MeOH with specific MS parameters (Sample Plate 0V, Extraction Grid 12V, Hexapole 9V and Aperture 3V). The samples were mixed with an equal volume of matrix solution, and for each sample, six technical replicates were spotted on a 96-well MALDI plate. Sulfadimethoxine (Sigma-Aldrich) was used as a lockmass compound during each run.

2.12 Metabolic profiling by MALDI-MS – data analysis.

Combination, background subtraction and centring of the raw data obtained from MALDI-MS analyses were done using MASSLYNX version 4.1 software (Waters, Manchester, UK). The resulting spectral data were binned in R (version 3.2.3) using the package 'MALDIQuant' (Gibb and Strimmer 2012) at a tolerance of 0.02 Da. Data were further corrected for the total ion current (TIC), the six technical replicates were averaged, and data corrected for dry weight of each biological replicate. The resulting matrix was imported into MeV software (Saeed et al. 2006), where data were normalised by row (*m/z* value; Value = [Value-RowMean]/RowStandardDeviation]) and column (sample; Value = [Value-ColumnMean]/ColumnStandardDeviation]. Detected *m/z* values showing statistically significant differences in intensity between samples were identified by ANOVA ($p \leq 0.01$, no FDR correction). These m/z values were clustered by Pearson correlation, using average linkage clustering. Clusters of m/z values displaying enhanced intensities only in Col-0 and not in *ibi1-1* after BABA pre-treatment and subsequent *Hpa* inoculation were selected and exported into a .csv (comma separated values) file. The MarVis software package was used for further analysis to predict metabolite masses (Kaever et al. 2009, 2015). To this end, MarVis filter was used to correct m/z values from positive and negative ionization mode for isotope and adducts (max. number of C^{13} isotopes per marker candidate = 2; min. cosine similarity = 0.75; m/z tolerance = 0.1). Predicted masses from both clusters where combined and analysed by MarVis Cluster prior to pathway analysis in MarVis Pathway. The latter step is necessary to retain information about isotope and adduct correction. To confirm putative metabolite identities, markers were matched to entries in the KEGG and BioCyc *Arabidopsis thaliana* databases using MarVis Pathway (m/z tolerance = 0.05Da).

2.13 Chlorophyll fluorescence measurements

Col-0 wildtype and *ibi1-1* plants were grown in 60 mL pots containing a mixture of Levington M3 soil and sand (2:1 v/v), at a density of 8 to 10 plants per pot. The soil was saturated with tap water, and seeds were stratified on soil at 4°C in the dark for 3 days. Plants were subsequently cultivated at 8.5 h light (160 µmol/m^2/s, 20°C) and 15.5 h darkness (20°C) at 70-80% relative humidity (RH). Four-week old seedlings were soil-drenched with BABA (racemic mixture of R- and S-BABA, final concentration 10 mg/L) or water. 2 days after priming induction, plants were inoculated with *H. arabidopsidis* by spraying with a spore suspension (1x10^5 conidiospores/mL) until run-off. Control plants were sprayed with a similar amount of distilled water. Photosynthetic parameters were measured at 1 and 2 days after inoculation using an AVT-D1 fluorometer (Walz, Germany). Measurements started approximately 1 hour after the beginning of the photoperiod. Due to the limitations of the AVT-D1 fluorometer, only one pot could be measured at a time. To minimise variation between genotypes that arises from circadian effects, similarly treated plants of each genotype were measured consecutively.

The following measurement protocol was used:

After a dark period of 5 minutes (minimal measurement light, intensity setting on AVT-D1 = 4), plants were exposed to a saturating light flash (intensity setting = 10). This was followed by blue actinic light (160 µmol/m^2/s, intensity setting = 14**)** over a period of 8 minutes. Measurements were taken every 20 seconds. The actinic light was then increased to 400 µmol/m^2/s (intensity setting = 18) for 3 minutes, and measurements taken every 20 seconds. Thereafter, the actinic light was turned off (intensity setting = 0), and measurements were taken every 20 seconds for 2 minutes, then once per minute for a further 6 minutes, then once more after another 5 minutes.

Chlorophyll fluorescence data were extracted from 5 to 8 leaves per condition (one leaf per plant). Only true leaves of similar size were included. To determine (near) steady-state non-photochemical quenching (NPQ) at 400 µmol/m^2/s, the last 4 measurements taken before the actinic light was switched off were averaged.

A similar experiment was performed using a split-pot set-up: 4 to 6 plants of Col-0 and *ibi1-1*, respectively, were sown in separate halves of a 60 mL pot. This allowed simultaneous measurement of similarly treated plants of each genotype. Three-week old plants were soil-drenched with 10 mg/L BABA or tap water, and two days later sprayed with a suspension of *H. arabidopsidis* spores or distilled water, as described above. Chlorophyll fluorescence measurements were taken one and two days after inoculation. The measurement protocol was as above, with the following modifications: after 5 minutes of dark adaptation, plants were kept at 160 µmol/m^2/s for 7:30 minutes, followed by measurements taken at 400 µmol/m^2/s over a period of 4:10 minutes.

Chapter 3

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Characterisation of IBI1 protein domains involved in BABA perception

3.1 Abstract

Perception of the chemical priming agent β–aminobutyric acid (BABA) is controlled by the aspartyl-tRNA synthetase (AspRS) enzyme IBI1. In this Chapter, a mutational approach was followed to identify protein domains of IBI1 that are critical for BABA perception. Site-directed mutagenesis of the L-aspartic acid (L-Asp)-binding region of IBI1 revealed that this domain is critical for both BABA-induced resistance (BABA-IR) and tolerance to otherwise phytotoxic concentrations of BABA. To identify additional protein sequences necessary for BABA-IR and BABA tolerance, chimeric gene constructs were generated between *IBI1* and its close gene homologue *HM1*, which does not play a role in BABA perception. While some chimeric gene constructs failed to complement the *ibi1-1* mutant phenotype, overexpression of the *HM1* gene completely restored BABA-IR and BABA tolerance in *ibi1-1*. This unexpected finding indicates that the ability of IBI1 to mediate BABA-IR and BABA tolerance is a general property of AspRS enzymes, which depends on the level of gene transcription, rather than IBI1-specific amino acid sequences.

3.2 Introduction

Aminoacyl-tRNA synthetases (aaRS) are evolutionarily conserved enzymes essential for protein biosynthesis. They are ubiquitous in all life forms, including prokaryotes and eukaryotes. Protein synthesis in plants takes place in several organelles of different evolutionary origin, but it is currently thought that cytoplasmic and organellar protein synthesis are mediated by a shared set of aaRSs (Duchêne et al. 2005). Accordingly, all aaRSs are encoded in the nuclear genome, and most are targeted to several subcellular localisations (Duchêne et al. 2005). The Arabidopsis genome contains three separate aspartyl-tRNA synthetase (AspRS) genes: At4g31180 (*IBI1*), At4g26870 (referred to as *HM1* in this Chapter) and At4g33760 (Duchêne et al. 2005). IBI1 and HM1 are close homologues, displaying 74.2% identity at the amino acid level. Both enzymes lack an organelle targeting sequence and are targeted to the cytosolic compartment (Duchêne et al. 2005; Luna et al. 2014). The third AspRS, At4g33760, resembles aaRS enzymes of cyanobacterial origin and contains a plastid-targeting sequence that signals for transportation into chloroplasts and mitochondria (Duchêne et al. 2005).

The AspRS IBI1 controls both β–aminobutyric acid-induced resistance (BABA-IR) and tolerance to phytotoxic concentrations of BABA (Luna et al. 2014). Due to its structural similarity to L-aspartic acid (L-Asp), it has been proposed that the bioactive R-enantiomer of BABA binds to the L-Asp-binding domain of IBI1, thereby 'jamming' the canonical AspRS function of the enzyme and priming it for an alternative defence function during pathogen attack (Luna et al. 2014; Chapter 1). This is supported by experimental evidence that R-BABA binds to the IBI1 protein *in planta*, as well as computational docking studies that indicated that R-BABA binds to the conserved L-Asp binding site of IBI1 in a similar manner as L-Asp. However, direct experimental evidence that the L-Asp binding domain of IBI1 is critical for BABA-IR and BABA tolerance is lacking.

Because single mutations in the *IBI1* gene (At4g31180) are sufficient to block BABA-IR against *Hyaloperonospora arabidopsidis* (*Hpa*) and increase stress sensitivity to BABA, Luna et al. (2014) concluded that the perception of BABA is solely determined by IBI1, while other AspRS isoforms have no contribution. In addition, a T-DNA mutant in *HM1* was found to be unaffected in BABA-IR and BABA tolerance, demonstrating that IBI1 alone is sufficient to mediate BABA-IR and BABA tolerance in Arabidopsis. Further evidence for a role of IBI1 in plant defence came from the finding that expression of *IBI1* is transiently induced during the early stages of *Hpa* infection, while the *HM1* gene is unresponsive to *Hpa* (Luna et al. 2014). Together, these findings justified the hypothesis that the non-canonical function of IBI1 in BABA-IR and BABA tolerance is determined by IBI1-specific amino acid sequences that are not present in HM1.

The study presented in this Chapter took a mutational approach to examine the importance of the IBI1 L-Asp-binding domain in BABA-IR and BABA tolerance and, secondly, to determine which IBI1-specific protein domains control these responses. The obtained results demonstrate that the L-Asp-binding domain is essential for both BABA-IR and BABA tolerance. Furthermore, based on the unexpected finding that over-expression of the *HM1* gene fully restored BABA-IR and BABA tolerance in the *ibi1-1* mutant, this Chapter concludes that the ability of IBI1 to mediate BABA perception does not depend on IBI1-specific amino acid sequences, but rather on the level of gene transcription.

3.3 Results

Binding of R-BABA to the L-Asp-binding domain of IBI1 is necessary for BABA-IR and BABA tolerance. To confirm the importance of the L-Asp-binding domain, site-directed mutagenesis was used to replace the polar glutamine residue at position 308 (GLN308) in this IBI1 domain with a non-polar alanine (IBI1^{SDM}). According to previous computational docking models (Luna et al., 2014; Chapter 1, Fig. 1.4), this mutation will disrupt the formation of a hydrogen bond between GLN308 and the amino group of L-Asp or R-BABA. The mutant *IBI*^{SDM} gene was cloned in the pEarleygate101 vector to encode a C-terminal fusion protein with YFP under control of the constitutive 35S CaMV promoter. Subsequently, the construct was transformed into the *ibi1-1* mutant background, and T1 transformants were selected for BASTA resistance (conferred by the *bar* gene on pEarleygate101). Segregating T2 plants expressing the *IBI1*^{SDM}*-YFP* were selected on the basis of YFP fluorescence and compared to wild-type plants (Col-0), un-transformed *ibi1-1* plants and *ibi1-1* plants overexpressing wild-type *IBI1* (35S:*IBI1-YFP* T3 generation) for BABA tolerance and BABA-IR against *Hpa*. As had been reported earlier (Luna et al. 2014), over-expression of the wild-type *IBI1* gene in *ibi1-1* plants (35S:*IBI1-YFP*; T3 generation) fully restored BABA tolerance, as evidenced by wild-type levels of seed germination and growth phenotypes on BABA-containing agar (50 mg/L). By contrast, T2 plants overexpressing mutant *IBI1*^{SDM}*-YFP* in the *ibi1-1* background showed a similar lack of seed germination as un-transformed *ibi1-1* plants (Fig. 3.1), which was apparent in two independently transformed lines. Furthermore, compared to wild-type and 35S:*IBI1-YFP* plants, fluorescent T2 plants expressing *IBI1*^{SDM}*-YFP* showed dramatically reduced levels of BABA-IR against *Hpa* after soil-drench treatment with 5 mg/L BABA. Hence, disruption of the L-Asp-binding domain in IBI1 compromises this protein's ability to mediate BABA tolerance and BABA-IR against *Hpa*.

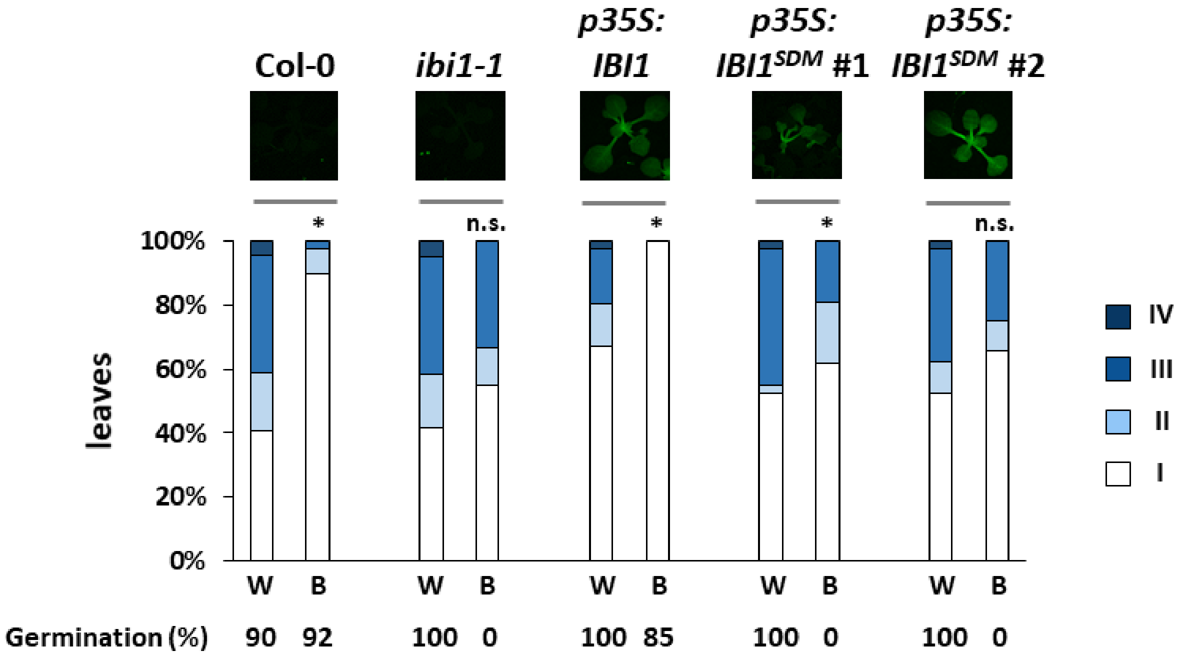


Figure 3.1: Targeted mutation of the L-Asp-binding pocket of IBI1 compromises BABA-IR to **Hpa** ***and BABA tolerance.*** *Plants (*ibi1-1*) were transformed with the p35S:*IBI1^{SDM}-YFP *construct carrying a point mutation that converts the polar glutamine residue (GLN308) in the L-Asp-binding domain into a non-polar alanine. To select plants expressing the* IBI1^{SDM}-YFP *gene, only YFP-fluorescent individuals (top inserts) in the segregating T2 generation (p35S:*IBI^{SDM}*) were collected for analysis and compared to wild-type plants (Col-0), un-transformed* ibi1-1 *plants and* ibi1-1 *plants overexpressing the wild-type* IBI1 *gene (p35S:*IBI1*; T3 generation; Luna et al. 2014). BABA-IR against* Hpa *was quantified in 3-week old plants after treatment with 5 mg/L BABA at 2 days before challenge inoculation with* Hpa *($1*10^5$ spores/mL).* Hpa *colonisation was determined in trypan blue-stained leaves at 5 dpi by assigning leaves to different* Hpa *colonisation classes (see chapter 2.4). Asterisks denote significant differences in class distributions between water- and BABA-treated samples (Fisher's Exact test; *: $p<0.05$; n.s.: not significant; n = 40 - 50). BABA tolerance was determined by measuring germination rate on ½ MS agar ± 50 mg/L BABA at 10 days after sowing (bottom line; n = 10 - 30).*

The non-canonical activity of IBI1 in BABA-IR and BABA tolerance is not determined by its unique amino acid domains. Based on the lack of BABA-IR and reduced BABA tolerance in independent *ibi1* mutants, Luna et al. (2014) concluded that the IBI1 homologue, HM1, does not have a contribution to BABA perception. Accordingly, it was assumed that the non-canonical function of IBI1 in BABA-IR is determined by its specific amino acid sequences that are absent in HM1 (Luna et al. 2014). *IBI1* encodes a protein of 558 amino acids, while *HM1* encodes a protein of 532 amino acids. Both proteins are 74% identical at the amino acid level (Fig. 3.2). The N-terminus of IBI1 is relatively poorly conserved. Conspicuously, IBI1 contains an N-terminal addition of 12 amino acids, as well as an insertion of 13 amino acids near the N-terminus, which are absent in the HM1 homologue. It has been suggested that N-terminal additions to aaRS enzymes confer novel (non-canonical) functions to these enzymes (Guo, Yang, and Schimmel 2010). Further dissimilarities, additions and deletions are distributed throughout the protein sequence of IBI1 and HM1, respectively (Fig. 3.2). On the other hand, 18 amino acids which have been shown to be essential for the functionality of AspRS enzymes (Cavarelli et al. 1994) are conserved in IBI1 and HM1.

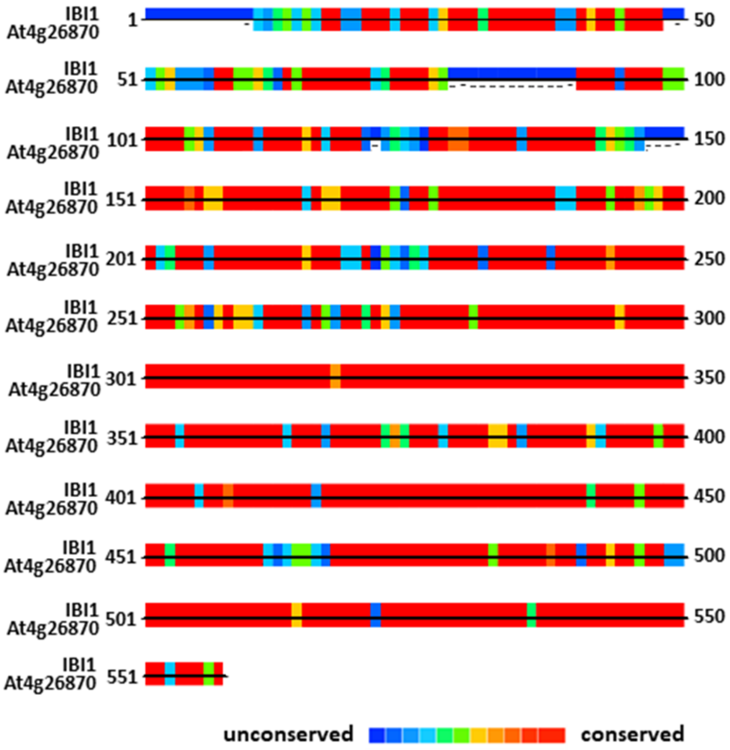


Figure 3.2: Alignment of amino acid sequences of IBI1 and its close homologue HM1 (At4g26870). *IBI1 and HM1 show 74% amino acid identity. Of note, IBI1 contains several unique amino acid sequences that are absent in the homologue. Colour codes indicate biochemical similarity between amino acid residues, ranging from red (identical) to blue (opposite in terms of molecular size and polarity). Alignment and similarity scoring were performed with the PRALINE sequence alignment tool (Simossis and Heringa 2005).*

Based on the assumption that IBI1 is unique in its ability to control BABA-IR and BABA tolerance, a domain-swapping approach between IBI1 and HM1 was undertaken, in order to map IBI1-specific sequences controlling BABA-IR and BABA tolerance. Chimeric gene constructs containing varying proportions of the coding sequences (CDS) from *IBI1* and *HM1* were cloned in the pEarleygate101 vector and transformed into the *ibi1-1* background for complementation analysis of BABA-IR and BABA tolerance. To achieve relatively high resolution, while keeping the number of possible chimeric proteins manageable, the domain-swapping between *IBI1* and *HM1* involved four regions of approximately similar size (Fig. 3.3). To facilitate primer design and avoid loss of functionality in regions with high degrees of sequence diversity, the exact fusion sites were selected in sequences of at least 8 identical nucleotides on either side of the centre of the fusion site.

Gene constructs encoding the chimeric fusion proteins were created by a PCR fusion/Gateway cloning strategy (Atanassov et al. 2009). *IBI1* and *HM1* coding sequences (CDS) without STOP-codons were cloned into pENTR/D-TOPO entry vectors. N- and C-terminal fragments were created by high-fidelity PCR, creating products with overlapping sequences at the CDS ends and Gateway-cloning extensions at the proximal N- or C-terminal ends. Fragments were then purified by agarose gel electrophoresis, after which matching fragments were fused by single-step PCR elongation fusion (Fig. 3.4). Subsequently, chimeric constructs, along with full-length *IBI1* and *HM1* CDSs, were cloned in pEarleygate101-YFP vectors and transformed into the *ibi1-1* background. YFP fluorescence was used as a selectable marker to ensure that only T2 plants expressing the chimeric constructs were included in the analysis.

Construct nr.	% IBI1	% HM1	IBI1 amino acids	HM1 amino acids	total length	BABA detoxification	BABA-IR
IBI1	100%	0%	1-558		558	YES	YES
HM1	0%	100%		1-532	532	YES	YES
Chim 1	28% N	72% C	1-158	133-532 (400)	558	YES	YES
Chim 2	50% N	50% C	1-284	259-532 (274)	558	NO	NO
Chim 3	73% N	27% C	1-409	384-532 (149)	558	NO	YES (weak)
Chim 4	29% C	71% N	407-558 (152)	1-380	532	INTERMEDIATE	NO (or very weak)
Chim 5	52% C	48% N	285-558 (274)	1-258	532	YES	YES
Chim 6	72% C	28% N	159-558 (400)	1-132	532	NO	NO (#1) /YES (#2)

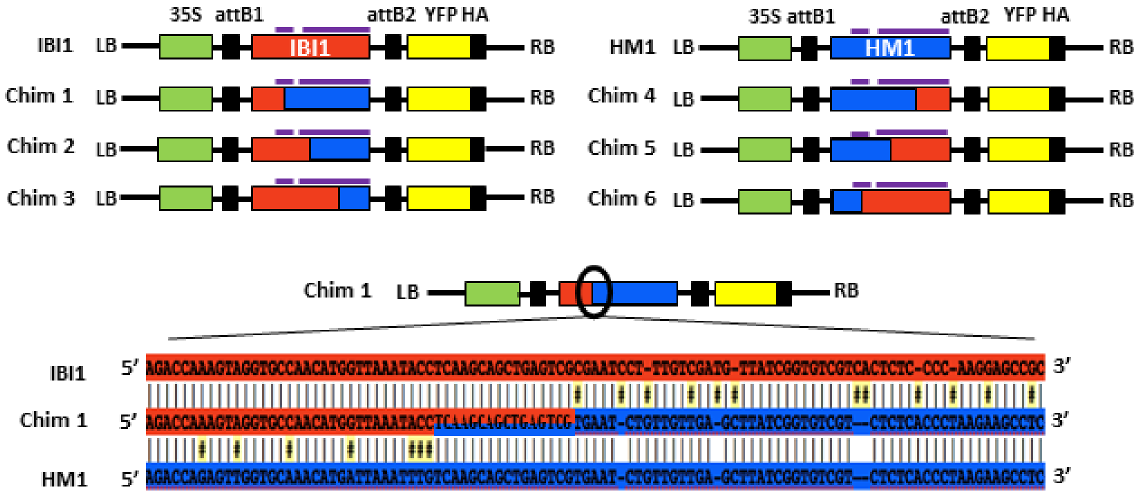


Figure 3.3: Design of chimeric proteins encoded by fusion constructs between **IBI1** ***and*** **HM1** ***gene fragments. Top:*** *The coding sequences of* IBI1 *and* HM1 *were divided into four regions of approximately equal length. Chimeric gene constructs were cloned in pEarleygate101 vectors to encode C-terminal fusion proteins with YFP that are under constitutive transcriptional control by the CaMV 35S promoter.* ***Middle:*** *Graphic representation of chimeric constructs in pEarleygate101-YFP vectors. The position of the conserved tRNA anticodon binding domain and tRNA synthetase class II domain are indicated by purple bars.* ***Bottom:*** *Fusion sites of the chimeric constructs were chosen in areas of high sequence homology to minimise the risk of mis-folding and to avoid loss of putative functionality by IBI1-specific regions.*

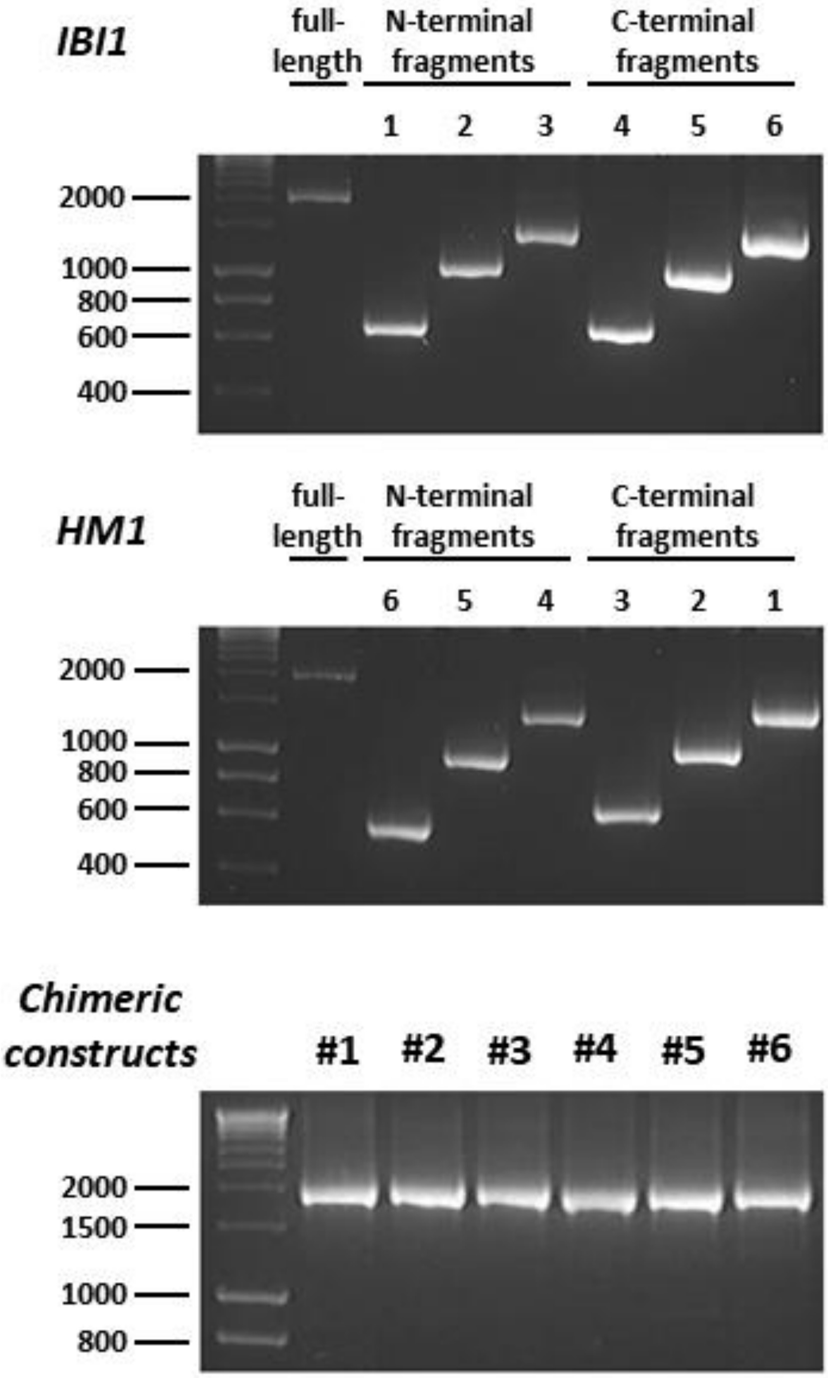
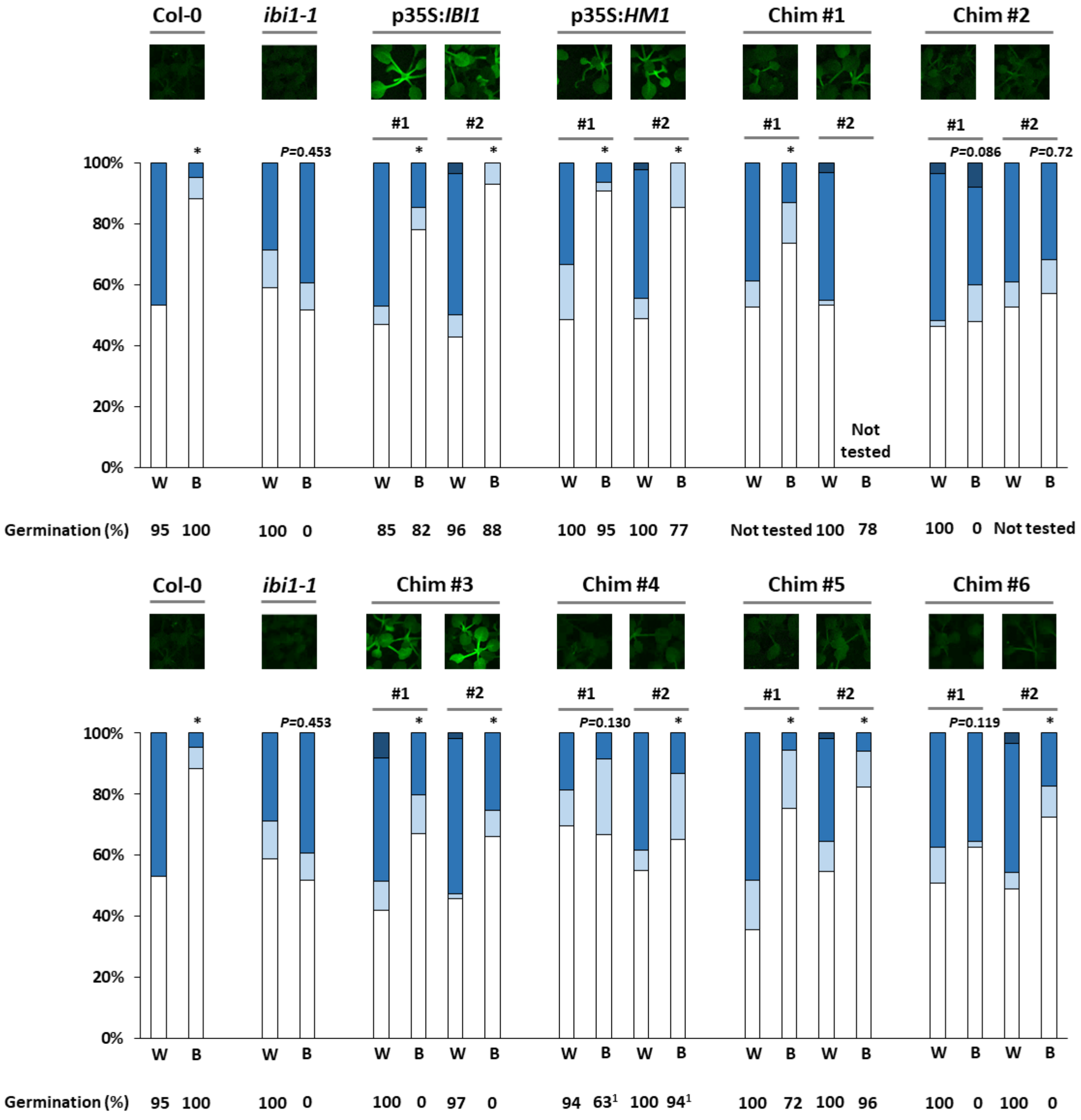


Figure 3.4: Generation of chimeric gene constructs between* IBI1 *and* HM1*. Top: *N- and C-terminal fragments of varying lengths were amplified by high-fidelity PCR from the* IBI1 *CDS. Shown are PCR products visualised on a 1.2% agarose gel.* ***Middle:*** *N- and C-terminal fragments of varying lengths were amplified by high-fidelity PCR from the* HM1 *CDS. Shown are PCR products visualised on a 1.2% agarose gel.* ***Bottom:*** *Chimeric gene constructs were generated by single step elongation fusion of* IBI1 *and* HM1 *fragments. Shown are the products of the elongation PCR on a 1.2% agarose gel.*

For most constructs, two independently transformed lines were analysed for BABA tolerance and BABA-IR against *Hpa*, and compared to Col-0, *ibi1-1* and 35S:*IBI1-YFP* plants. As evidenced by germination and growth phenotypes on ½ MS agar medium with and without BABA (50 mg/L), overexpression of *IBI1,* construct #1 and construct #5 rescued BABA tolerance in the *ibi1-1* mutant (Fig. 3.5). This indicates that the chimeric proteins encoded by these constructs are fully capable of conferring BABA tolerance. T2 plants over-expressing construct #4 mostly germinated, but showed reduced growth compared to Col-0 and 35S:*IBI1-YFP*, indicating that this chimera is partially affected in its ability to mediate BABA tolerance. Finally, T2 plants expressing constructs #2, #3 and #6 were similarly affected in germination as were *ibi1-1* plants, indicating that these chimeric proteins are completely impaired in their ability to mediate BABA tolerance. Interestingly, over-expression of *HM1* also rescued BABA tolerance, demonstrating that HM1 is fully functional in conferring BABA tolerance.

To determine the ability of the chimeric proteins to mediate BABA-IR, YFP-fluorescent T2 plants were analysed for BABA-IR against *Hpa*. As expected, soil-drench treatment with 5 mg/L BABA induced relatively high levels of resistance in Col-0 plants and 35S:*IBI1-YFP* plants, while the un-transformed *ibi1-1* mutant failed to show statistically significant levels of BABA-IR (Fig. 3.5). T2 plants overexpressing constructs #1 and #5 expressed comparable levels of BABA-IR as wild-type plants, indicating that the encoded chimeric proteins can mediate BABA-IR. Furthermore, T2 plants over-expressing constructs #3, #4 and #6 showed statistically significant levels of BABA-IR in at least one of the two lines tested, but the extent of BABA-IR compared to Col-0 and 35S:*IBI1-YFP* plants was lower. Finally, T2 plants expressing construct #2 resembled the phenotype of the *ibi1-1* mutant in that they failed to express statistically significant levels of BABA-IR. These results suggest that chimeras #1 and #5 are fully functional in BABA-IR, chimeras #3, #4, and #6 are partially impaired in mediating BABA-IR, and chimera # 2 is completely impaired in its ability to mediate BABA-IR. However, it has to be noted that the level of YFP fluorescence in T2 plants expressing constructs #2 and #6 was relatively low. Therefore, it cannot be excluded that the lack of complementation by these gene constructs is due to the relatively low levels of expression and/or corresponding protein accumulation. Surprisingly, T2 plants over-expressing the *HM1* gene displayed wild-type levels of BABA-IR, indicating that the HM1 protein is fully capable of mediating BABA-IR. This unexpected finding indicates that the previously reported lack of redundancy of the *IBI1* gene for BABA-IR and BABA tolerance in Col-0 Arabidopsis is due to the low transcription level of *HM1*, rather than IBI1-specific amino acid sequences. Hence, the non-canonical role of IBI1 in BABA-IR and BABA tolerance appears to be a general feature of AspRS enzymes that is dependent on the level of corresponding gene transcription.



[1] Plants germinated, but showed severely stressed phenotype

Figure 3.5: Complementation analysis of BABA-IR against* Hpa *and BABA tolerance by IBI1, HM1 and chimeric proteins. *Mutant* ibi1-1 *plants were transformed with constructs encoding* IBI1 *(p35S:*IBI1*),* HM1 *(p35S:*HM1*) or chimeric constructs of these two proteins. Expression of the gene constructs, which carry a C-terminal YFP tag, was assessed by fluorescence microscopy (top inserts). BABA-IR against* Hpa *was analysed in 3-week old Arabidopsis plants. For each construct, two independently transformed lines were treated with 5 mg/L BABA two days prior to inoculation with* Hpa *($1*10^5$ spores/mL).* Hpa *colonisation was determined at 5 dpi by assigning trypan blue-stained leaves to different* Hpa *colonisation classes (see chapter 2.4). Asterisks denote significant differences in class distributions between water- and BABA-treated samples (Fisher's Exact test; *: p<0.05; n = 30 - 70). Germination rate on ½ MS agar ± 50 mg/L BABA was analysed 10 days after sowing (n = 10 - 30).*

3.4 Discussion

This study has addressed the function of structural IBI1 features in BABA-IR and BABA tolerance. Based on docking models between the conserved L-Asp-binding domain of IBI1 and R-BABA, it was predicted that the polar glutamine residue GLN308 interacts with the amino group of (R)-BABA (Luna et al., 2014; Chapter 1, Fig 1.4). To test this hypothesis, a site-directed mutant variant of IBI1 was created (IBI1^{SDM}), in which the GLN308 residue was replaced by a non-polar alanine residue. Over-expression of the corresponding *IBI1^{SDM}* gene failed to restore BABA-IR and BABA tolerance in the *ibi1-1* mutant (Fig. 3.1), providing first experimental evidence that the L-Asp-binding pocket of IBI1 is necessary for BABA-IR and BABA tolerance. Direct evidence that this mutation prevents physical binding of R-BABA to IBI1 would require further protein immunoprecipitation experiments from control- and BABA-treated plants, as described previously (Luna et al. 2014). Nevertheless, the lack of functionality of IBI1^{SDM} in BABA perception strongly supports the notion that R-BABA binds to the conserved L-Asp-binding pocket of IBI1.

Based on the defence priming activity of BABA and confocal laser microscopy studies of subcellular IBI1 localisation, Luna et al. (2014) proposed that denial of the canonical AspRS activity of IBI1 primes the protein for augmented translocation to the cytoplasm following pathogen attack, where it activates defence through non-canonical cellular activity. It has been proposed that blocking of the AspRS function of the enzyme could be achieved by either lack of substrate (L-Asp depletion), or disruptive binding to R-BABA (Schwarzenbacher, Luna, and Ton 2014, Chapter 8.1). This could explain why *IBI1* overexpression can lead to constitutive defence and enhanced basal resistance, since this increases the amount of IBI1 enzyme lacking L-Asp substrate. Similarly, selective uptake of L-Asp by biotrophic parasites, such as downy mildew, would also deprive IBI1 from its canonical AspRS activity, and could point to a mechanism by which IBI1 contributes to basal defence against biotrophic pathogens. This latter hypothesis provides biological relevance to the non-canonical defence function of IBI1 (Schwarzenbacher, Luna, and Ton 2014; Chapter 8, Fig. 8.1). However, the results in this Chapter showed that deprivation of IBI1 from primary AspRS activity through site-directed mutagenesis of its L-Asp-binding domain did not enhance basal resistance against *Hpa* (Fig. 3.1). This indicates that priming of the non-canonical defence activity of IBI1 involves additional, yet unknown, mechanisms, such as changes in tertiary protein structure. The two-step AspRS reaction requires not only L-Asp as a substrate, but also ATP and tRNA^{Asp} (Guo and Schimmel 2013). Accordingly, it is possible that priming of the protein's non-canonical defence activity is induced by specific changes in the stoichiometry of the AspRS reaction that do not occur by mutagenesis of the L-Asp-binding domain.

Although the amino acid sequences between AspRS isoforms are highly conserved, mutation of the *IBI1* gene specifically blocks BABA-IR and BABA tolerance in Arabidopsis (Luna et al. 2014). Hence, the ability of *IBI1* to mediate BABA-IR is unique in the Arabidopsis genome and absent in genes encoding other AspRS isoforms. Based on that conclusion, it seemed plausible that IBI1-specific protein sites are responsible for the non-canonical defence function of IBI1. To identify those protein features, chimeric gene constructs between *IBI1* and its close homologue At4g26870 (*HM1*) were tested for their ability to complement the *ibi1-1* mutant for BABA-IR and BABA tolerance. Unexpectedly, overexpression of the full-length *HM1* gene in independent T2 lines completely restored BABA-IR (Fig. 3.5). Similarly, T2 plants expressing the *HM1-YFP* gene showed wild-type germination rates and growth phenotypes on BABA-supplemented agar. This demonstrates that the ability of IBI1 to mediate BABA-IR and BABA tolerance is not unique, but rather a general feature of AspRS enzymes that is determined by the transcription level of the corresponding genes. *HM1* shows more than 10-fold lower levels of transcription than *IBI1* (Luna et al. 2014). Apparently, this relatively low level of *HM1* expression is insufficient to confer BABA-IR and BABA tolerance in *ibi1-1*. However, as is recurrent throughout this thesis, BABA treatment often leads to small but statistically insignificant increases in resistance in *ibi1-1*. While this weak resistance response could be explained by the mutant's exaggerated stress response to BABA, which could make the plant less attractive for biotrophic pathogens like *Hpa*, the findings presented in this Chapter make it plausible that this trend is due to a weak residual contribution of *HM1* to BABA-IR.

Considering that both IBI1 and HM1 can mediate BABA-IR and BABA tolerance, it follows that chimeras of these two proteins should rescue BABA-IR and BABA tolerance in the *ibi1-1* mutant. Interestingly, however, this was not the case for all chimeras. Only two out of six chimeric proteins (encoded by constructs #1 and #5) fully rescued BABA-IR and BABA tolerance in the *ibi1-1* mutant (Fig. 3.5). While constructs #3, #4 and #6 rescued BABA-IR to varying degrees, the levels of BABA-IR in these lines were generally lower than those in Col-0 and stable 35S:*IBI1-YFP* plants (Fig. 3.5), suggesting compromised ability of the constructs to mediate BABA-IR. Constructs #3 and #6 also failed to rescue BABA tolerance in the *ibi1-1* mutant, while construct #4 partially restored BABA tolerance. Finally, construct #2 completely failed to complement both BABA-IR and BABA tolerance (Fig. 3.5). It is possible that the chimeric proteins encoded by constructs #2, #3, #4 and #6 are altered in tertiary structure due to mis-folding, which would affect their functionality in BABA-IR and BABA tolerance. Mis-folding of proteins often leads to active degradation by the proteasome (Howell 2013), which could explain the loss of functionality in BABA-IR and BABA tolerance. Indeed, with the exception of construct #3, T2 plants affected in BABA-IR and BABA tolerance showed overall weaker levels of YFP fluorescence. Alternatively, the observed correlation between YFP fluorescence and BABA responsiveness could be explained by the genomic insertion sites of the constructs, which can lead to variation in transgene expression.

In general, the chimeric proteins failing to restore BABA-IR in *ibi1-1* were also impaired in their ability to confer BABA tolerance. However, T2 plants over-expressing construct #3 were a noticeable exception to this correlation. Although these plants showed only relatively weak reductions in BABA-IR compared to Col-0 and 35S:*IBI1-YFP* plants, they were fully impaired in BABA tolerance, as evidenced by a complete lack of germination on BABA-supplemented agar (Fig. 3.5). Interestingly, T2 plants over-expressing construct #3 also displayed relatively high levels of YFP fluorescence, excluding the possibility that the mildly reduced levels of BABA-IR and complete lack of BABA tolerance are caused by low levels of transcription and/or protein accumulation. This makes chimera #3 an interesting mutant protein as it partially uncouples BABA-IR from BABA tolerance.

The results in this Chapter have important translational implications. It has been suggested that overexpression of homologues of IBI1 could enhance the responsiveness of crop plants to BABA, while at the same time increasing tolerance to this priming agent (Schwarzenbacher, Luna, and Ton 2014). The insights gained from this Chapter suggest that time-consuming analyses to separate defence-capable AspRSs from non-defence-capable isoforms might not be necessary. Genetically modified (GM) crops are tightly regulated in the European Union, and consumer acceptance of GM crops is low (Lucht 2015). This is particularly true for crops containing transgenes. Increasing BABA responsiveness via insertion of foreign DNA might thus not be a viable option to exploit the broad-spectrum effectiveness of BABA-IR in crops. However, since most plant species possess several isoforms of AspRS (Peeters et al. 2000; Duchêne et al. 2005), it should be relatively straightforward to select for crop varieties with enhanced AspRS gene expression through marker-assisted breeding. Furthermore, new breeding techniques, such as CRISPR-cas9, have recently been developed that allow gene modifications that are nearly indistinguishable from naturally occurring mutations (Woo et al. 2015). It is feasible that such gene editing techniques can enhance the transcription of endogenous AspRS genes in crop species. The legal status of such ‘gene-edited’ crops is not clear at the moment, but several European countries have tentatively ruled that gene editing does not necessarily require permanent integration of foreign DNA into the plant’s genome, and this form of genetic modification may thus not fall under the existing GMO legislation (Nature Editorials 2017). A decision by the European Court of Justice is not expected until 2018, but if it confirms the favourable ruling on gene editing, augmentation of AspRS gene expression might become a viable tool for obtaining crop varieties that are more responsive to BABA and develop higher levels of broad-spectrum disease resistance upon treatment with lower (non-toxic) concentrations of this chemical.

Chapter 4

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Identification of proteins that interact with the BABA receptor IBI1

4.1 Abstract

Treatment with β-aminobutyric acid (BABA) increases biotic and abiotic stress resistance in a wide range of plant species, a phenomenon known as BABA-induced resistance (BABA-IR). In the model plant species *Arabidopsis thaliana*, the aspartyl-tRNA synthetase IBI1 acts as a receptor for BABA. Binding of BABA to the IBI1 protein sensitises the enzyme for an alternative cytoplasmic defence activity that becomes active after pathogen attack. Since defence signalling pathways typically involve protein interactions, a yeast two-hybrid (Y2H) screen was performed to identify putative defence signalling proteins that interact with the IBI1 receptor. Two independent Y2H screens identified various defence regulatory proteins that are produced in leaves of Arabidopsis. The screen also identified various proteins that are exclusively produced in flower tissues, suggesting a role for IBI1 in previously reported BABA-induced female sterility. Four candidates, with predicted transcriptional expression in the leaves and previously described links to plant defence, were confirmed for *in planta* interaction with IBI1 in *Nicotiana benthamiana*, using bimolecular fluorescence complementation (BiFC) analysis. The findings presented in this chapter open up the possibility to dissect further the defence signalling pathways initiated upon BABA perception.

4.2 Introduction

Plants and plant pathogens have co-existed for millions of years. Over the course of their shared evolutionary history, an evolutionary arms-race has resulted in complex mechanisms of defence and attack, respectively. The plant immune system is controlled by hundreds of interacting proteins (Mukhtar et al. 2011), and plant pathologists are only beginning to understand the connectivity of proteins contributing to the defence-signalling network of the plant cell. The importance of protein-protein interactions to plant immunity is underscored by the fact that adapted plant pathogens employ effector proteins that preferably target highly connected hubs in the plant immune network (Mukhtar et al. 2011), which allows them to manipulate or de-regulate the defence-signalling network and induce susceptibility. Some plants counteract the deployment of effectors through so-called resistance (R) genes, which recognise the presence or activity of an effector protein and subsequently induce an exaggerated immune response termed effector-triggered immunity (ETI; Jones and Dangl 2006). However, pathogens can break or circumvent ETI relatively easily as it relies on recognition by single R proteins. More durable resistance can be obtained by augmentation of components of basal immunity (also referred to as horizontal or quantitative resistance), which relies on a multitude of genes. This so-called plant acquired immunity, or induced resistance, can be triggered in naïve plants by a variety of specific environmental stimuli, such as localised pathogen attack, colonisation by plant-growth promoting rhizobacteria, or specific chemicals. Plant acquired immunity consists of two components: direct induction of defence mechanisms and priming of defence (Conrath et al. 2006). In the primed state, plants do not execute a full defence response before infection, but react to attack with a stronger and faster basal immune response.

Priming can be induced by treating plants with the non-proteinogenic amino acid β-aminobutyric acid (BABA). This priming agent has attracted considerable interest, because it induces resistance against a wide range of biotic and abiotic stresses (Cohen, Vaknin, and Mauch-Mani 2016). A considerable body of research documents that BABA treatment primes the activation of a multitude of defence pathways, including SA- and JA-dependent defence, as well as cell wall-mediated defence (Hamiduzzaman et al. 2005; Ton and Mauch-Mani 2004; Ton et al. 2005). Recent findings have shown that BABA accumulates as a naturally occurring stress-induced metabolite in several plant species (Thevenet et al. 2016), which supports the earlier identification of a specific plant receptor of BABA, the aspartyl-tRNA synthetase (AspRS) IBI1 (Luna et al. 2014; Thevenet et al. 2017). While it seems surprising that an AspRS, whose main function is provision of charged tRNA for protein biosynthesis, mediates the first step of a defence signalling cascade, aminoacyl-tRNA synthetases (aaRSs) are known to have crucial functions beyond protein translation (Guo and Schimmel 2013). For example, human tryptophanyl-tRNA synthetase regulates blood vessel growth, while lysyl-tRNA synthetase regulates immune responses by mast cells in humans (Yannay-Cohen et al. 2009; Guo, Yang, and Schimmel 2010). These 'moonlighting' functions of aaRSs are mediated by the addition of novel protein domains, which facilitate complex protein-protein interactions (Guo, Yang, and Schimmel 2010).

To gain a better mechanistic understanding of signalling events mediating the IBI1-dependent resistance, a screen for IBI1-interacting proteins in the model plant species *Arabidopsis thaliana* (Arabidopsis) was performed. Considering the importance of protein-protein interactions in defence signalling (Mukhtar et al. 2011; Alves et al. 2014; Zhang, Chen, and Harmon 2016), identifying IBI1-interacting partners may generate important insights to decode the early molecular events by which IBI1 mediates defence. Aminoacyl tRNA synthetases are known to be promiscuous proteins that form multi-protein complexes in mammalian and yeast cells (Guo, Yang, and Schimmel 2010). During basal and primed defence, IBI1 translocates from the endoplasmic reticulum (ER) membrane to the cytoplasm (Luna et al., 2014), which brings the enzyme into a new protein environment. It is plausible that this translocation enables different protein interactions, which may initiate the downstream defence response. This Chapter describes two independent yeast two-hybrid (Y2H) screens with different cDNA libraries and reporter constructs. From a selection of 25 candidates, 4 proteins that are expressed in the leaves with previously reported links to plant defence were confirmed for *in planta* interaction with IBI1, using bimolecular fluorescence complementation (BiFC) analysis in *N. benthamiana*.

4.3 Results

Yeast two-hybrid screening identifies 25 putative protein interactors of IBI1. To identify proteins that interact with the BABA receptor IBI1, a yeast two-hybrid (Y2H) screen was performed using the full-length coding sequence of *IBI1* (At4g31180) as bait (Fig. 4.1). Due to the technical complexity and time requirements of Y2H screens, the procedure was carried out by Hybrigenics Services, S.A.S., Paris, France (http://www.hybrigenics-services.com). The first screen was performed with a cDNA library from one-week old Arabidopsis seedlings, grown on ½ MS agar at 24°C with 16 hours light per day. Bait and prey fragments were fused to the DNA-binding domain and activation domain of the bacterial transcription factor (TF) LexA, respectively (Table 4.1). Protein interactions were identified by screening for complementation of histidine auxotrophy. Interactors were assigned to different classes indicating confidence in the putative protein interaction (Predicted Biological Score [PBS], A, B = high confidence interactor; D = medium confidence interactor; see Chapter 2 for details). This first screen yielded only three putative IBI1 interactors: IBI1 itself (PBS B), PED1 (PBS D), and TCP4 (PBS D) (Tables 4.1 & 4.2). Since aminoacyl tRNA synthetases are known to be highly interactive proteins (Guo, Schimmel, and Yang 2010), the small number of protein interactions identified in this screen was rather surprising. Therefore, a second screen was performed, using the Clontech Mate & Plate™ Library of Arabidopsis, which consists of cDNA from 11 different tissues of Arabidopsis, chosen to represent a broad range of expressed genes. In contrast to the cDNA library used for the first screen, this Clontech Mate & Plate™ Library has been normalised to remove highly abundant cDNAs from the libraries and increase the representation of low-abundance cDNAs. While the mix of tissues used in this library increases the chance of detecting interactors that are not relevant for IBI1-mediated defence against foliar pathogens, this drawback is offset by augmenting the chances of detecting lowly expressed interactors, especially because the expression of key signalling genes is often relatively low. Furthermore, for this second screen, bait and prey fragments were fused to the DNA-binding domain of the yeast Gal4 transcription factor, instead of LexA (Table 4.1). Gal4 and LexA each have distinct advantages and disadvantages for use in Y2H screens, and sometimes interactions can be picked up more easily with one system compared to the other (Van Criekinge and Beyaert 1999). This second Y2H screen resulted in the identification of 23 putative IBI1 interactors (7 high confidence interactors, 16 moderate confidence interactors, Table 4.2). The only protein identified in both screens was IBI1, indicating auto-interaction through formation of homodimers.

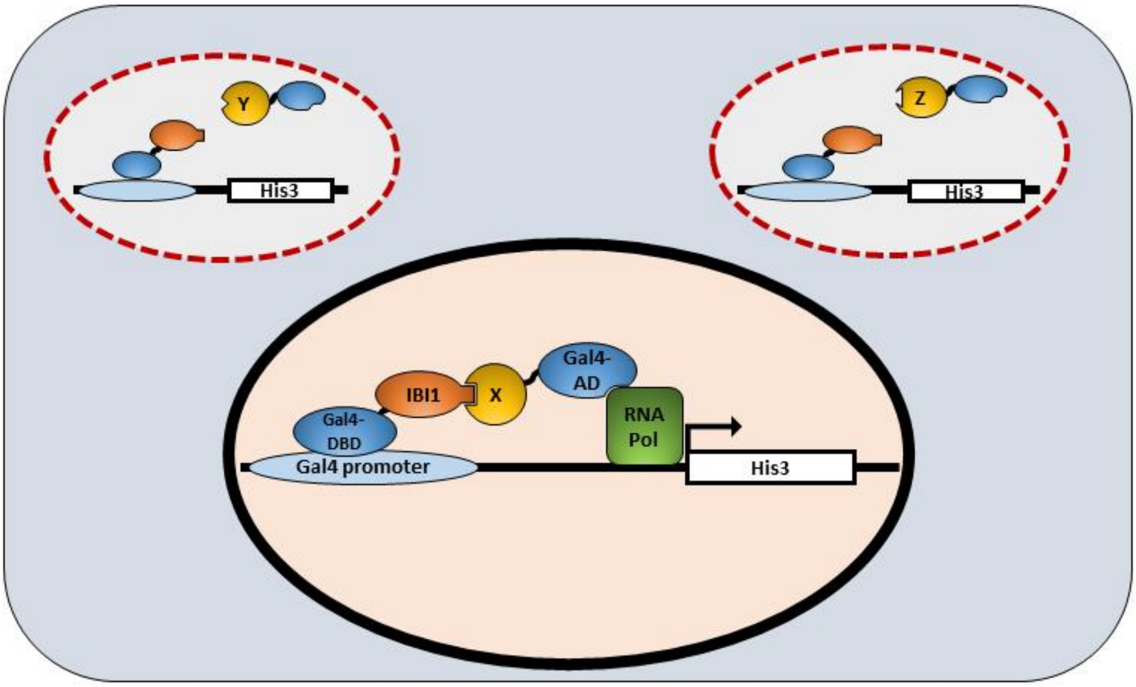

Figure 4.1: Model of yeast two-hybrid (Y2H) screens. *Y2H screens are based on the modular nature of transcription factors, which consist of a DNA-binding domain (DBD) and a transcription-activating (AD) domain, which do not have to be encoded in the same protein. A functional transcription factor (TF) can be formed by two interacting proteins that provide a DBD and AD. To identify protein interaction partners of a gene of interest, a chimeric fusion protein between the gene of interest and the DBD of a TF (most commonly Gal4 or LexA) is encoded by a recombinant plasmid. A library of protein-coding genes (or gene fragments) from the tissue(s) of interest, fused to the AD, is encoded by a second recombinant plasmid. Both plasmids are co-transformed into a suitable yeast strain. In cells expressing two interacting fusion proteins, a functional TF is reconstructed, activating expression of a reporter gene. In the depicted example, the coding sequence of IBI1 is fused to the DBD of the Gal4 TF, while the interacting protein ('protein X') is fused to the AD of the Gal4 TF. Interaction between IBI1 and protein X reconstructs an active Gal4 TF, which activates transcription of the* His3 *gene that allows the yeast to grow on medium without histidine. In contrast, IBI1 does not interact with proteins Y and Z, and cells receiving these plasmid combinations cannot grow on medium without histidine supplementation. Extraction and sequencing of the plasmids from the cells growing on the selective medium will reveal the identity of the gene that encodes for the interacting protein X. Note: The interaction between the two fusion proteins takes place in the nucleus and does not necessarily reflect* in planta *interactions. Schematic not drawn to scale.*

Table 4.1: Yeast two-hybrid screens used to identify putative IBI1-interacting proteins.

	Bait	Prey library	Good confidence interactors (A, B, C)	Moderate confidence interactors (D)
1st screen	N-terminal fusion of full length IBI1 to DNA-binding domain of bacterial TF LexA	cDNA from 1-week old Arabidopsis seedlings, grown on ½ MS at 16h light/day (Hybrigenics)	1	2
2nd screen	N-terminal fusion of full length IBI1 to DNA-binding domain of yeast TF Gal4	Normalised mRNA from 11 different Arabidopsis tissues (Clontech Mate&Plate)	7	16

Two independent screens were performed, using different cDNA libraries and different bait and prey constructs. The two screens resulted in the identification of 25 putative IBI1 interaction partners (IBI1 auto-interaction was identified in both screens).

Putative interactors of IBI1 in different plant tissues. To assess which of the 25 putative IBI1-interacting proteins identified in the Y2H screens could play a role in IBI1-dependent defence signalling, it was verified whether the corresponding genes are expressed in Arabidopsis leaves, using the public AtGenExpress Visualization Tool (AVT; http://jsp.weigelworld.org/expviz/expviz.jsp). Fifteen putative IBI1 interactors showed robust expression in the leaves (Table 4.2). These included proteins that have previously been linked to defence, and genes that are transcriptionally responsive to (a)biotic stress. Three putative IBI1 interactors, *SWEET9, PSG1* and *PIPK11*, were not expressed in leaves (AVT intensity < 10), and a further seven (*SWEET5, UCLACYANIN2, At1g32120, At1g48100, At4g25150, At2g17710, At1g12810*) were expressed in leaves only at relatively low levels (AVT intensity < 200, with much higher expression levels in other organs). Of the ten putative IBI1 interactors that are not/lowly expressed in leaves, seven showed high expression levels in flowers or pollen (*PSG1, PIPK11, SWEET5, SWEET9, At4g25150, At1g48100, At1g12810)*.

Table 4.2: Putative IBI1-interacting proteins.

Confidence (PBS score)	Gene	Alternative names	Expressed in leaves?	Notes
AB	At4g31180	IBI1	+	IBI1 auto-interaction
AB	At1g28520	VOZ1	+	VOZ1 and VOZ2 are transcription factors involved in regulation of abiotic and biotic stress resistance. *voz1/2* double mutant impaired in SAR.
AB	At2g42400	VOZ2	+	
AB	At3g47520	plNAD-MDH	+	Plastidic NAD(H)-dependent malate dehydrogenase
AB	At1g26650		+	7 trans membrane-helix protein
AB	At5g26610		+	
AB	At5g62850	SWEET5 (VEX1)	+/-	Sucrose transporter, mainly expressed in floral tissue
AB	At3g01620	PSG1	-	Pollen-specific gene involved in pollen germination and pollen tube growth
D	At4g20870	FAH2	+	Fatty acid hydroxylase, required for the BI-1-mediated suppression of programmed cell death. Downregulated during biotic stress.
D	At4g18800	RAB11B	+	Located in ER and trans-Golgi network. Involved in root hair growth.
D	At4g38430	ROPGEF1	+	Interacts with Rop1, which is involved in pollen tube growth. Other Rop proteins are also involved in defence.
D	At3g11400	eIF3G1	+	Translation initiation factor
D	At1g12810	Proline-rich family protein	+	Expressed in leaves, but 5x higher expression in pollen
D	At1g52220	CURT1C	+	
D	At4g15040	TCP4	+	TCP family transcription factor, possible artefact?

D	At2g33150	PED1, KAT2	+	Organellar 3-ketoacyl-CoA thiolase, involved in fatty acid β-oxidation during germination and subsequent seedling growth.
D	At2g17710		+	Much higher expression in seeds than leaves (~30x)
D	At2g44790	UCLACYANIN2	+/-	
D	At1g48100		+/-	Putative polygalacturonase, induced by wounding, but not *Hpa*
D	At4g25150	HAD SUPERFAMILY IIIB ACID PHOSPHATASE	+/-	Mostly expressed in pollen
D	At1g32120		+/-	
D	At1g01460	PIPK11	-	Expressed in pollen
D	At2g39060	SWEET9	-	
D	At1g10657		?	
D	GI:339773249	Hypothetical mitochondrial protein	?	

Proteins identified in the Y2H screen against IBI1 are listed according to their confidence score (AB = high confidence; D = medium confidence). Since the prey library consisted of cDNA extracted from a mix of different tissues, not all IBI1 interactors are expressed in the leaves, which is where BABA-primed defence expression against foliar pathogens takes place. Out of the 25 putative interactors, 15 show robust expression in the leaves. Of the remaining 10 proteins, 7 are highly expressed in pollen or flower tissue.

Confirmation of IBI1-protein interactions via Bimolecular Fluorescence Complementation assay. Yeast two-hybrid screens typically yield a significant number of false-positive interactions. Complementary approaches can be used to confirm biologically relevant *in planta* interactions. One such method is bimolecular fluorescence complementation (BiFC). BiFC assays are based on fusions of putatively interacting proteins to separate (non-fluorescent) halves of a fluorescent protein (e.g. yellow fluorescent protein, YFP). Interaction will reconstitute a functional fluorophore, resulting in detectable levels of fluorescence. This can be achieved *in planta* after transformation of plants with expression vectors encoding the complementary BiFC constructs. For instance, transient *Agrobacterium tumefaciens* transformation of *Nicotiana benthamiana* leaves to express proteins with complementing N-terminal and C-terminal parts of YFP, respectively, can be used to confirm putative interactions from Y2H assays, using epifluorescence microscopy analysis after excitation with blue light (excitation peak at 514nm).

To focus attention on protein interactions contributing to BABA-induced resistance, four putative interactors were selected that *i.* are transcriptionally expressed in leaves (where the resistance is expressed) and *ii.* have previously been linked to plant immunity. These four proteins were IBI1, VOZ2, plNAD-MDH and FAH2. Full-length coding sequences (CDS) of the respective genes were cloned into BiFC-compatible vectors (pEarleyGate201-YN, pEarleyGate202-YC), and leaves of 5- to 6-week old *Nicotiana benthamiana* plants were co-infiltrated with combinations of transformed *Agrobacterium* strains to transiently co-express *IBI1* with its putative interactor. The plasma membrane-bound protein OAK was used as a negative control. At 2 – 3 days after co-infiltration, protein interactions were analysed via epi-fluorescence microscopy. As is shown in Figure 4.2, all protein candidates showed BiFC, although to varying extents. IBI1 and VOZ2 showed comparably high levels of complementation, followed by FAH2 and plNAD-MDH (Fig. 4.2). The negative control, OAK, did not yield detectable levels of fluorescence (Fig 4.2). Hence, all four IBI1-interacting candidates from the Y2H screen could be confirmed as *in planta* interactors of IBI1.

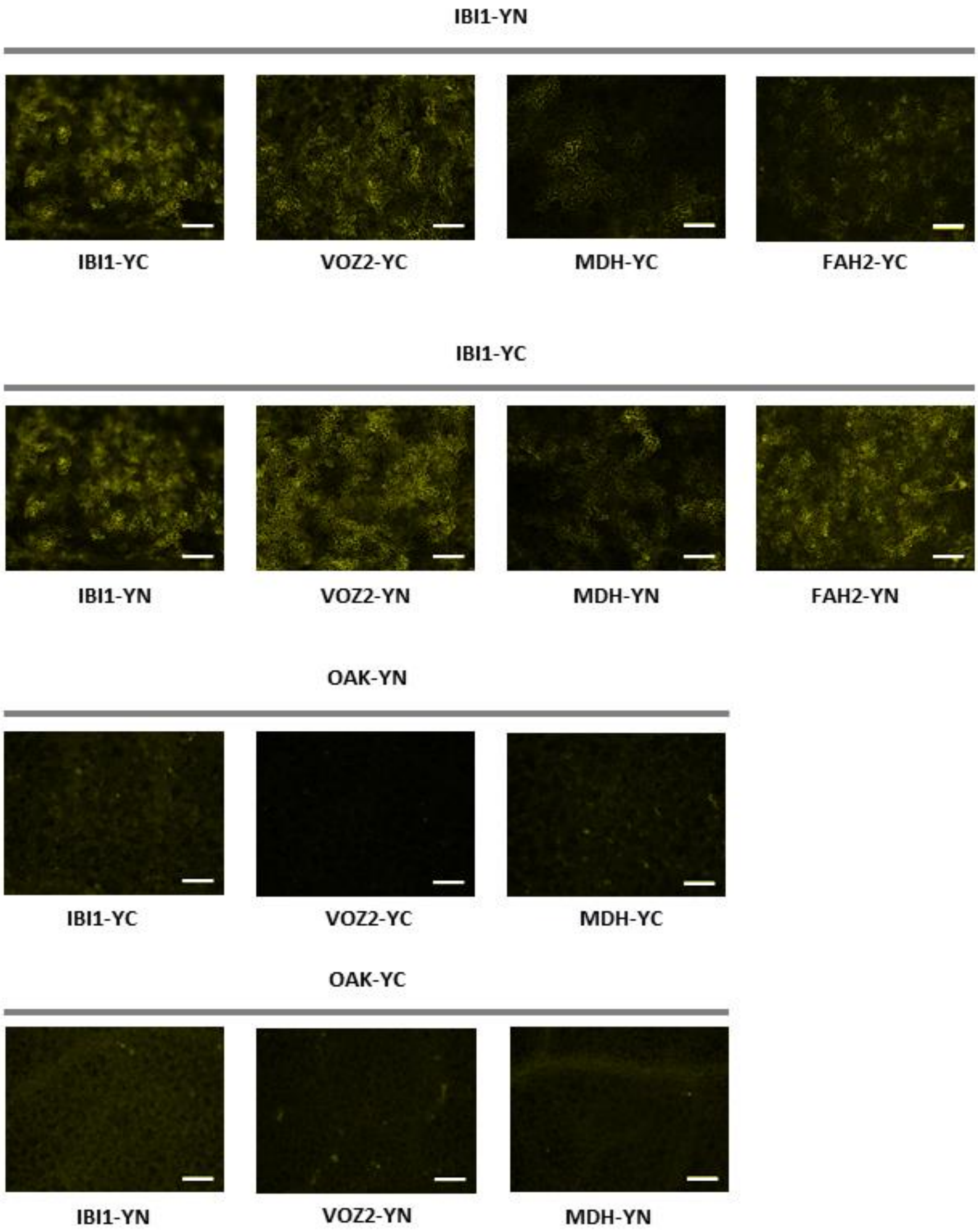

Figure 4.2: Bimolecular Fluorescence Complementation (BiFC) analysis of* in planta *protein interactions with IBI1. *Four putative interacting proteins identified in the Y2H screen (IBI1, VOZ2, plNAD-MDH and FAH2) were confirmed via BiFC. Full-length constructs of the respective proteins with a C-terminal fusion to the amino- or carboxyl-terminal half of yellow fluorescent protein (YFP) were transiently expressed in 5-6 week old* N. benthamiana *via leaf infiltration with* Agrobacterium tumefaciens*. For each putative interactor, fusion proteins with both the amino- and carboxyl-terminal half of YFP were generated, and all reciprocal combinations tested. Specific interactions between constructs were determined by epi-fluorescence analysis of YFP fluorescence at 3 days post infiltration. The plasma membrane-bound protein OAK was used as a negative control. Scale bar = 200µm.*

4.4 Discussion

The defence capacity of plants can be augmented by pre-treatment with chemical priming agents, which increases their resistance to subsequent stress exposure (Conrath et al. 2006). One such priming agent is β-aminobutyric acid (BABA). Plants primed with BABA show increased resistance to a wide range of biotic and abiotic stresses (Cohen, Vaknin, and Mauch-Mani 2016). However, high doses of BABA lead to negative non-target effects, such as growth inhibition and female sterility (Ton et al. 2005; Wu et al. 2010; Luna et al. 2014). To gain a better understanding of the mechanisms leading to broad-spectrum disease resistance by BABA, two independent Y2H screens were performed to identify putative protein interaction partners of the BABA receptor IBI1. The first screen only identified three putative IBI1-interacting proteins, which came as a surprise, considering that aminoacyl-tRNA synthetases are known to interact with a multitude of proteins in yeast and mammalian cells (Guo, Yang, and Schimmel 2010). Besides technical errors or random fluctuations during the screen, a possible reason for the low yield of putative interactors could be sub-optimal representation of interactors in the screened library, potentially because of low expression of interactors in etiolated young seedlings. Therefore, a second screen was performed, using a Mate & Plate™ library of Arabidopsis that consists of cDNA from 11 different tissues of Arabidopsis. In contrast to the cDNA library from etiolated Arabidopsis seedlings, the abundance of cDNAs has been normalised in the second library to remove highly abundant cDNAs and increase the representation of low-abundance cDNAs. The second Y2H screen with the Mate & Plate™ library resulted in the identification of an additional 23 candidates, yielding a total of 25 putative IBI1-interacting proteins from both screens together (Tables 4.1 & 4.2). Bioinformatic analysis using publicly available transcriptome data revealed that seven of these proteins are mainly expressed in pollen, stamina and other floral tissues. This relatively large fraction (28%) suggests that BABA perception by IBI1 not only controls BABA-IR in leaves, but that it can also regulate BABA responses in other tissues. For instance, BABA is known to induce female sterility in Arabidopsis when applied at relatively high doses. This BABA-induced sterility has a genetic basis, as indicated by the identification of Arabidopsis mutants that produced seeds even after treatment with high doses of BABA (Ton et al. 2005). Analysis of three *ibs* mutants (*impaired in BABA-induced sterility)* revealed reduced levels of BABA-induced resistance against biotic and abiotic stress in all tested mutant lines, demonstrating that induction of resistance and female sterility by BABA share common pathways. Indeed, BABA-induced female sterility is caused by excessive callose deposition that blocks pollen tube growth (Jakab et al. 2001).

The only putative IBI1-interacting protein that was detected in both Y2H screens was IBI1 itself. This result is not surprising, because homodimerisation is a general feature of class II aminoacyl tRNA synthetases and has been shown to be necessary for Asp-tRNA synthetase activity (O'Donoghue and Luthey-Schulten 2003). Whether formation of homodimers is also necessary for the non-canonical defence function of IBI1 remains unclear. In yeast AspRS, mutations in the dimeric interface resulted in lower affinity for $tRNA^{Asp}$, but did not change Km values for aspartic acid or ATP, even though ATP has been implicated in stabilising the dimeric interaction (Eriani et al. 1993). Thus, the study of mutated IBI1 constructs affected in binding of ATP or $tRNA^{Asp}$ might give further clues about the importance of homodimerisation for IBI1 defence function.

High-confidence putative IBI1 interaction partners with robust foliar expression patterns included the transcription factors VASCULAR PLANT ONE-ZINC FINGER 1 (VOZ1) and VOZ2. These highly conserved proteins play a role in biotic and abiotic stress resistance, as evidenced by the increased susceptibility of *voz1-2/voz2-2* (*voz1/2-2*) Arabidopsis double mutants to fungal and bacterial pathogens (Nakai, Nakahira, et al. 2013). Conversely, the *voz1/2-2* mutant shows increased resistance to freezing and drought (Nakai, Nakahira, et al. 2013). The interaction between IBI1 and VOZ1 / VOZ2 could therefore provide a mechanism through which BABA primes resistance to both biotic and abiotic stresses.

The Y2H screen also identified plastidic NAD-dependent malate dehydrogenase (plNAD-MDH, At3g47520) as an interactor of IBI1, which could be confirmed by BiFC analysis. In a previous study, IBI1 was reported to be associated with the endoplasmic reticulum and translocate to the cytosol during pathogen attack (Luna et al. 2014). Protein biosynthesis in plants takes place in the cytosol, mitochondria and chloroplasts, but all aminoacyl tRNA synthetases are nuclear encoded proteins. Hence, aminoacyl tRNA synthetases are shared between different plant cell organelles (Duchêne et al. 2005). Arabidopsis possesses three Asp-tRNA synthetases: *IBI1* (At4g31180), its close homologue At4g26870, and the cyanobacteria-like enzyme At4g33760. While it has been suggested that Asp-tRNA synthetase activity in chloroplasts and mitochondria is provided by At4g33760 (Duchêne et al. 2005), three out of four bioinformatics tools tested (AtSubP, TargetP, distill) predicted that IBI1 may also be targeted to the chloroplasts; only Plant-mPLoc predicted cytoplasmic localisation of IBI1 (Baú et al. 2006; Emanuelsson et al. 2007; Chou and Shen 2010; Kaundal, Saini, and Zhao 2010). Accordingly, it is not unthinkable that some IBI1 proteins can be imported into chloroplasts, where they interact with plNAD-MDH. The fact that chloroplastic localisation of IBI1-YFP was not reported by Luna et al. (2014) could be due to the masking effects of chloroplast autofluorescence. This raises the possibility that BABA-induced defence relies partially on *in planta* interactions between plNAD-MDH and IBI1. MDH catalyses the reversible oxidation of malate to oxaloacetate that occurs simultaneously with the reduction of NAD^+ to NADH (An, Cao, and Xu 2016). Accordingly, *miR-mdh-1* knockdown mutants of Arabidopsis expressing reduced levels of *MDH1* accumulate twice as much malate at night as wild-type plants (Beeler et al. 2014). The malate – glutamate/2-oxoglutarate antiporter in the chloroplast membrane regulates the balance between glutamate and 2-oxoglutarate in the plastid stroma, which act as donors and acceptors of amino groups in the cyclic photorespiration pathway, respectively. Hence, plastidic MDH activity can indirectly influence photorespiration rates, as has been demonstrated for mitochondrial MDH (Lindén et al. 2016). In most plants, photorespiration is a major source of ROS (Foyer et al. 2009), which act as signalling molecules during defence responses (Rojas et al. 2012). Alleles of the key photorespiration enzyme glycolate oxidase (GOX) have been identified as 'eR' (enzymatic resistance) genes (Taler et al. 2004), and *gox/haox* mutants affected in photorespiration are impaired in non-host resistance (Rojas et al. 2012), a multigenic defence response that shares similarities with BABA-induced resistance. Considering the possibility that IBI1 influences photorespiration rates through the interaction with plNAD-MDH, an increased generation of reactive oxygen species (ROS) may contribute to BABA-induced resistance after pathogen attack. MDH activity has been reported to increase during cold acclimation, suggesting a contribution of MDH to abiotic stress adaptation (Savitch et al. 2001). Moreover, disturbance in MDH, along with deficiencies in ascorbic acid or alternative oxidase, have been reported to cause increases in photorespiration components, such as catalase, P-protein of glycine decarboxylase complex (GDC) and glycine content (Voss et al. 2013). Hence, a disruptive interaction of MDH with IBI1 may indeed enhance the production of photorespiratory ROS. While homozygous *mdh* mutations are embryo-lethal (Beeler et al. 2014), a possible role for photorespiration can be tested further by studying the contribution of H_2O_2-generating glycolate oxidase (GOX) genes to BABA-IR (see Chapter 6).

The present study also identified an interaction between IBI1 and a fatty acid 2-hydroxylase (FAH2). The Arabidopsis genome contains two fatty acid 2-hydroxylases, *FAH1* and *FAH2,* which catalyse the hydroxylation of fatty acid chains in sphingolipids. Despite high sequence homology (70% identity at amino acid level), the two proteins encoded by these genes differ in substrate specificity: FAH1 preferentially hydroxylates very long chain fatty acids (VLCFAs), whereas FAH2 seems to favour palmitic acid (C16:0) as a substrate in Arabidopsis (Nagano et al. 2012). The exact extent of this specificity, however, remains unclear, and it seems that mutations in either gene disturb the sphingolipids composition by reducing the pool of glucosylceramides (and possibly glucosyl inositol phosphoryl ceramides), while increasing the pool of long chain bases and simple ceramides (König et al. 2012). Interestingly, *fah1/fah2* double mutants showed increased accumulation of salicylic acid and SA derivatives, which leads to increased resistance against biotrophic powdery mildew (*Golovinomyces cichoracearum*; König et al. 2012). Notably, both FAH1, and FAH2 interact indirectly via Cytochrome *b5* with the At-Bax Inhibitor 1 (At-BI1), a negative regulator of cell death (Nagano et al. 2009, 2012). The physiological importance of this interaction is underscored by the finding that non-hydroxylated ceramides induce programmed cell death (PCD), whereas hydroxylated ceramides do not (Townley et al. 2005). It is tempting to speculate that IBI1 could interact with FAH2 (and possibly FAH1) to modify sphingolipid pools, which subsequently leads to increased SA-dependent defences and/or PCD. Analysis of changes to lipid content during BABA-IR via mass spectrometry could help to clarify the role of sphingolipids in BABA-induced resistance.

In summary, the results described in this chapter show that IBI1 interacts with proteins that are involved in both defence and reproduction, two processes that are directly influenced by BABA (Jakab et al. 2001). This outcome opens promising avenues for further investigation into the molecular mechanisms behind IBI1-mediated BABA-IR, particularly the role of VOZ1/2, photorespiration and sphingolipid metabolism in induced defence.

4.5 Acknowledgements

I would like to thank Dr. Stéphanie Blachon (Hybrigenics Services) for excellent supervision and execution of the Y2H screens.

Chapter 5

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The IBI1-interacting transcription factors VOZ1 and VOZ2 control SA-independent cell wall defence during BABA-induced resistance

5.1 Abstract

Plants respond to β-aminobutyric acid (BABA) by sensitising their immune system, resulting in faster and/or stronger defence induction upon subsequent stress exposure. Broad-spectrum induced resistance by BABA is based on priming of distinct immune responses, including SA-dependent defence and SA-independent cell wall-mediated defence. The previous Chapter showed that the BABA receptor IBI1 interacts with the defence-regulatory transcription factors VOZ1 and VOZ2. In this Chapter, analysis of the *voz1/2* double mutant revealed that these transcription factors are critical for augmentation of defence-related callose deposition in BABA-primed plants, which provides SA-independent resistance against filamentous pathogens, such as the biotrophic pathogen *Hyaloperonospora arabidopsidis.* BABA-induced priming of SA-dependent gene expression, on the other hand, was not impaired in the *voz1/2* double mutant. Mutant complementation analysis showed that the contribution to BABA-induced resistance and basal resistance requires nuclear localisation of VOZ2, presumably by activating transcription of defence-related genes. Although VOZ2 directly interacts with phytochrome B, changes in red/far-red light ratio did not affect BABA-induced resistance, nor was the Arabidopsis *phyA/B* double mutant impaired in BABA-induced resistance. The presented results reveal novel insights into the molecular mechanisms by which IBI1 mediates BABA-induced resistance.

5.2 Introduction

Plants are constantly threatened by pathogenic microbes and sub-optimal growth conditions such as drought and cold. In the face of such adverse conditions, plants have evolved a complex immune system that protects them from the vast majority of these environmental pressures. One such adaptation is the capacity of plants to sensitise their immune system in response to certain stimuli, such that subsequent encounters with biotic or abiotic stress lead to stronger and faster activation of the plant's defence arsenal. This so-called 'priming' of defence provides plants with efficient immunity at low fitness costs, and can be induced by diverse stimuli, such as beneficial microbes, phytohormones and small chemical compounds (van Hulten et al. 2006; Conrath et al. 2006). Amongst the multitude of priming agents identified over the years, the non-proteinogenic amino acid β-aminobutyric acid (BABA) has raised particular interest, because it is effective against a large number of abiotic and biotic stresses in a range of plant species, including several important crops (Cohen, Vaknin, and Mauch-Mani 2016).

The resistance-inducing properties of BABA were first demonstrated in 1963 (Papavizas and Davey 1963). A considerable body of literature has since accumulated reporting BABA-induced resistance (BABA-IR) in a wide range of plant-pathogen interactions (Cohen 2002, 1994; Liljeroth et al. 2010; Zimmerli et al. 2000; reviewed in Cohen, Vaknin, and Mauch-Mani 2016). In addition, BABA enhances tolerance of drought, high temperatures and salinity (Jakab et al. 2005; Zimmerli et al. 2008). This broad-spectrum effectiveness of BABA can be attributed to its capacity to prime distinct defence pathways, including those controlling SA- and JA-dependent defences, as well as phytohormone-independent cell wall-mediated defences (Zimmerli et al. 2000; Hamiduzzaman et al. 2005). However, the initial signalling steps following molecular perception of BABA towards the priming of the various immune pathways remains elusive. The recent identification of the BABA receptor, IBI1, in *Arabidopsis thaliana* (Arabidopsis) has provided a unique opportunity to further unravel the defence signalling events culminating in broad-spectrum defence priming and resistance (Luna et al. 2014). As was shown in the previous Chapter, a yeast two-hybrid screen followed by bimolecular fluorescence complementation (BiFC) analysis established that IBI1 interacts with the transcription factors (TFs) VASCULAR PLANT ONE ZINC-FINGER 1 (VOZ1) and VOZ2 (Chapter 4). These two homologous proteins, which are evolutionarily conserved in land plants, have previously been shown to regulate biotic and abiotic resistance pathways (Nakai, Nakahira, et al. 2013). The present Chapter describes a further characterisation of the role of these TFs in BABA-induced resistance.

5.3 Results

VOZ1 and VOZ2 partially contribute to BABA-induced resistance. In addition to its alternative function as a receptor of BABA, the IBI1 protein functions primarily as an aspartyl-tRNA synthetase (AspRS) during protein biosynthesis (Luna et al. 2014). Therefore, the protein interactions described in the previous Chapter do not necessarily contribute to BABA-IR. To test whether the interaction between IBI1 and VOZ1 and/or VOZ2 has a role in BABA-induced resistance, wild-type plants and homozygous *voz1-2/voz2-2* double mutant plants (further called *voz1/2-2;* first described in Yasui et al. 2012; Fig. 5.1A) were soil-drenched with 5 mg/L BABA and two days later challenged with the downy mildew pathogen *Hyaloperonospora arabidopsidis* (*Hpa*). While the BABA receptor mutant *ibi1-1* was completely deficient in BABA-IR, the *voz1/2-2* mutant was partially affected in BABA-IR against *Hpa*, as evidenced by significantly higher levels of *Hpa* colonisation in BABA-treated *voz1/2-2* compared to BABA-treated wild-type plants (Col-0; Fig. 5.1B).

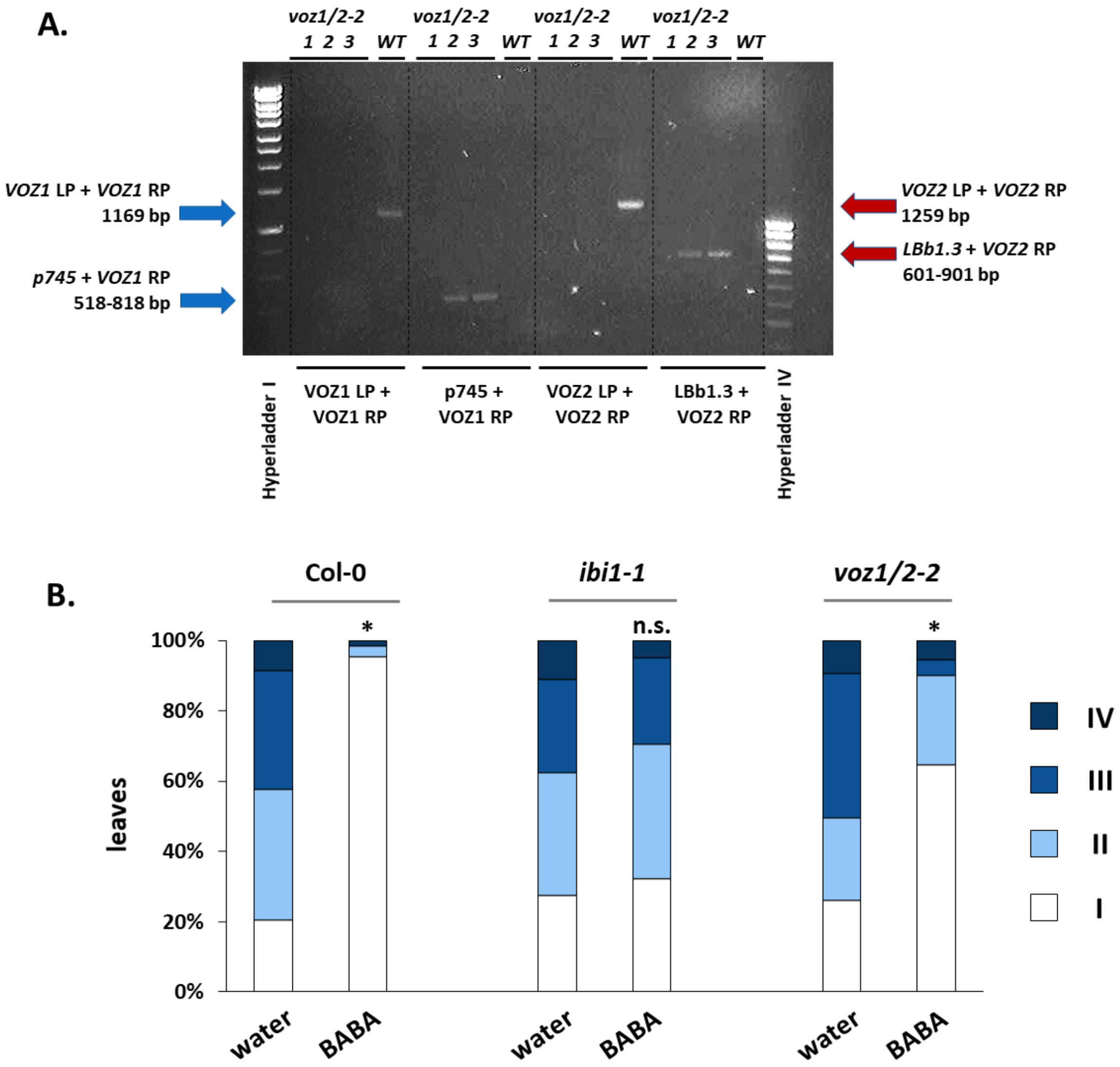

Figure 5.1: The Arabidopsis double mutant in the IBI1-interacting proteins VOZ1 and VOZ2 is affected in BABA-induced resistance (BABA-IR). A. *PCR confirmation of homozygous T-DNA insertions in* VOZ1 *and* VOZ2 *of the* voz1-2/voz2-2 *double mutant (*voz1/2-2*). Genomic T-DNA insertions were verified using T-DNA-specific left border (LB) primers (p745 and LBb1.3) and gene-specific right border (RB) primers. Gene-specific LB and RB primers were used to confirm absence of PCR product in* voz1-2/voz2-2 *lines, with Col-0 wild-type (WT) as positive control.* voz1/2-2 *lines 2 and 3 were bulked and analysed for BABA-induced resistance (BABA-IR). The* voz1-2 *mutation corresponds to* WISCDSLOX489-492O10; the *voz2-2* mutation corresponds to SALK_115813. ***B.*** *Quantification of BABA-IR in 3-week old wild-type (Col-0),* ibi1-1 *and* voz1/2-2 *plants against* Hpa. *2-week old plants were pre-treated with water or 5 mg/L BABA by soil-drenching, and 2 days later spray-inoculated with* Hpa *spore suspension (1*10*^{5} *spores/mL). Disease progression was assessed at 5 days post inoculation (dpi) by assigning trypan-blue-stained leaves to different* Hpa *colonisation classes (see chapter 2.4). Asterisks denote significant differences in class distributions between water- and BABA-pretreated samples (Fisher's Exact test; *: p<0.05; n.s.: not significant; n=60 – 150).*

VOZ1 and VOZ2 control SA-independent expression of cell wall defence during basal and BABA-induced resistance. BABA-IR against *Hpa* in Arabidopsis is based on priming of SA-dependent and SA-independent defence (Mauch-Mani and Slusarenko 1996; Zimmerli et al. 2000). The partial increase in resistance against *Hpa* after BABA treatment in the *voz1/2-2* double mutant suggested that only one of these pathways is impaired in the mutant. The effect of mutations in *VOZ1* or *VOZ2* on priming of SA-dependent defence was tested by spraying wild-type (Col-0) and *voz1/2-2* plants with 0.01% Silwet solution containing 1 mM SA or mock solution (0.01 % Silwet only) at 2 days after soil-drenching with water (control) or BABA (5 mg/L). At 4, 8 and 24 hours after the SA challenge, the level of induction of the SA marker gene *PR1* was tested by RT-qPCR. No significant differences in basal or induced expression of *PR1* could be detected in the *voz1/2-2* mutant compared to wild-type plants at any time-point (Fig. 5.2A, only 8 h timepoint shown), indicating that this double mutant is neither impaired in basal nor BABA-primed expression of salicylic acid (SA)-dependent defence. To test a role for the VOZ TFs in the BABA-augmented expression of cell wall defence, callose deposition in water- and BABA-pretreated Col-0 and *voz1/2-2* plants was quantified at 3 days after inoculation with *Hpa*, using aniline blue/calcofluor double staining. Epi-fluorescence microscopy analysis of the amount of callose-arrested germ tubes emerging from *Hpa* spores revealed that BABA augmented the efficiency of callose-rich papillae in Col-0, whereas the effectiveness of cell wall defence was not increased by BABA treatment in the *voz1/2-2* mutant (Fig. 5.2B). Interestingly, basal levels of callose efficiency in un-primed (water-treated) *voz1/2-2* plants were also reduced in comparison to un-primed wild-type plants, suggesting a contribution of one or both VOZ TFs to basal defence. Taken together, these results indicate that the interaction between IBI1 and VOZ1/VOZ2 contributes to SA-independent cell well defence during basal and BABA-induced resistance.

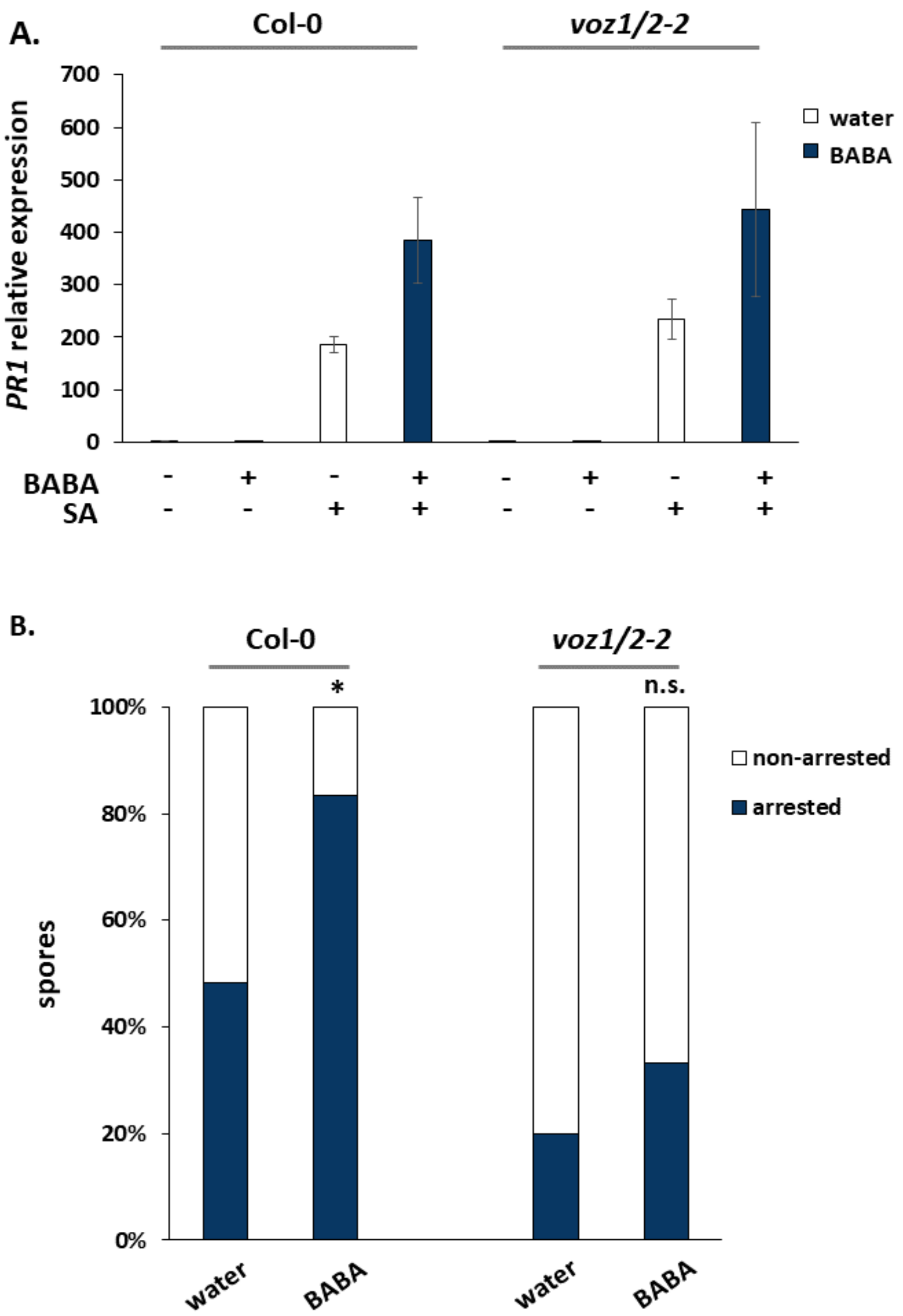

Figure 5.2: VOZ1 and VOZ2 are required for induction of cell wall-mediated defence by BABA. A. *Levels of basal and SA-induced* PR1 *gene expression in un-primed and BABA-primed Col-0 and* voz1/2-2 *plants. Leaves were sprayed with 1 mM salicylic acid (SA) at 2 days after treatment with BABA or water. Samples were harvested at 8 h after SA treatment.* PR1 *expression was analysed by RT-qPCR. Data shown are mean levels (± SEM) of* PR1 *expression relative to water-treated mock-sprayed Col-0 plants.* ***B.*** *Quantification of BABA-induced priming of defence-contributing callose deposition against* Hpa*. Effectiveness of callose deposition was analysed 3 dpi by aniline blue/ calcofluor double staining of leaves from Col-0 and* voz1/2-2 *plants. Shown are percentages of callose-arrested and non-arrested germ tubes of* Hpa*. Asterisks indicate statistically significant differences between water- and BABA-treated plants within each genotype (Fisher's Exact test; *: p<0.05; n.s. = not significant).*

***VOZ2 requires nuclear localisation for basal and BABA-induced resistance against* Hpa**. The above results indicate that the transcription factors VOZ1 and VOZ2 contribute to both basal and BABA-induced resistance. While VOZ transcription factors are predominantly localised in the cytoplasm, they require translocation to the nucleus to mediate transcriptional induction of target genes (Yasui et al. 2012). To investigate whether nuclear translocation occurs during expression of BABA-IR, the subcellular localisation of VOZ2 was studied in stably transformed *voz1/2-2* overexpressing GFP-tagged VOZ2 (*voz1/2-2 /* 35S:*GFP-VOZ2,* first described in Yasui et al. 2012) upon pre-treatment with water or 5 mg/L BABA and after subsequent mock or *Hpa* challenge (i.e. the four conditions of the 4-stage model; see Chapter 1). Confocal microscopy revealed GFP fluorescence in the cytoplasm under all 4 conditions tested (Fig. 5.3A). This demonstrates that VOZ2 is present in the cytoplasm during expression of BABA-IR, where it can interact with IBI1. Additionally, GFP fluorescence was detected in structures that resemble the tonoplast. However, no nuclear signal was detected under any condition tested.

VOZ1 and VOZ2 have been reported to control the expression of defence-related genes (Nakai, Nakahira, et al. 2013). However, direct binding of VOZ2 has only been demonstrated to a 38bp *cis*-acting region of the *AVP1* gene, which encodes a pollen-specific proton pump involved in auxin transport (Mitsuda et al. 2004). Since the confocal microscopy analysis of VOZ2-GFP did not reveal nuclear localisation of VOZ2 during BABA-IR (Figure 5.3A), the possibility arose that VOZ2 regulates BABA-IR in the cytoplasm, for example by mediating interactions between IBI1 and other defence signalling proteins. To test the relevance of the subcellular localisation of VOZ2, BABA-IR was tested in *voz1/2-1* plants overexpressing *VOZ2* fused to either a nuclear localisation sequence (*VOZ2-NLS,* Yasui et al. 2012), or a nuclear export signal (*VOZ2-NES*, Yasui et al. 2012). While wild-type Col-0 and *VOZ2-NLS* plants expressed similarly high levels of BABA-IR against *Hpa*, the level of BABA-IR in *VOZ2-NES* plants was significantly lower (Fig. 5.3B) and comparable to the reduced BABA-IR response of *voz1/2-2* plants (Fig. 5.1A). Hence, despite the fact that the confocal microscopy analysis failed to detect nuclear VOZ2-GFP during expression of BABA-IR (Figure 5.3A), nuclear translocation of VOZ2 is necessary to mount a full BABA-IR response. Interestingly, overexpression of VOZ2-NLS increased basal resistance, while the *voz1/2-1* double mutant complemented with VOZ2-NES showed reduced basal resistance in comparison to water-treated Col-0 plants. It can therefore be concluded that nuclear VOZ2 localisation contributes to both BABA-IR and basal resistance against *Hpa*.

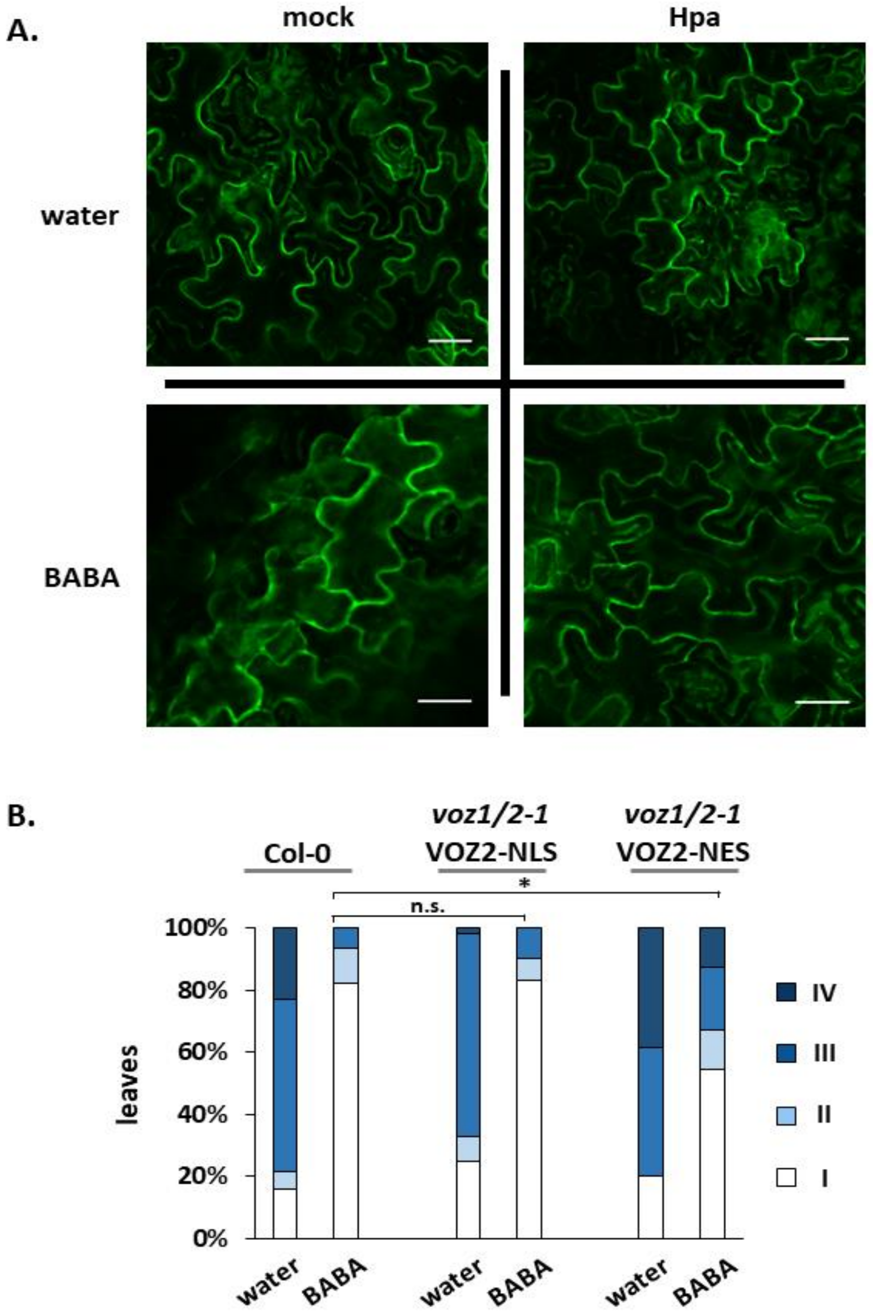

Figure 5.3: Subcellular localisation of VOZ2 and importance of nuclear localisation for BABA-IR. A. *Confocal laser scanning microscopy of GFP-VOZ2 in the 35S:*GFP-VOZ2 *overexpression line of Arabidopsis. Shown is GFP fluorescence in epidermal leaf cells of 3-week old un-primed and BABA-primed plants (5 mg/L) at 3 dpi with* Hpa *or mock solution. Scale bars = 20 µm.* ***B.*** *BABA-IR against* Hpa *in 3-week old Col-0 and* voz1/2-2 *complemented with VOZ2 fused to a nuclear localisation signal (VOZ2-NLS) or nuclear exportation sequence (VOZ2-NES). Plants were treated with 5 mg/L BABA two days prior to inoculation with* Hpa *(1*10^5 spores/mL).* Hpa *colonisation was determined in trypan blue-stained leaves at 5 dpi by assigning leaves to different* Hpa *colonisation classes. Asterisks denote significant differences in class distributions (Fisher's Exact test; *: p<0.05; n.s.: not significant; n = 60 – 150).*

BABA-induced resistance is not dependent on phytochrome signalling. Yasui *et al.* reported that VOZ2 interacts with cytoplasmic phytochrome B (PhyB; Yasui et al. 2012). PhyB exists in in two distinct forms: the red-light absorbing, cytoplasmic P_r form, and the far-red-light absorbing, nuclear P_{fr} form. Absorption of red light by cytoplasmic P_r leads to photoconversion into the nuclear P_{fr} form. *Vice versa*, absorption of far-red light by nuclear P_{fr} leads to photoconversion into the cytoplasmic P_r form (de Wit et al. 2013). It is generally accepted that the nuclear P_{fr} form of phyB is biologically active (Smith 2000). Interestingly, phyB has been reported to be critical for expression of systemic acquired resistance (SAR; Griebel and Zeier 2008), which, like BABA-IR, is based on priming of inducible defences (Kohler, Schwindling, and Conrath 2002). To explore the role of phytochrome signalling in BABA-IR, Col-0 plants were grown under standard light or far-red supplemented light, while maintaining similar levels of photosynthetic photon flux density (PPFD). The supplementation with far-red light resulted in a red/far-red ratio of 0.31 (compared to 7.75 under standard light), which was sufficient to induce an etiolation response (Fig. 5.4A). However, despite this physiological response, the differences in red/far-red ratios did not result in differences in BABA-IR (after pre-treatment with 5 mg/L BABA) or basal resistance against *Hpa* (Fig. 5.4B), indicating that the red/far-red ratio does not majorly influence BABA-IR and basal resistance to *Hpa*. To exclude a non-canonical role for phyB in *Hpa* resistance, the phytochrome A/B double mutant *phyA-211 phyB-9* (*phyA/B*) was tested for BABA-IR and basal resistance against *Hpa*. As is shown in Fig. 5.4C, the *phyA/B* mutant was not affected in BABA-IR or basal resistance. This indicates that the previously reported interaction between VOZ2 and phyB is not of direct relevance to basal resistance or BABA-IR against *Hpa* (Fig. 5.4B).

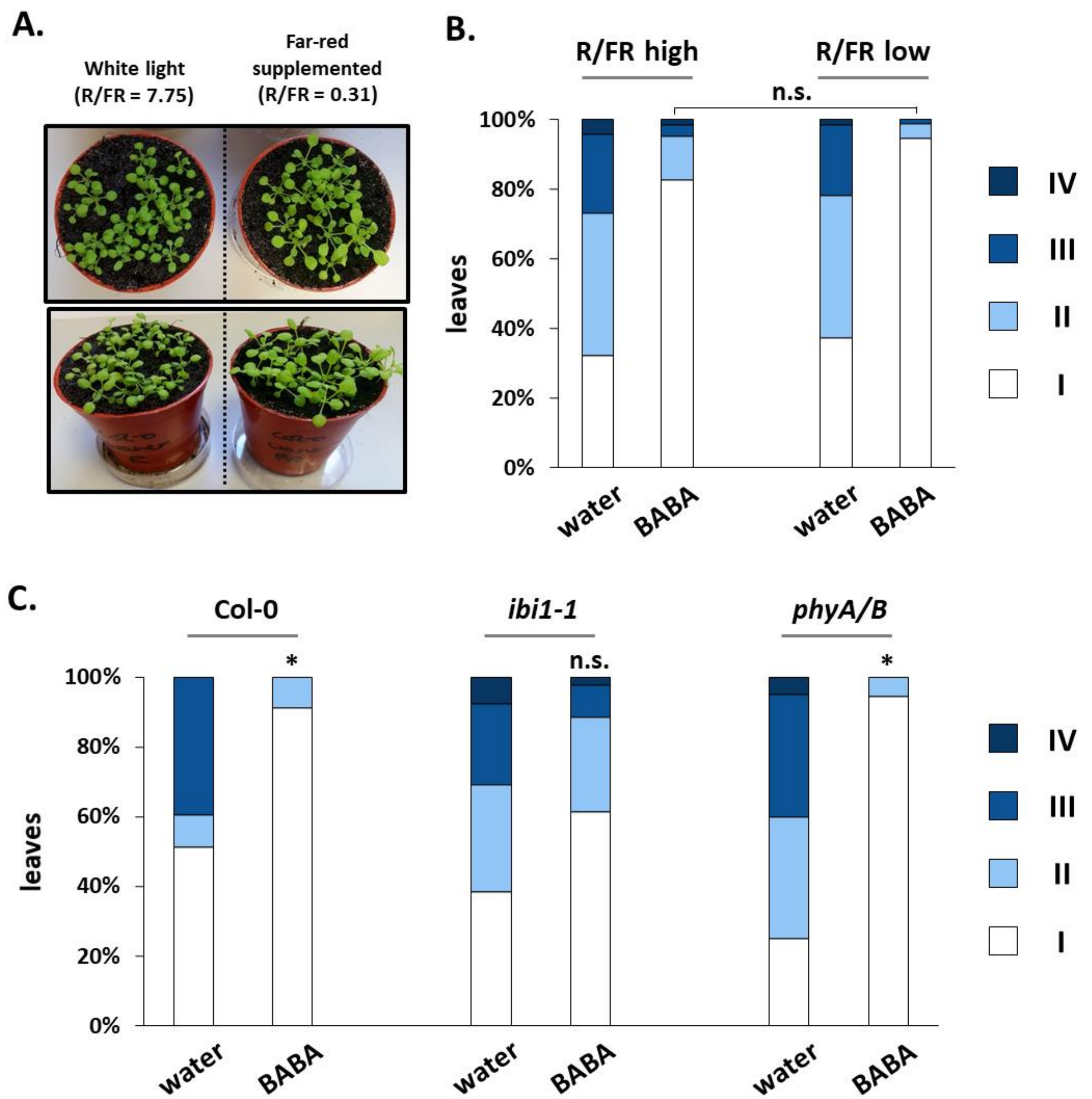

Figure 5.4: Phytochrome B does not play a role in BABA-IR against downy mildew. A. *Growth phenotypes of wild-type Arabidopsis (Col-0) under standard and far-red supplemented light conditions (R/FR = 7.75 and 0.31, respectively). Plants grown under low R/FR conditions showed longer hypocotyls and petioles.* ***B.*** *BABA-IR (5 mg/L) against* Hpa *in 3-week old wild-type plants (Col-0) grown under standard light conditions with or without far-red enrichment. Plants were treated with 5 mg/L BABA two days prior to inoculation with* Hpa *($1*10^5$ spores/mL).* Hpa *colonisation was determined in trypan blue-stained leaves at 5 dpi by assigning leaves to different* Hpa *colonisation classes. Asterisks denote significant differences in class distributions (Fisher's Exact test; *: p<0.05; n = 55 – 70).* ***C.*** *BABA-IR (5 mg/L) against* Hpa *in 3-week old Col-0,* ibi1-1 *and* phyA/B *plants that are impaired in phytochrome signalling. Asterisks denote significant differences in class distributions (Fisher's exact test; p<0.05; n.s. = not significant; n = 20 – 40).*

5.4 Discussion

VOZ1 and VOZ2 belong to the NAC family of TFs and show 53% sequence similarity at the amino acid level (Mitsuda et al. 2004). Orthologues of VOZ1 and VOZ2 have been found in various higher plants and the moss *Physcomitrella patens* (Mitsuda et al. 2004). VOZ1 and VOZ2 seem to have mutually redundant activities in the plant cell. This is evidenced by the fact that a *voz1/voz2* double mutant, but neither single mutant, shows a delayed-flowering phenotype (Yasui et al. 2012). Furthermore, single mutants in either *voz1* or *voz2* show only a mild reduction in primary root growth, which is exacerbated in a *voz1/voz2* double mutant; expression of either gene under the control of its native promotor prevents this growth phenotype (Nakai, Nakahira, et al. 2013). As described in Chapter 4 of this thesis, yeast two-hybrid analysis identified both VOZs as interaction partners of the BABA receptor IBI1 (Chapter 4; Table 4.2). Furthermore, the *in planta* interaction between VOZ2 and IBI1 could be confirmed by BiFC assays in *N. benthamiana* (Chapter 4; Figure 4.2). Based on the contrasting resistance phenotypes of Arabidopsis knock-out and overexpressing lines of *VOZ1* and *VOZ2* to biotic and abiotic stresses (Nakai, Nakahira, et al. 2013; Nakai, Fujiwara, et al. 2013; Koguchi et al. 2017), it is commonly assumed that VOZ1 and VOZ2 regulate the balance between plant responses to biotic and abiotic stress. For instance, *voz1/2-2* plants show increased cold tolerance, which is associated with enhanced expression of abiotic stress-responsive genes, such as the ABA-inducible and cold-responsive genes *CBF4* and *RD29B* (Nakai, Nakahira, et al. 2013). Despite the fact that *voz1/2* plants contain wild-type levels of ABA, the enhanced abiotic stress tolerance and ABA-responsive gene expression in *voz1/2* plants indicates that VOZs suppress ABA signalling (Nakai, Nakahira, et al. 2013). In contrast to abiotic stress resistance, resistance of *voz1/2-2* against adapted and unadapted pathogens is severely reduced (Nakai, Nakahira, et al. 2013), indicating a role for VOZs in both PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI)-related defence mechanisms. The *voz1/2* mutant is also affected in systemic acquired resistance (SAR) against *P. syringae* pv. *tomato* (*Pst*) DC3000 after priming treatment with the avirulent strain *Pst* DC3000/avrRpt2 (Nakai, Nakahira, et al. 2013). Hence, VOZs increase plant immune responses against pathogens, but antagonise ABA-dependent defence against abiotic stress. This positive role for VOZs in biotic stress resistance is in agreement with the outcome of this Chapter, which has established that IBI1-interacting VOZs play an important role in basal and BABA-induced resistance against *Hpa* (Figs. 5.1 – 5.3).

Unlike the BABA receptor mutant *ibi1-1*, *voz1/2-2* plants still showed enhanced resistance against *Hpa* after BABA pre-treatment (Fig 5.1A). However, the level of BABA-IR in *voz1/2-2* plants was substantially reduced in comparison to Col-0 wild-type plants, indicating partial impairment of BABA-IR in *voz1/2-2* plants. BABA-IR against *Hpa* is based on priming of at least two distinct defence pathways: the SA-dependent signalling pathway that results in augmented expression of SA-inducible defence genes, and an SA-independent signalling pathway that regulates augmented deposition of defence-related callose (Zimmerli et al. 2000; Ton et al. 2005). The latter pathway involves regulation by ABA (Ton and Mauch-Mani 2004; Ton et al. 2005), although the exact mechanisms by which ABA controls this primed callose response remain unknown (Ton et al. 2009). Analysis of both priming responses suggested that priming of SA-dependent gene expression is intact in the *voz1/2-2* mutant, but that BABA-induced priming of defence-related callose deposition is abolished in the *voz1/2-2* mutant (Figs. 5.1B and 5.1C). It can thus be concluded that the reduced BABA-IR response in *voz1/2-2* is caused by impaired priming of SA-independent callose deposition. The interaction between VOZ-dependent signalling and ABA signalling is congruent with the regulatory role of ABA during BABA-induced priming of defence-related callose (Ton and Mauch-Mani 2004; Ton et al. 2005). Exactly how ABA signalling and VOZ TFs interact during expression of BABA-IR, however, requires further investigation.

It has previously been reported that enhanced disease susceptibility of *voz1/2-2* plants to the hemi-biotrophic fungus *Colletotrichum higginsianum* is associated with reduced expression of the SA-dependent *PR1* gene (Nakai, Nakahira et al. 2013), while overexpression of *VOZ2* results in augmented *PR1* induction during *C. higginsianum* infection (Nakai, Fujiwara et al. 2013). These results contrast the *PR1* expression profiles presented in Fig. 5.2A, which show that SA-induced *PR1* induction is unaffected in *voz1/2-2* plants (Fig. 5.2A). Furthermore, in contrast to Nakai et al. (2013), who reported enhanced disease susceptibility in *voz1/2-2* to *C. higginsianum* (Nakai, Nakahira et al. 2013), the *voz1/2-2* mutant did not show statistically enhanced levels of susceptibility to *Hpa* (Fig. 5.1). This discrepancy could be explained by the different lifestyles of hemibiotrophic *C. higginsianum* and the obligate biotroph *Hpa.* While biotrophic pathogens suppress host defences in order to establish host parasitisation, hemibiotrophic *Colletotrichum* species do not reduce expression of defence mechanisms during their biotrophic growth stage (Vargas et al. 2012). Accordingly, it is possible that the suppression of SA-dependent basal defence by *Hpa* in wild-type Col-0 masks the enhanced disease susceptibility phenotype of *voz1/2* plants. Nonetheless, Fig. 5.3B showed that overexpression of *VOZ2-NLS* in the background of *voz1/2* boosts basal resistance against *Hpa*, indicating that nuclear localisation of VOZ2 contributes to basal resistance against *Hpa.* Whether this increased basal resistance relies on SA-dependent dependent defence or enhanced deposition of SA-independent callose remains to be answered. However, the unaffected *PR1* response of *voz1/2* plants to SA (Fig. 5.2A), in combination with the reduced callose effectiveness in *voz1/2* plants, strongly suggests that VOZs regulate SA-independent callose defence in Arabidopsis.

Despite their nuclear activity, VOZ TFs are predominantly localised in the cytoplasm (Yasui et al. 2012). Recently, it was shown that VOZ2 congregates into cytosolic stress granules during heat stress, of which a small fraction translocates to the nucleus, where it is immediately degraded by the ubiquitin/26S proteasome system (Koguchi et al. 2017). Hence, only a small fraction of VOZ2 is physically localised in the nucleus during the regulation of VOZ-dependent genes. In agreement with these findings, the confocal microscopy results presented in Fig. 5.3 showed that GFP-VOZ2 is mostly present in the cytoplasm, irrespective of BABA treatment or *Hpa* challenge. The cytoplasmic localisation of VOZ2 creates opportunity for VOZ2 to interact with IBI1, which translocates from the ER to the cytoplasm during expression of BABA-IR (Luna et al. 2014). It should be noted that during downy mildew infection, only a relatively small number of host cells is in direct contact with oomycete hyphae, and the presence of haustoria in the imaged cells was not determined in this experiment. Therefore, increased translocation of VOZ proteins to the nucleus in infected cells cannot be excluded. To investigate whether nuclear localisation of VOZ2 is necessary for BABA-IR, levels of BABA-IR were compared between *voz1/2* plants overexpressing VOZ2 fused to a nuclear localisation sequence (*VOZ2-NLS*) or a nuclear export signal (*VOZ2-NES*), respectively. *VOZ2-NLS* restored BABA-IR against downy mildew to wild-type levels, whereas the *VOZ2-NES* line showed partial impairment in BABA-IR (Fig. 5.3B), similar to the *voz1/2-2* mutant (Fig. 5.1). Of note, constitutive overexpression of *VOZ2-NLS* and *VOZ2-NES* also enhanced and reduced basal resistance to *Hpa*, respectively, indicating that nuclear VOZ activity is important for both BABA-IR and basal resistance.

Apart from regulating plant responses to biotic and abiotic stresses, VOZ1 and VOZ2 also control the timing of transition to flowering (Yasui et al. 2012). This process is influenced by the red/far-red light receptor phytochrome B, which represses flowering by suppressing the key flowering regulator *FLOWERING LOCUS T* (*FT*). Consequently, *phyB* mutants flower earlier than wild-type Arabidopsis. The early-flowering phenotype of the *phyB* mutant is reversed in the *phyB/voz1/voz2* triple mutant, indicating that VOZ1 and VOZ2 mediate phytochrome B-dependent regulation of flowering (Yasui et al. 2012). In support of this, VOZ1 and VOZ2 have been identified as direct interaction partners of phyB in yeast two-hybrid assays (Yasui et al. 2012). The importance of light signalling, and specifically the ratio of red/far-red light, for defence is well established (Ballaré 2014). Low red/far-red ratios, as well as low light intensities (e.g. shading), increase susceptibility to necrotrophic and biotrophic pathogens, and weaken defences against herbivores. This repressive effect of low light is not fully explained by decreased photosynthesis and involves an active regulatory mechanism that allows plants to prioritise defences in photosynthetically active, and thus valuable, tissues. Plants also prioritise growth over defence: while far-red light and shading suppress disease resistance, defence induction by pathogen inoculation does not inhibit shade-avoidance syndrome (SAS) (de Wit et al. 2013). Perception of low red/far-red light compromises both SA- and JA-dependent defence pathways (de Wit et al. 2013; Ballaré 2014). In the case of JA-dependent immunity, this repression is caused by a complex interplay between several hormonal pathways. JAZ proteins are negative regulators of JA signalling. Low R/FR ratios reduce resistance to *Botrytis cinerea* in a JAZ-dependent manner (Cerrudo et al. 2012), possibly via increased transcription and stability of JAZ proteins (Ballaré 2014). Part of this repressive action of low R/FR is mediated by DELLA proteins, which act as negative regulators of gibberellic acid (GA) signalling (Eckardt 2007). JAZ and DELLAs mutually antagonise each other (Hou et al. 2010; Yang et al. 2012). Under low light conditions (e.g. low R/FR), GA activity is increased, leading to degradation of DELLA proteins, which stabilises JAZ proteins and represses JA-dependent resistance (Ballaré 2014 and references therein). Light-controlled changes in other phytohormone pathways, including auxin and brassinosteroid signalling, could further influence JA responses (Ballaré 2014). The effects of low R/FR ratios or shading on SA-dependent defences are less well understood. Low R/FR ratios decrease the expression of SA-induced genes, even though monomerisation and nuclear translocation of the key SA-response regulator NPR1 are enhanced under these conditions (de Wit et al. 2013). A possible explanation could be that low light signals prevent phosphorylation, nuclear translocation, ubiquitination or subsequent degradation of NPR1, which are essential mechanisms for full induction of SA-dependent gene expression (Fu et al. 2012; Ballaré 2014). As with JA signalling, the effects of light signalling on other hormonal pathways likely influence SA responses. The fact that GAs, which appear to be positive regulators of SA signalling in Arabidopsis (Navarro et al. 2008; Alonso-Ramírez et al. 2009), are more active under low R/FR or shading conditions underscores the complexity of this regulatory network (Ballaré 2014). Nevertheless, it is clear that low R/FR increases susceptibility to (hemi)biotrophic pathogens (de Wit et al. 2013). The importance of phytochromes for acquired immunity is further evidenced by the previous report that *phyA/B* double mutant plants are compromised in pathogen-induced expression of SA-dependent SAR (Griebel and Zeier 2008). Based on this role of phytochrome signalling in acquired immunity, and because the IBI1-interacting protein VOZ2 interacts with phytochrome B (Yasui et al. 2012), the role of R/FR signalling in BABA-IR was investigated (Fig. 5.4). To this end, Col-0 wild-type plants were exposed to similar light intensities with and without FR enrichment (corresponding to R/RF ratios of 0.31 and 7.75, respectively). Despite the fact that FR enrichment was sufficient to induce mild etiolation, as evidenced by elongated hypocotyls and petioles (Fig. 5.4A), BABA-IR against *Hpa* was unaffected by this treatment (Fig. 5.4B). Furthermore, in a separate experiment, the *phyA/B* double mutant was fully capable of expressing wild-type levels of BABA-IR against *Hpa* (Fig. 5.4C). Thus, despite the previously reported interaction between VOZ2 and phyB and the role of R/FR signalling in disease resistance (Griebel and Zeier 2008;

Yasui et al. 2012; de Wit et al. 2013), the results presented in Fig. 5.4 clearly demonstrate that phyB does not play a role in BABA-IR against *Hpa*.

In summary, the results presented in this Chapter have identified VOZ TFs as novel signalling components in callose-mediated BABA-IR against *Hpa*. The previously demonstrated role of ABA in callose-mediated BABA-IR and transcriptional repression of VOZs during ABA-dependent abiotic stress (Ton and Mauch-Mani 2004; Nakai, Nakahira et al. 2013) suggest that VOZ-dependent repression of ABA-inducible plant responses is important for expression of IBI1-dependent callose-mediated defence. Curiously, apart from BABA-induced defence priming against *Hpa*, BABA has been reported to augment ABA-dependent pathways for increased protection against abiotic stress (Jakab et al. 2005) and necrotrophic pathogens (Ton and Mauch-Mani 2004). The role of ABA in BABA-IR thus remains enigmatic. Future studies are needed to determine the role of VOZs in BABA-IR against abiotic stress and necrotrophic pathogens, as well as the exact signalling role of ABA in IBI1-dependent defence against *Hpa.* Future experiments should also focus on the biochemical modifications mediating VOZ2-dependent cell wall defence, such as post-translational modifications of VOZs that enhance VOZ stability and/or nuclear activity.

5.5 Acknowledgements

Arabidopsis *voz1-2/voz2-2* seeds were kindly provided by Prof. Masa Sato (Kyoto Prefectural University, Japan). Arabidopsis *voz1-1/voz2-1/*Pro35S:*GFP-VOZ2, voz1-1/voz2-1/*35S:*GFP-VOZ2-NLS* and *voz1-1/voz2-1/*35S:*GFP-VOZ2-NES* seeds were kindly provided by Prof. Takayuki Kohchi (Kyoto University, Japan).

Chapter 6

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The role of photorespiration in BABA-induced resistance

6.1 Abstract

The plastidic NAD-dependent malate dehydrogenase plNAD-MDH was identified as an interactor of the β-aminobutyric acid (BABA) receptor IBI1 by yeast two-hybrid screening (Chapter 4). MDH catalyses the reversible reduction of oxaloacetic acid to malate. This activity enables exchange of reducing equivalents and amino-group receptors between the chloroplast stroma and the cytosol, thereby fulfilling a critical role in the functioning of the cyclic photorespiration pathway. Photorespiration is a major intracellular source of reactive oxygen species (ROS) and has been reported to contribute to non-host resistance and effector-triggered immunity in Arabidopsis. The present study explores the contribution of photorespiration to BABA-induced resistance. Eliminating photorespiration by keeping plants in the dark abolished BABA-induced resistance. Moreover, knock-down mutants in glycolate oxidase (*GOX*), which is the ROS-generating enzyme within the photorespiration cycle, displayed reduced levels of BABA-IR against the downy mildew pathogen *Hyaloperonospora arabidopsidis*. This chapter thus provides evidence that BABA-IR partially depends on GOX-derived ROS produced during photorespiration.

6.2 Introduction

The plastidic NAD-dependent malate dehydrogenase plNAD-MDH was identified in a yeast two-hybrid screen as an interactor of the β-aminobutyric acid (BABA) receptor IBI1 (Chapter 4). This chloroplast-located enzyme catalyses the NAD+/NADH-consuming and reversible interconversion between malate and oxaloacetic acid (OAA), which plays an important role in nitrogen metabolism. For instance, plNAD-MDH participates in nitrogen metabolism through regeneration of NADH that is consumed by NADH-dependent glutamate-oxoglutarate amino transferase (GOGAT) in roots during (non-photorespiratory) ammonium assimilation (Konishi et al. 2014). In addition, plNAD-MDH provides the cytosolic enzyme nitrate reductase (NR) with chloroplastic NADH equivalents (Taniguchi and Miyake 2012), which can generate defence-regulatory nitric oxide (NO; Delledonne et al. 1998; Salgado et al. 2013). Furthermore, plNAD-MDH-derived malate acts as an essential antiporter substrate to ensure export of glutamate and import of 2-oxoglutarate from and into the chloroplast, respectively (Weber and Flügge 2002). This exchange of glutamate and 2-oxoglutarate is crucial for recycling of ammonium that is released during photorespiration by the GS-GOGAT system (Bauwe, Hagemann, and Fernie 2010). Malate levels are co-regulated by chloroplastic plNAD-MDH and NADP-dependent MDH. Expression levels of plNADP-MDH in the leaves are two- to three-fold higher than those of plNAD-MDH. However, in contrast to the constitutively active plNAD-MDH, plNADP-MDH is only active in the light (Berkemeyer, Scheibe, and Ocheretina 1998; Beeler et al. 2014). While the relative contribution of these two enzymes to the regulation of malate/OAA balance in illuminated leaves remains unknown, it stands to reason that both enzymes could work in tandem to adjust malate levels to the changing metabolic needs of the leaf.

Redox homeostasis of the chloroplast requires tight regulation to prevent photoinhibition by stromal overreduction from excess electron absorption. Reducing equivalents can be shuttled between the chloroplast and the cytosol via the oxaloacetate/malate antiporter *pOMT1*, a process called the 'malate valve' (Kinoshita et al. 2011). As explained above for Glu/2OG antiport, this process is mediated by redox-regulated plastidic MDH enzymes. Accordingly, it is likely that plNAD-MDH and NADP-dependent MDH co-regulate redox homeostasis of the chloroplast. In addition to the malate valve and nitrogen assimilation, plNAD-MDH could also play a role in fatty acid biosynthesis (Berkemeyer, Scheibe, and Ocheretina 1998). Plastids are the main sites of *de novo* biosynthesis of fatty acids in plants (Lichtenthaler and Golz 1995). The acetyl-CoA necessary for this process is largely generated by NAD-dependent pyruvate decarboxylase complex (Lin and Oliver 2008). The plNAD-MDH enzyme could generate malate which is then de-carboxylated to pyruvate by NADP-malic enzyme to serve this pathway. Indeed, malate has been shown to act as a precursor in fatty acid biosynthesis in castor bean endosperm (Smith et al. 1992).

In addition to the above functions of MDH, the enzyme also regulates photorespiration. Photorespiration occurs in illuminated leaves of C3 plants, resulting in O_2 consumption and CO_2 production. Unlike cellular respiration, photorespiration is light dependent. Photorespiration is caused by the catalytic properties of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), which catalyses the biochemical assimilation of atmospheric CO_2 (Ellis 1979). Although Rubisco has a strong preference for CO_2, the much higher atmospheric concentration of O_2 can lead to significant substrate competition between CO_2 and O_2 (Foyer et al. 2009). As a result, it has been estimated that a quarter of the reactions catalysed by Rubisco under standard growth conditions consume O_2 (Sharkey 1988; Peterhansel and Maurino 2011). The incorporation of CO_2 into ribulose 1,5-bisphosphate (RuBP, a C5 compound) leads to the production of two molecules of 3-phosphoglycerate (PGA, a C3 compound), which is subsequently used for synthesis of C6 sugars and regeneration of ribulose 1,5-bisphosphate. By contrast, when Rubisco incorporates O_2 into RuBP, the products are one molecule of PGA (C3) and one molecule of 2-phosphoglycolate (C2). Phosphoglycolate is a potent inhibitor of phosphofructokinase and triosephosphate isomerase, and is quickly metabolised into toxic glycolate (Peterhansel and Maurino 2011 and references therein). The photorespiration pathway allows plants to generate one molecule of PGA from two molecules of glycolate, thereby recovering 3 out of 4 carbon atoms that were incorporated into glycolate due to the oxygenation reaction of RuBP (Peterhansel and Maurino 2011). Regulation of photorespiration is complex as it requires coordination of biochemical reactions between four cell compartments: chloroplasts, peroxisomes, mitochondria and the cytosol (Bauwe, Hagemann, and Fernie 2010).

The role of plNAD-MDH in photorespiration is two-fold (Fig. 6.1). Firstly, together with NADP-MDH, it ensures continuous Glu/2OG antiport across the chloroplast membrane, which is necessary for re-fixation of ammonium released during the conversion of glycine to serine by the glycine decarboxylase complex (GDC). It has been estimated that re-fixation of ammonium from photorespiration exceeds nitrate reduction in C3 plants by 5- to 10-fold (Wingler et al. 2000). Secondly, the reduction of pyruvate to glycerate in the peroxisome requires NADH (Schmitt and Edwards 1983; Reumann, Heupel, and Heldt 1994). Since NADH cannot cross the peroxisomal membrane, it must be generated in the organelle itself through activity by peroxisomal NAD-MDH, which in turn uses malate that is provided by chloroplastic MDH (Fig. 6.1). Photorespiration significantly reduces the efficiency of carbon fixation. Furthermore, the regeneration of PGA and recycling of ammonium requires ATP and NADPH. It is therefore often thought that the oxygenase activity of Rubisco is an evolutionary relic from pre-historic times, when atmospheric oxygen levels were so low that the incorporation of oxygen into

RuBP would have been inconsequential for overall photosynthetic output (Peterhansel and Maurino 2011). However, recent evidence indicates that photorespiratory flux is important for a variety of other processes, including nitrate assimilation and responses to biotic and abiotic stress (Betti et al. 2016; Voss et al. 2013). With regards to biotic stress resistance, it has been found that photorespiration mutants in glycolate oxidase (GOX) are impaired in non-host resistance and effector-triggered immunity (Rojas et al. 2012). Furthermore, naturally occurring hyper-active alleles of the photorespiratory enzyme glyoxylate aminotransferase have been shown to confer resistance in melon against downy mildew (*Pseudoperonospora cubensis*), which is associated with an increased capacity for photorespiratory ROS production (Taler et al. 2004). Since photorespiration is a major source of intracellular ROS, which can act as second messengers for plant defence (Sharma et al. 2012; Pastor et al. 2013), photorespiration flux is increasingly regarded as an important contributor to plant defence. Based on the earlier finding that plNAD-MDH interacts with the BABA receptor IBI1 (Chapter 3), together with the emerging role of photorespiration in broad-spectrum disease resistance, this Chapter explores the contribution of photorespiration in BABA-induced resistance.

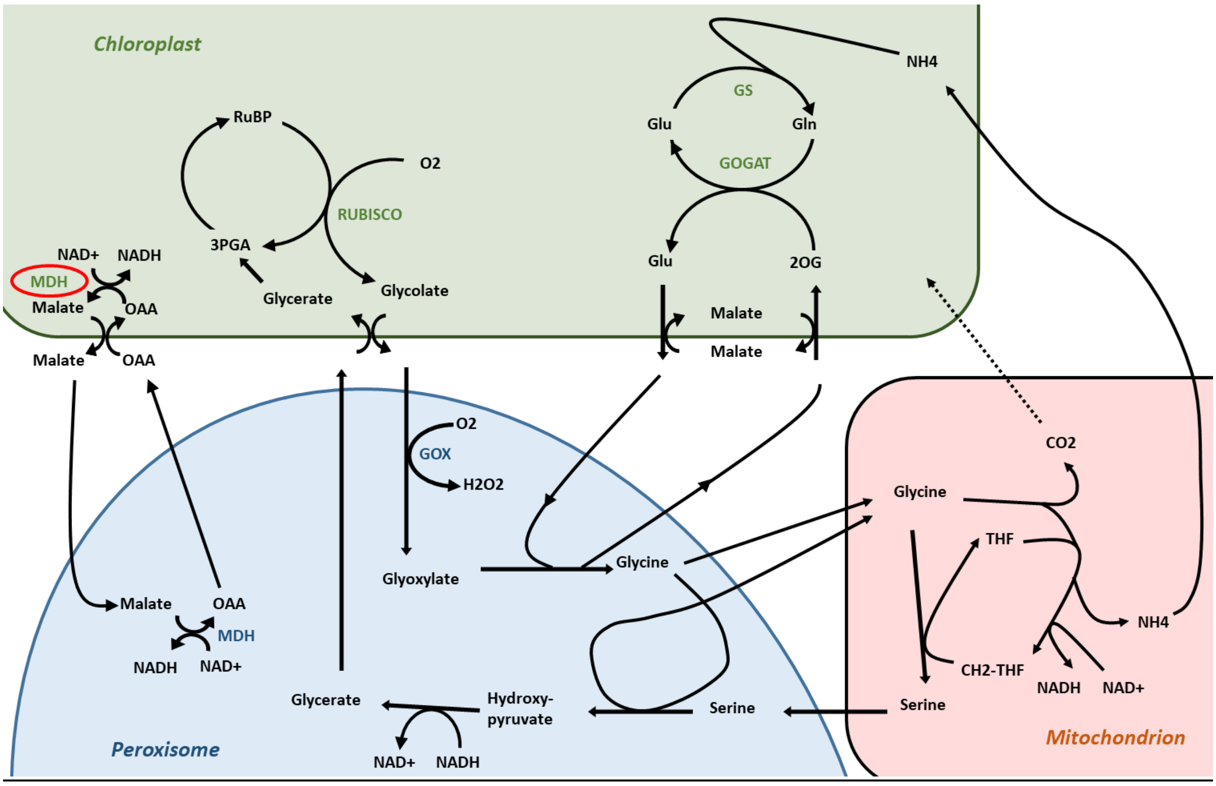

Figure 6.1: The photorespiration pathway. *Photorespiration occurs when RUBISCO incorporates* O_2 *into ribulose 1,5-bisphosphate. This oxygenation reaction results in chloroplastic accumulation of glycolate, which is exported to the peroxisomes, where it is oxidised into glyoxylate by glycolate oxidase (GOX). This step uses* O_2 *and generates* H_2O_2 *that can cascade into other reactive oxygen species (ROS). Glyoxylate is aminated into glycine and exported to the mitochondria, where it is converted into serine. The latter step releases* CO_2 *and ammonium, which is re-assimilated in the chloroplast by the activity of the GS-GOGAT cycle. Serine is imported back into the peroxisomes, where it is converted via hydroxypyruvate into glycerate, which re-enters the chloroplast and the Calvin cycle. The reduction of hydroxypyruvate into glycerate consumes reducing power in the form of NADH. Since NADH cannot cross membranes directly, NADH equivalents are imported from chloroplasts and mitochondria in the form of malate after redox conversion from oxaloacetic acid by malate dehydrogenase (red-circled). Inside the peroxisome, malate is oxidised into oxaloacetic acid by peroxisomal MDH, generating NADH. Figure adapted from Bauwe, Hagemann, and Fernie (2010).*

6.3 Results

BABA-IR is inhibited by darkness. Homozygous knock-out mutations in *plNAD-MDH* are embryo-lethal, while plants with reduced levels of *plNAD-MDH* show severe pleiotropic effects (Beeler et al. 2014). Accordingly, *plNAD-MDH* mutants cannot be used to study the role of this gene in BABA-IR. Instead, a different approach was used to test the hypothesis that the interaction between IBI1 and plNAD-MDH contributes to BABA-IR by modulating photorespiratory ROS production. Photorespiration, in contrast to dark respiration (cellular respiration), is a light-dependent process that is completely inhibited in the dark. To gain support for the hypothesis that BABA-IR requires photorespiration, wild-type plants (Col-0) were grown for 2.5 weeks under short-day conditions at a light intensity of approx. 120µmol/m^2/s and transferred to different light conditions, ranging from complete darkness to 20µmol/m^2/s, and 120µmol/m^2/s at 2 days after soil-drenching and immediately after challenge inoculation with the downy mildew pathogen *Hyaloperonospora arabidopsidis* (*Hpa*). At 5 days post inoculation (dpi), disease progression was quantified by microscopy analysis of trypan blue-stained leaves. Strikingly, priming treatment with 5 mg/L BABA did not enhance *Hpa* resistance when plants were kept in the dark after inoculation (Fig. 6.2A). In contrast, BABA induced statistically significant levels of resistance in plants that were kept at low or medium light intensities after inoculation, compared to the respective water-treated controls. Notably, *Hpa* colonisation in control-treated plants was lower in darkness-kept plants compared to light-kept plants, which is probably due to the fact that colonisation by the obligate biotroph *Hpa* requires photosynthates from its host.

BABA-IR is augmented under high light. Under otherwise constant conditions, photorespiration increases with light intensity, due to the increase in O_2 production from the light reaction of photosynthesis (Kangasjärvi et al. 2012). To test if the correlation between light and BABA-IR at reduced light also holds true at higher light intensity, plants were grown at 120 µmol/m^2/s for two weeks, then soil-drenched with 5 mg/L BABA or water. Two days after pre-treatment, plants were inoculated with *Hpa,* and immediately moved to 120 µmol/m^2/s or 200 µmol/m^2/s for 5 days before quantification of *Hpa* colonisation (Fig. 6.2B). While the increase in light intensity from 120 to 200 µmol/m^2/s did not augment basal resistance, the level of BABA-IR was significantly enhanced in plants kept under 200 µmol/m^2/s light. Together with the lack of BABA-IR under complete darkness, these results are in line with the hypothesis that light-dependent photorespiration contributes to BABA-IR.

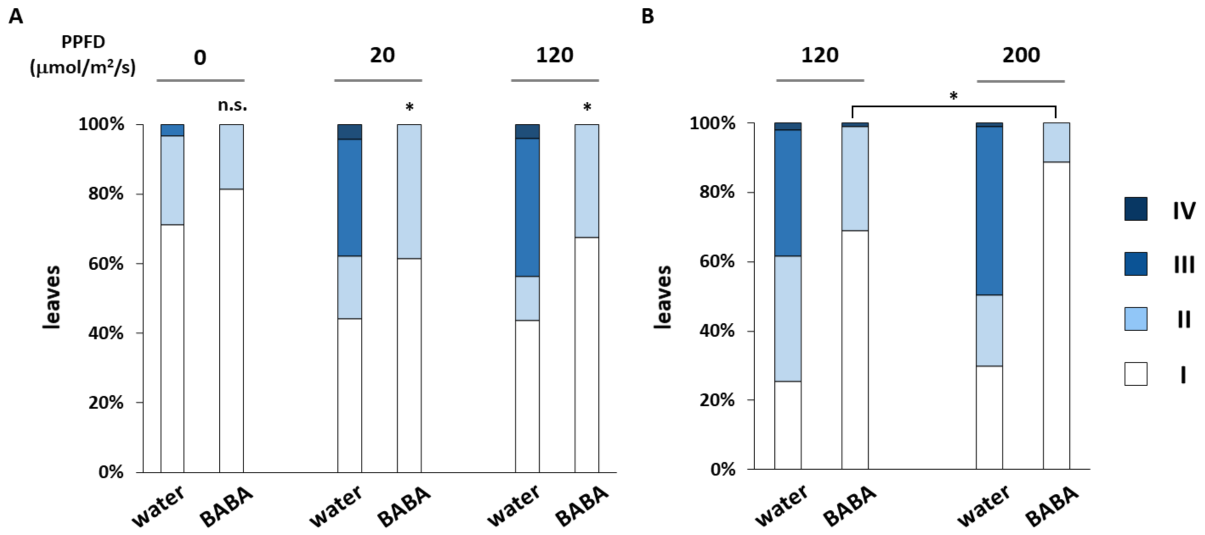

Figure 6.2: BABA-induced resistance (BABA-IR) is dependent on light. *Plants (2.5-week old) were spray-inoculated with* Hpa *(10^5 spores/mL) at 2 days after soil-drenching with 5 mg/L BABA or water. Immediately after inoculation, plants were transferred to different light intensities. At 5 days post inoculation (dpi), plants were assessed for* Hpa *colonisation by microscopy analysis of trypan blue-stained leaves. Asterisks denote statistically significant differences in distribution over four different* Hpa *colonisation classes (see chapter 2.4) ($p<0.01$; Fisher's exact test; n=60-120) n.s. = not statistically significant.* ***A.*** *Effects of decreasing light intensities (120, 20 and 0 µmol/m^2/s) on BABA-IR.* ***B.*** *Effects of increased light intensity (200 versus 120 µmol/m^2/s) on BABA-IR. PPFD: photosynthetic photon flux density*

Knock-down mutations reducing* GOX *gene expression affect BABA-IR. In addition to photorespiration, changes in light intensity affect numerous other physiological processes (Ballaré 2014). To obtain more direct evidence for a specific role of photorespiration in BABA-IR, levels of induced protection were tested in mutants that are affected in peroxisomal GOX, which catalyses the H_2O_2-generating conversion of glycolate into glyoxylate. The Arabidopsis genome encodes five GOX isoenzymes (*GOX1, GOX2, GOX3, HAOX1, HAOX2;* Rojas et al. 2012). *GOX1* and *GOX2* are the main forms expressed in leaves (Dellero et al. 2016). *HAOX1*, *HAOX2* and *GOX3* show much lower abundance in the leaves, but their expression is upregulated five- to nine-fold during non-host resistance (Rojas et al. 2012). Since loss-of-function mutations in photorespiration lead to severe growth defects or lethality at ambient CO_2 conditions (Timm and Bauwe 2013), and reduction of GOX activity can significantly affect primary metabolism (Dellero et al. 2016), which would complicate the interpretation of *Hpa* patho-assays, single knock-down mutants were selected that carry T-DNA insertions in the promotor or coding regions, respectively, of *GOX1* or *HAOX1* (*gox1-2* and *haox1-2*). Both *GOX1* and *HAOX1* have previously been implicated in biotic stress resistance in Arabidopsis (Rojas et al. 2012). Despite the fact that the T-DNA mutations reduced *GOX1* and *HAOX1* expression by 42.6% and 75.4%, respectively (Fig. 6.3), *gox1-2* and *haox1-2* displayed wild-type growth phenotypes (data not shown), indicating that the remaining glycolate oxidase activity in these knock-down mutants is sufficient for normal development under standard growth conditions. To evaluate whether photorespiration contributes to BABA-IR, 2.5 week-old plants were soil-drenched with increasing BABA concentrations (0, 5, 10 mg/L BABA) two days prior to infection with *Hpa.* Disease progression was assessed by trypan blue staining at 5 days post inoculation (Fig. 6.4). Basal resistance to the downy mildew pathogen was not affected in either photorespiration mutant. However, while Col-0 plants responded to 5 mg/L BABA with a statistically significant increase in *Hpa* resistance*,* neither *gox1-2* nor *haox1-2* developed statistically significant levels of resistance upon pre-treatment with 5 mg/L BABA. Although both mutants showed statistically significant levels upon pre-treatment with 10 mg/L BABA, their levels of BABA-IR were still lower than that of Col-0 plants (Col-0 vs *gox1-2*: $p<0.01$; Col-0 vs *haox1-2*: $p<0.05$; Fisher's exact test). Thus, reduction in plant *GOX* gene expression affects BABA-IR against *Hpa*, which supports the notion that photorespiration contributes to light-dependent BABA-IR.

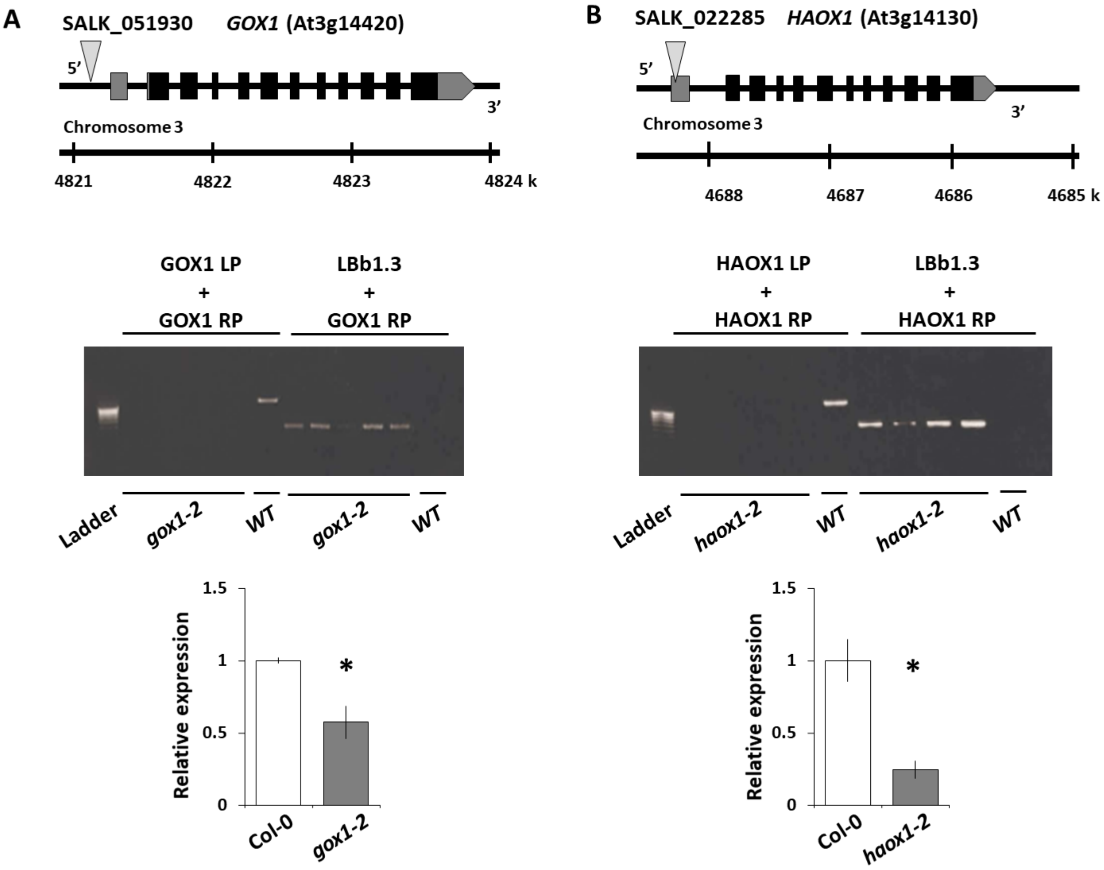

Figure 6.3: Selection of homozygous knockdown mutants in the glycolate oxidase genes* GOX1 *(A) and* HAOX1 *(B). *Gene models on the top show locations of T-DNA insertions in promoter of GOX1 (SALK_051930) and the 5' UTR of HAOX1 (SALK_022285). Gel pictures in the middle show PCR products from i.) mutant DNA with LP + RP primers (no band); ii.) Col-0 DNA with LP + RP primers; iii.), mutant DNA with LBb1.3 + RP primers; and iv.) Col-0 DNA with LBb1.3 + RP primers. Graphs on the bottom show the impacts of mutations on transcriptional levels in homozygous mutant plants. Data presented are mean values of relative transcript levels (± SEM; n = 5) in shoot tissues of 3-week old plants. Asterisks indicate statistically significant reductions in relative transcript level compared to wild-type plants (Col-0; Student's t-test, P < 0.05).*

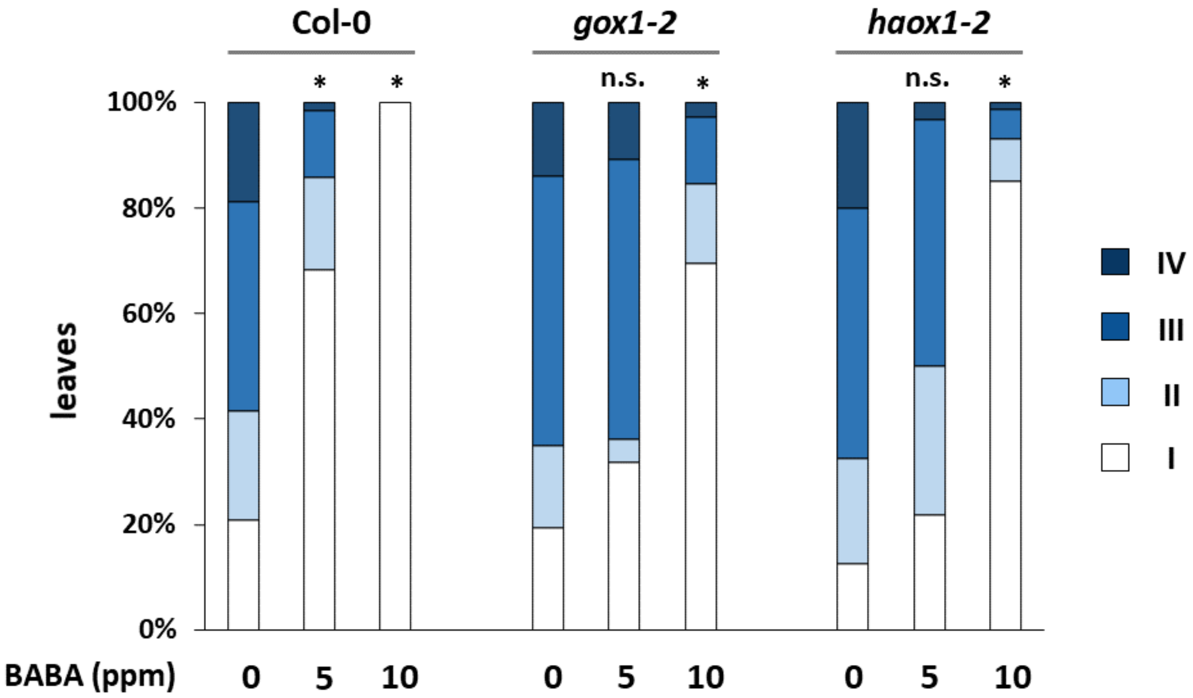

Figure 6.4: BABA-IR is reduced by knockdown mutations in glycolate oxidase. *Wild-type (Col-0),* gox1-2 *and* haox1-2 *plants (2.5-week old) were inoculated with* Hpa *(10^5 spores/mL) at 2 days after soil-drenching with water, 5 mg/L, or 10 mg/L BABA.* Hpa *colonisation was assessed in trypan blue-stained leaves at 5 dpi by assigning leaves to different* Hpa *colonisation classes. Asterisks denote statistically significant differences in class distribution from the water-treated sample of the same genotype (Fisher's exact test; p<0.05; n = 30 – 70).*

6.4 Discussion

Chapter 4 identified the plastidic NAD-dependent malate dehydrogenase plNAD-MDH as an interactor of the BABA receptor IBI1. This enzyme catalyses the reversible reduction of oxaloacetic acid to malate, using NADH as electron donor (Berkemeyer, Scheibe, and Ocheretina 1998). Homozygous *Arabidopsis* null mutants are non-viable, as embryo development is arrested at the globular-to-heart transition stage (Beeler et al. 2014), and reduced levels of plNAD-MDH lead to severe pleiotropic effects, including dwarfism, reduced photosynthesis and disordered chloroplast ultrastructure (Beeler et al. 2014). While these findings suggest that plNAD-MDH is critical for primary plant metabolism, the exact function of plNAD-MDH remains difficult to pinpoint. This can be attributed to two factors: Firstly, malate is a central metabolite of primary metabolism, and is involved in a range of essential metabolic pathways. Secondly, plants possess several isoforms of MDH. In addition to plastidic NAD-MDH, Arabidopsis encodes three cytosolic, two mitochondrial and two peroxisomal MDH isoenzymes (Beeler et al. 2014). Furthermore, chloroplasts contain an additional NADP-dependent MDH, which uses the electron acceptor NADP+ (Berkemeyer, Scheibe, and Ocheretina 1998). For these reasons, the exact contribution of plNAD-MDH to the malate valve remains controversial. To prevent photoinhibition under excessive light conditions by overreduction of the chloroplast stroma, reducing equivalents are exported to the cytosol in the form of malate. Traditionally, it was assumed that this mechanism was conferred exclusively by NADP-dependent MDH, but the discovery of plNAD-MDH challenged this notion (Berkemeyer, Scheibe, and Ocheretina 1998). NAD-MDH is active in illuminated and non-illuminated chloroplasts, whereas NADP-MDH is strictly redox-regulated and light-dependent (Berkemeyer, Scheibe, and Ocheretina 1998). While the relative contributions of NAD- and NADP-dependent MDH to malate production in illuminated chloroplasts have not been measured, it is plausible that both enzymes work together to ensure swift adaptation of malate content and redox status to the ever-changing needs of the plant. The finding that the BABA receptor IBI1 interacts with plNAD-MDH (Chapter 4), in combination with the results that BABA-IR is light-dependent (Fig. 6.1) and partially dependent on *GOX1* and *HAOX1* gene expression (Fig. 6.3), provides further evidence that plNAD-MDH plays a role in redox regulation of the chloroplast.

Appropriate regulation of malate levels is critical to ensure the operation of the photorespiration cycle. Malate participates in this cycle as an antiporter substrate that shuttles glutamate, oxo-glutarate and reduction equivalents across the chloroplast membrane. Photorespiration has long been viewed as an energy-wasting process that has evolved as a consequence of the oxygenation reaction of Rubisco. Considerable effort has gone into engineering plants with reduced photorespiration to improve crop yields (Foyer et al. 2009; Peterhansel and Maurino 2011; Betti et al. 2016). However, recent evidence indicates that plants have co-opted photorespiration for other purposes, such as nitrogen assimilation and defence responses to biotic and abiotic stress (Wingler et al. 2000; Voss et al. 2013; Rojas et al. 2012). For example, mutants in the key photorespiration enzyme glycolate oxidase (*GOX*) are impaired in non-host resistance and effector-triggered immunity (Rojas et al. 2012). Based on the recent evidence that photorespiration plays a role in (a)biotic stress resistance and that the BABA receptor IBI1 interacts with plNAD-MDH (Chapter 4), this Chapter explored a possible contribution of photorespiration to BABA-IR. Since photorespiration is directly linked to the Calvin-Benson cycle, this process only occurs in the light. Interestingly, BABA-IR was abolished when plants were transferred to the dark after inoculation with the biotrophic pathogen *Hpa* (Fig. 6.2A). Conversely, photorespiration increases under relatively high light irradiance, (Kangasjärvi et al. 2012; Sharkey 1988), and exposure to high light immediately after *Hpa* inoculation significantly increased levels of BABA-IR (Fig. 6.2B). This increase in BABA-IR efficiency was not due to an unspecific increase in basal immunity, since high light did not increase basal resistance to *Hpa* (Fig. 6.2B). This suggests that photorespiration is specifically recruited by primed plants to mediate an augmented defence response to pathogen attack. However, light impacts on a wide range of physiological processes (Ballaré 2014), and the light dependency of BABA-IR does not necessarily mean that BABA-IR is partially dependent on photorespiration.

To gain direct evidence for the hypothesis that photorespiration enables the augmented defence of BABA-primed plants, BABA-IR was analysed in photorespiration mutants. During the oxidation of glycolate to glyoxylate, molecular O_2 is irreversibly reduced to H_2O_2 by glycolate oxidase (GOX) activity. The resulting intracellular accumulation of H_2O_2 could act as a second messenger for plant defence. While the highly expressed *GOX1* and *GOX2* genes are responsible for the majority of glycolate oxidase activity in Arabidopsis leaves, their expression is downregulated upon pathogen infection (Rojas et al. 2012; Dellero et al. 2016). By contrast, three other enzymes (*GOX3, HAOX1, HAOX2*) show low constitutive expression, but are inducible by non-host pathogen inoculation (Rojas et al. 2012). While mutations in photorespiratory genes often result in severe (and sometimes lethal) phenotypes under normal growth conditions, no such effects have been reported for mutations in *GOX* genes (Timm and Bauwe 2013; Dellero et al. 2016). However, mutants with reduced GOX activity are affected in the abundance of primary metabolites, specifically in the accumulation of TCA cycle organic acids (Dellero et al. 2016). To reduce the disturbance of primary metabolism, which could complicate the interpretation of the *Hpa* patho-assays, knock-down mutations were selected that reduce *GOX1* and *HAOX1* expression due to T-DNA insertions in the promoter and 5’-UTR regions, respectively, of these genes (Fig. 6.3). Neither mutant showed severe growth reductions and/or stress symptoms under normal growth conditions, and basal resistance against *Hpa* was unaffected. However, *gox1-2* and *haox1-2* plants did show substantially reduced levels of BABA-IR. Thus, while photorespiration might not be essential for BABA-IR, it at least contributes to the augmented resistance of BABA-primed plants. Since both knock-down mutants should have a substantial amount of residual GOX activity, these results also suggest that relatively subtle reductions in photorespiration metabolism are sufficient to modulate the intensity of BABA-IR.

Mutants impaired in *GOX1* and *GOX2* show reduced accumulation of malate (Dellero et al. 2016). Conversely, perturbation of malate/oxaloacetate homeostasis through repression of mitochondrial MDH activity affects photorespiration (Tomaz et al. 2010). In light of the results shown in this Chapter, it is tempting to speculate that the interaction between IBI1 and plNAD-MDH recruits photorespiratory H_2O_2 into the augmented defence response of BABA-primed plants. Future measurements of intra-cellular ROS production at different light and atmospheric CO_2 regimes in both wild-type and *ibi1-1* plants could quantify the contribution of photorespiratory ROS to BABA-IR. Nonetheless, despite this Chapter's evidence that photorespiration contributes to BABA-IR, these results do not necessarily demonstrate that the interaction between IBI1 and plNAD-MDH is responsible for this contribution. Alternatively, based on the possible role of MDH in nitrogen assimilation (Temple, Vance, and Gantt 1998; Foyer, Noctor, and Hodges 2011), the interaction between IBI1 and plNAD-MDH could influence nitrogen homeostasis. Nitrogen metabolism and plant defence seem to be tightly linked (Fagard et al. 2014). In this context, it is interesting to note that plants lacking the high affinity nitrogen reporter *NRT2.1* show constitutively primed defence against *Pseudomonas syringae* (Camañes et al. 2012). In addition, the nitrate reductase-deficient *nia1nia2* Arabidopsis mutant shows altered expression of core ABA signalling components and impaired ABA-induced stomatal closure (Zhao et al. 2016). Another possibility is that plNAD-MDH provides substrates for the biosynthesis of fatty acids, which are known to play a regulatory role in immune responses (Kachroo and Kachroo 2009). Notably, IBI1 was also found to interact with the fatty acid hydroxylase FAH2, suggesting that fatty acid metabolism could have another regulatory contribution to IBI1-dependent defence and BABA. The contributions of these pathways to *IBI1*-mediated BABA-IR do not have to be mutually exclusive, but could act in a synergistic or complementary manner. If this proves to be the case, the interaction between IBI1 and plNAD-MDH would constitute an impressive example of efficiency, where the BABA receptor targets a central regulator of multiple pathways to induce a robust, broad-spectrum resistance response.

6.5 Acknowledgements

I would like to thank Alex Williams for his help with characterising the *gox1-2* and *haox1-2* mutant lines, and for helpful discussion on the role of photorespiration in plant defence. I am also grateful to Stephan Turner for his technical help in setting up the growth chambers for experiments with modulated R/FR ratios.

Chapter 7

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Analysis of transcriptional and metabolic changes during IBI1-mediated BABA-induced resistance

7.1 Abstract

The resistance of a plant to an attacking pathogen depends on complex molecular interactions that play out on various levels. Perception of an invading microbe leads to activation of the immune system, which entails coordinated activation of cellular defence pathways at the transcriptional, proteomic and metabolic level. Priming can alter the dynamics of this immune response, and render the plant more resistant to disease. This chapter describes the results of an integrated transcriptome/metabolome analysis aimed at identifying pathways that mediate BABA-induced resistance in Arabidopsis against the downy mildew pathogen *Hyaloperonospora arabidopsidis*. By including the BABA receptor mutant *ibi1-1,* which is impaired in BABA-induced resistance (BABA-IR), but hyper-susceptible to BABA-induced stress, the experimental setup was designed to distinguish the BABA-augmented defence response from the BABA-induced stress response. Genome-wide transcriptome analysis with Arabidopsis Gene ST 1.1 arrays revealed one gene, *MIOX4*, displaying IBI1-dependent augmented expression during BABA-IR. However, analysis of T-DNA insertion and overexpression lines of *MIOX* genes revealed only a marginal contribution to BABA-IR against *Hpa*. The transcriptome also revealed IBI1-dependent repression of abscisic acid (ABA) response genes during expression of BABA-IR. Metabolome analysis of the same samples by Matrix-Assisted Laser Desorption Ionisation Mass Spectrometry (MALDI-MS) pointed to IBI1-dependent regulation of pathways converging at the xanthophyll cycle and chlorophyll metabolism. Molecules of the xanthophyll cycle are not only involved in ABA biosynthesis, but are also critical for non-photochemical quenching (NPQ). Direct measurements of chlorophyll fluorescence confirmed differences in NPQ between Col-0 wild-type plants and the *ibi1-1* mutant, suggesting involvement of IBI1-dependent reduction in NPQ in BABA-IR. Thus, several lines of evidence point to modulation of ABA-dependent signalling and NPQ during expression of IBI1-dependent BABA-IR.

7.2 Introduction

Plants possess a complex, multi-layered immune system that protects them against the vast majority of potentially hostile microbes (Dangl and Jones 2001; Thordal-Christensen 2003; also Chapter 1). A pre-requisite for an effective immune response is the fast and timely induction of defence mechanisms upon perception of the invading microbe. At the same time, constant activation of defence responses has deleterious consequences on growth and development (van Hulten et al. 2006; Göhre et al. 2012). The selective pressure to avoid the negative effects of constitutive defence, while maintaining the capacity for fast and strong immune responsiveness when needed, has led to the evolution of defence priming (Conrath et al. 2006; Ahmad et al. 2010). In the absence of a stress stimulus, primed plants show no or low expression of defence genes. However, upon perception of an attack, primed plants exhibit enhanced induction of immune responses, including augmented ROS production, increased defence efficacy of callose deposition, and a faster and/or stronger induction of defence genes (Zimmerli et al. 2000; Pastor et al. 2013).

Priming agents, i.e. stimuli that elicit a primed state in plants, include pathogenic and beneficial microbes, certain mutations and chemical compounds (see Chapter 1). One example of a priming-inducing chemical is the non-proteinogenic amino acid β-aminobutyric acid (BABA). BABA primes SA-dependent and SA-independent defence mechanisms, and induces effective resistance against a wide range of biotrophic and necrotrophic microbes, including many important crop pathogens that are difficult to control by conventional methods (Cohen, Vaknin, and Mauch-Mani 2016). However, high doses of BABA can lead to stunted plant growth and sterility (Jakab et al. 2001; Wu et al. 2010; Luna et al. 2014).

While the resistance-inducing properties of BABA have been known for several decades (Cohen 1994; Papavizas 1964), the identity of a BABA receptor protein in the model plant species *Arabidopsis thaliana* (Arabidopsis) was only described relatively recently (Luna et al. 2014). This study showed that perception of BABA in Arabidopsis requires the aspartyl-tRNA synthetase (AspRS) *IBI1*. This class of enzyme catalyses the linkage of L-aspartic acid (L-Asp) with its cognate tRNA, producing charged Asp-tRNA^{Asp} that is used for protein synthesis. Based on evidence that the active R-enantiomer of BABA binds to IBI1 (Luna et al. 2014) and on the structural similarity between L-Asp and R-BABA, it was hypothesised that R-BABA binds to the L-Asp-binding domain of IBI1 (see also chapters 1 and 3). In support of this, chapter 3 showed that site-directed mutagenesis of this domain in IBI1 blocks BABA tolerance and BABA-IR. The current model of IBI1 activity predicts that R-BABA blocks the canonical AspRS activity of IBI1, which primes the protein for defence, presumably by loosening its attachment to the endoplasmic reticulum membrane (see also the 4-stage model, Fig. 1.3, Chapter 1). At the same time, when high concentrations of R-BABA block cellular AspRS activity, the resulting accumulation of uncharged tRNA^{Asp} activates the protein kinase GCN2 (Luna et al. 2014). The subsequent phosphorylation of eIF2α leads to strong reduction in global protein biosynthesis and ultimately, growth inhibition (Lageix et al. 2008; Luna et al. 2014). Interestingly, and in agreement with this model, the BABA receptor mutant *ibi1-1* is impaired in BABA-induced resistance, but is hypersensitive to BABA-induced stress. This exaggerated stress response of *ibi1-1* can be explained by an overall reduction in AspRS capacity (Luna et al., 2014). Of note, IBI1 controls the BABA-induced stress response via a pathway separate to the IBI1-dependent resistance response. This conclusion is based on the observation that the *gcn2* mutant of Arabidopsis shows strongly reduced growth inhibition after treatment with high doses of BABA (Luna et al. 2014), but is unaffected in BABA-IR. Hence, BABA-IR and BABA-induced stress are controlled by distinct IBI1-dependent pathways. Despite this knowledge about the molecular perception of BABA, the downstream signalling pathways leading to IBI1-dependent BABA-IR have remained poorly understood. Chapters 3 to 6 have revealed novel signalling components in BABA-IR, largely stemming from the identification of IBI1-interacting proteins, such as the VOZ transcription factors. This Chapter takes a top-down approach by comparing transcriptome and metabolome responses of wild-type and *ibi1-1* Arabidopsis plants during expression of BABA-IR against *Hyaloperonospora arabidopsidis* (*Hpa*).

Perception of pathogen attack causes large-scale transcriptional and metabolic reprogramming in the plant, which is a consequence of both susceptibility-inducing pathogen effectors and the plant's own immune response (Doehlemann et al. 2008; Windram et al. 2012). During expression of augmented defence in primed plants, the dynamics of these changes are altered, increasing the activity of defence-related responses at the expense of changes that are associated with effector-induced susceptibility. While transcriptional and metabolic changes correlate to an extent, their analysis yields complementary information (Tohge et al. 2005). One of the advantages of transcriptome analysis in genomically well-annotated species like *Arabidopsis thaliana* is that changes in transcript abundance can be accurately and unambiguously attributed to specific genes. The metabolome, on the other hand, is more proximal to the phenotype, and captures changes in the abundance of metabolites that are caused by post-transcriptional and -translational changes. Matrix-Assisted Laser Desorption Ionisation Mass Spectrometry (MALDI-MS) allows the measurement of tens of thousands of compounds from a single sample, giving a comprehensive snapshot of the metabolic status of a sample. However, since multiple metabolites share the same m/z value (mass/charge ratio; ion), and MALDI-MS is not preceded by chromatographic separation of compounds or post-ionisation fragmentation, the measured ion abundance peaks can only be putatively assigned to specific metabolites. Simultaneous measurement of transcriptional and metabolic changes enables a more accurate global impression of the physiological mechanisms contributing to BABA-IR. Since the *ibi1-1* mutant is deficient in BABA-IR, but hypersensitive to BABA-induced stress, comparison between Col-0 wild-type and *ibi1-1* mutant plants should allow to differentiate between pathways that are activated by these separate BABA responses. The current chapter describes the results of a combined transcriptome/metabolome analysis aimed at identifying pathways that contribute to IBI1-mediated BABA-induced resistance.

7.3 Results

Transcriptional differences between Col-0 and* ibi1-1 *are dominated by the exaggerated stress response of* ibi1-1 *to BABA. To obtain a global impression of the non-canonical defence activity of IBI1, the transcriptome of 3-week old wild-type and *ibi1-1* mutant plants was studied after pre-treatment with water or BABA (10 mg/L) and subsequent inoculation with downy mildew (*Hpa*) or a mock solution (water; Fig. 7.1). Three biological replicates per treatment-genotype combination, each consisting of pooled above-ground parts from ~20 plants, were collected at 2 days post inoculation (dpi) for RNA extraction. IBI1-dependent BABA-IR was confirmed in the experiment from which the samples were collected by analysing deposition of effective callose at 2 days post inoculation (dpi; Fig. 7.2A) and by quantification of *Hpa* colonisation at 5 dpi (Fig. 7.2B). RNA was hybridised to Arabidopsis Gene ST 1.1 arrays (Affymetrix). Analysis of gene expression focused on genotypic differences between the Col-0 wild-type and *ibi1-1* mutant under four different conditions: water-pretreated & mock-inoculated; BABA-pretreated & mock-inoculated; water-pretreated & *Hpa*-inoculated; and BABA-pretreated & *Hpa*-inoculated.

Figure 7.1: Scheme of plant material sampling conditions for transcriptome and metabolome analysis. *For both genotypes (Col-0 and* ibi1-1*), sample material was collected under four different conditions: plants were pre-treated with water or BABA (10 mg/L), and two days later spray-inoculated with* Hyaloperonospora arabidopsidis *(*Hpa*) or mock (water).*

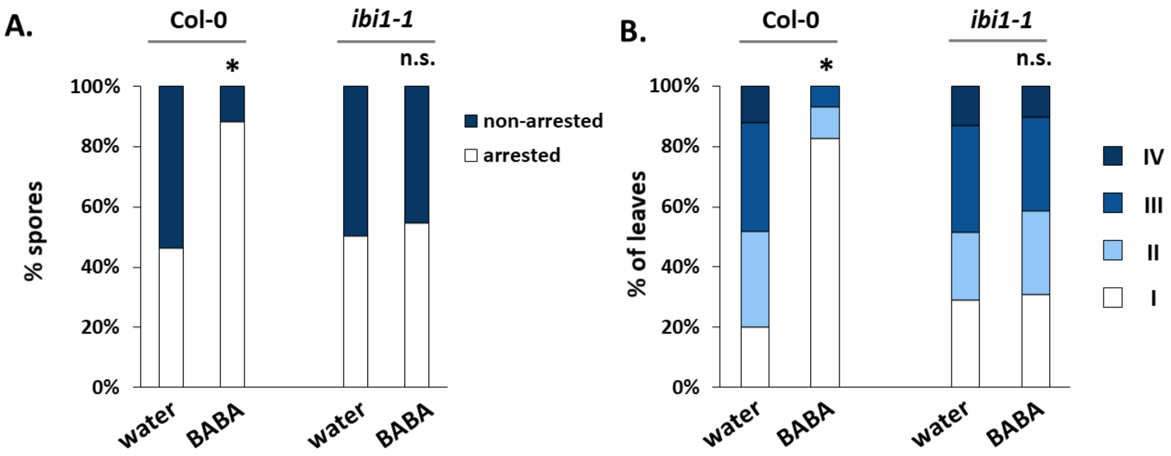

Figure 7.2: Confirmation of IBI1-dependent defence priming and BABA-induced resistance in the experiment used for the transcriptome-metabolome analysis*. Plants (two-week old) were soil-drenched with 10 mg/L BABA or water at 2 days before inoculation with* Hpa*.* ***A****. BABA-induced priming of defence-contributing callose deposition against* Hpa*. Effectiveness of callose deposition was analysed 2 days post inoculation (dpi) by aniline blue/ calcofluor double staining of leaves from Col-0 and* ibi1-1 *plants. Shown are percentages of callose-arrested and non-arrested germ tubes of* Hpa*. Asterisk indicates statistically significant differences between water- and BABA-treated plants within each genotype (Fisher's Exact test; *: p<0.01; n.s.: not significant; n = 110-130).* ***B.*** *BABA-induced resistance (BABA-IR) against* Hpa*. Pathogen colonisation was assessed at 5 dpi by assigning trypan-blue-stained leaves to different* Hpa *colonisation classes (see chapter 2.4). Asterisks denote significant differences in class distributions. (Fisher's Exact test; *: p<0.05; n.s.: not significant; n = 25 - 30).*

Gene expression data were extracted from the 24 microarrays (8 conditions with 3 replicates each), quality-checked and normalised (using Robust Multichip Average [RMA]) via the R Bioconductor software package (Gautier et al. 2004; Carvalho and Irizarry 2010; Huber et al. 2015). After those initial steps, a 2-dimensional principal component analysis (PCA) of gene hybridisation signals was constructed, which showed clustering of biologically replicate samples (Fig. 7.3A). This clustering pattern demonstrates that both genotype and treatment have profound impacts on global transcriptional patterns. Interestingly, the differences in global transcription between Col-0 and *ibi1-1* were most pronounced after pre-treatment with BABA with or without subsequent challenge with *Hpa* (Fig. 7.3A), which confirms the essential role of IBI1 in detoxification responses to BABA and BABA-primed defence.

To confirm that this experimental setup produced valid data, and that the downstream analysis was sensitive enough to detect biologically meaningful changes in defence-related gene expression, an initial analysis focussed on identification of genes that responded to inoculation with *Hpa* with a significant up- or down-regulation in Col-0 (Welch's t-test; $p \leq 0.05$, fold change ≥ ±2). Comparison of transcript levels in (water-pretreated) mock- and *Hpa*-inoculated Col-0 samples resulted in the identification of 477 differentially expressed genes (DEGs), of which 446 showed at least 2-fold up-regulation in response to inoculation with downy mildew, whereas 31 genes showed transcriptional repression (Appendix 2). The number of identified DEGs is thus roughly comparable to previous reports of *Hpa*-responsive DEGs (Huibers et al. 2009; López Sánchez et al. 2016). DEGs included well-known *Hpa*-inducible genes (Appendix 2), like NDR1 (Century, Holub, and Staskawicz 1995), PAD3 and PAD4 (J. Glazebrook et al. 1997) and members of the WRKY transcription factor family (Kalde et al. 2003). Moreover, GO term analysis of these 477 DEGs revealed highly significant enrichment of genes that are associated with plant defence and immunity, including defences that are known to provide resistance to *Hpa* (Table 7.1).

Table 7.1: GO term enrichment among Hpa*-responsive genes in Col-0*

GO term[1]	Associated differentially expressed genes / Enrichment[2]	FDR-q value
Response to salicylic acid	33 / 11.18	1.28E-22
response to chitin	29 / 11.88	1.48E-20
response to wounding	27 / 7.34	1.34E-13
response to jasmonic acid	21 / 7.16	3.00E-10
defense response to bacterium, incompatible interaction	12 / 16.07	7.89E-10
leaf senescence	15 / 9.57	5.84E-09
systemic acquired resistance	11 / 15.10	1.21E-08
plant-type hypersensitive response	12 / 10.29	1.65E-07
protein phosphorylation	42 / 2.83	1.74E-07
induced systemic resistance	7 / 27.45	1.89E-07
response to molecule of bacterial origin	8 / 13.72	6.32E-06
response to salt stress	30 / 3.04	6.53E-06
salicylic acid mediated signaling pathway	8 / 11.87	1.95E-05
camalexin biosynthetic process	5 / 24.95	5.46E-05
response to ethylene	13 / 4.69	2.43E-04
defense response to fungus, incompatible interaction	7 / 9.15	5.14E-04

[1] only the most specific significant GO terms (without further children GO terms) are reported. [2] Enrichment was calculated as (% of DEGs associated with GO term) / (% of genes associated with GO term on microarray).

To identify differentially expressed genes (DEGs) between Col-0 and *ibi1-1* under the different (pre)treatment conditions, pairwise comparisons were performed between similarly (pre)treated Col-0 and *ibi1-1* samples, using Welch's t-tests ($p \leq 0.05$, fold change ≥ 2). This analysis revealed hardly any differences under basal (water-mock) conditions (14 DEGs), which is consistent with the notion that *IBI1* and its homologue At4g26870 act redundantly with regards to canonical aspartyl-tRNA synthetase activity under stress-free conditions (Luna et al. 2014). Similarly, the transcriptional differences between un-primed Col-0 and *ibi1-1* after *Hpa* inoculation (water-*Hpa*) were relatively marginal (39 DEGs), supporting the observation that *ibi1-1* is not majorly affected in basal resistance (Luna et al. 2014). As was already clear from the PCA (Fig. 7.3A), the transcriptional differences after pre-treatment with BABA, followed by either mock or *Hpa* inoculation (BABA-mock or BABA-*Hpa*), were more substantial, amounting to 243 and 159 DEGs, respectively (Fig. 7.3B). These differences can be attributed to two factors: i) the augmented expression of defence genes in primed Col-0 plants, which is absent in *ibi1-1,* and ii) increased transcription of stress-related genes in *ibi1-1*, due to its hypersensitivity to BABA-induced stress. Gene ontology (GO) term analysis of the 243 DEGs after BABA treatment alone (BABA-mock) revealed highly significant enrichment of genes associated with hydrogen peroxide metabolism (FDR $q<1.7*10^{-5}$), suberin biosynthesis (FDR $q<1.7*10^{-5}$) and induced systemic resistance (FDR $q<0.003$). However, cluster analysis (Pearson correlation) of these 243 genes showed that replicate samples from both water- and BABA-treated, mock-inoculated Col-0 plants clustered closely to samples from water-treated, mock-inoculated *ibi1-1* plants (Fig. 7.3D). This indicates that resistant BABA-treated Col-0 plants show similar expression profiles for these genes as susceptible water-treated Col-0 and *ibi1-1*, suggesting that these genes are un-related to BABA-IR. Secondly, samples from all BABA-treated *ibi1-1* plants (BABA-mock and BABA-*Hpa*) formed a common outgroup to all other samples (Fig. 7.3D). Since *ibi1-1* plants do not express BABA-IR and are hypersensitive to BABA-induces stress (Luna et al., 2014), this further confirms that the differential expression of these 243 genes is related to the enhanced stress in BABA-treated *ibi1-1*, rather than BABA-IR in Col-0 plants.

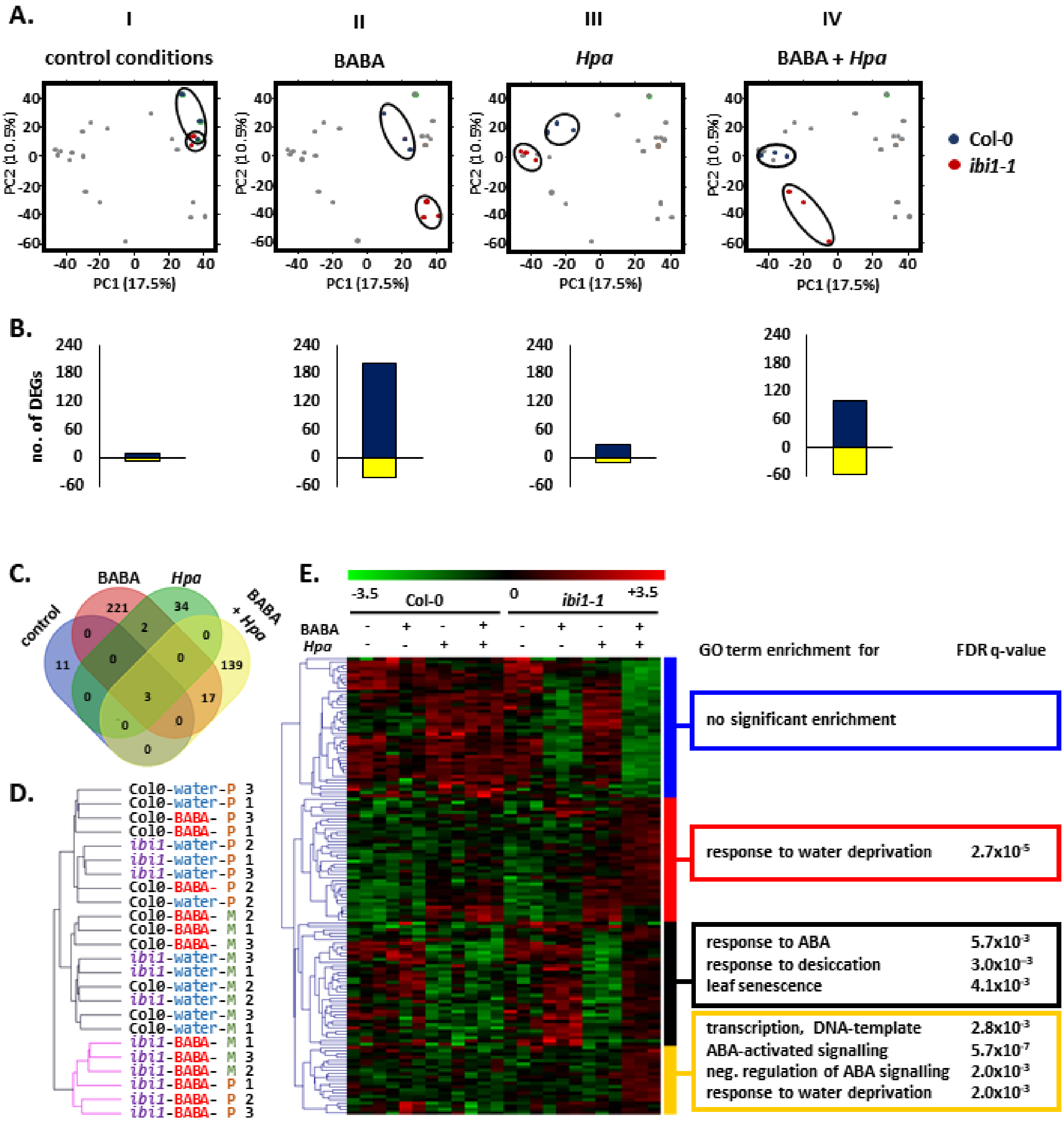

Figure 7.3: Global impacts of IBI1 on the Arabidopsis transcriptome. *Shoot samples for transcriptome analysis were collected from water- and BABA-pre-treated Col-0 and* ibi1-1 *at 2 dpi with* Hpa *or mock solution. See legend to Figure 7.1 for details.* ***A.*** *Principal Component Analysis (PCA) shows separation of samples according to genotype and treatment. PCA was performed across all 24 samples. For each condition of the 4-stage model of BABA-induced resistance (see chapter 1), the six samples representing the respective condition are highlighted in colour.* ***B.*** *Numbers of up- (blue) and down-regulated (yellow) genes in* ibi1-1 *compared to Col-0 under the 4 different (pre)treatment conditions (Welch's t-test; p ≤ 0.05; fold change ≥ 2).* ***C:*** *Venn diagram showing numbers of differentially expressed genes (DEGs) between Col-0 and* ibi1-1 *under the 4 different sampling conditions.* ***D.*** *Hierarchical clustering (Pearson correlation) of replicate samples based on 243 DEGs upon BABA treatment without*

subsequent infection (BABA-mock). ***E:*** *Hierarchical clustering (Pearson correlation) and expression levels of 139 DEGs that are differentially expressed between BABA-treated,* Hpa*-inoculated Col-0 and* ibi1-1 *(BABA-*Hpa*), but not between BABA-treated, mock-inoculated Col-0 and* ibi1-1 *(BABA-mock). Shown are the differences of RMA-processed (log2-transformed) expression values from the mean expression after row- and column-normalisation (see chapter 2.10). Coloured boxes on the right indicate distinct clusters of DEGs with gene ontology (GO) terms and corresponding enrichment values (hypergeometric distribution; FDR: Benjamini-Hochberg; Eden et al. 2009). FDR: false discovery rate correction.*

Expression of IBI1-dependent BABA-IR against **Hpa** ***is associated with repressed transcription of ABA-dependent abiotic stress-related genes.*** The transcriptomic differences between BABA-treated, *Hpa*-inoculated Col-0 and *ibi1-1* (BABA-*Hpa*) are associated with IBI1-dependent expression of BABA-IR, as well as differences in BABA-induced stress sensitivity. To enrich this selection for defence-related genes, DEGs were selected whose expression only differed between BABA-treated Col-0 and *ibi1-1* plants after *Hpa* challenge treatment (BABA-*Hpa*), but not after BABA treatment alone (BABA-mock). This subtraction resulted in 139 DEGs (Fig. 7.3C). Hierarchical clustering of these genes over all experimental conditions (Pearson correlation) revealed four distinct expression patterns (Fig 7.3E). The largest cluster was characterised by genes whose expression was largely unaffected in Col-0 by BABA treatment, but repressed in *ibi1-1* upon BABA treatment, with or without subsequent pathogen infection. Therefore, expression of these genes correlates with increased stress of *ibi1-1* after BABA treatment*.* GO term analysis showed an enrichment of genes in this cluster involved in the response to oxidative stress, however, this was no longer significant after false discovery rate (FDR) correction ($q \leq 0.01$; Fig. 7.3E). A second cluster included genes that were only marginally induced in Col-0 by *Hpa* inoculation (either control- or BABA-treated), but more so in the *ibi1-1* mutant (Fig 7.3E). A third cluster consisted of genes showing repressed transcript levels after *Hpa* inoculation in water- and BABA-treated Col-0 and water-treated *ibi1-1*, but not in BABA-treated *ibi1-1* plants. Finally, a fourth cluster of genes showed strong induction only in BABA-treated *ibi1-1* plants after inoculation and *Hpa.* Although none of these clusters contained genes that are exclusively differentially expressed between Col-0 and *ibi1-1* after BABA treatment and subsequent *Hpa* inoculation (i.e. during expression of IBI1-dependent BABA-IR), a common pattern between clusters 2, 3 and 4 is that the transcriptional levels are repressed during expression of IBI1-dependent BABA-IR against *Hpa*. Genes in these clusters also shared strong statistical enrichment for GO terms related to water deprivation and/or ABA signalling (Fig. 7.3E; Appendix 7), indicating that expression of BABA-IR against *Hpa* is associated with a repression of ABA-dependent response to abiotic stress. However, the vast majority of genes in clusters 2, 3, and 4 showed similar transcriptional repression in susceptible water-treated Col-0 after *Hpa* inoculation. Hence, the apparent repression of ABA signalling may contribute to BABA-IR, but by itself is not sufficient to mediate an augmented defence response.

Expression of* MYO-INOSITOL OXYGENASE 4 *shows IBI1-dependent priming, but contributes only marginally to BABA-IR. None of the gene clusters in the selection of 139 DEGs showed a pattern that correlates positively and exclusively with IBI1-dependent expression of BABA-IR in Col-0 (BABA-*Hpa*-Col). However, there was one gene, *MYO-INOSITOL OXYGENASE 4* (*MIOX4;* At4g26260) showing elevated expression exclusively during BABA-IR in Col-0, which was confirmed via RT-qPCR (Fig. 7.4A). *MIOX4* encodes a myo-inositol oxygenase that catalyses the oxygenative cleavage of myo-inositol into D-glucuronate (Kanter et al. 2005) (Fig. 7.4B). Myo-inositol oxygenase activity has been associated with reduced resistance to the beet cyst nematode *Heterodera schachtii* (Siddique et al. 2014), while *MIOX4*-overexpressing Arabidopsis lines show enhanced tolerance to abiotic stresses, such as cold and salt stress, presumably due to elevated ascorbate contents (Lisko et al. 2013). To test whether *MIOX4* plays a positive role in BABA-IR against *Hpa*, initial experiments aimed to quantify basal and BABA-induced resistance in two independent 35S*:MIOX4* overexpression lines. Both 35S:*MIOX4* lines were unaffected in basal resistance, but showed a small increase in the level of BABA-IR after treatment with 5 mg/L BABA, which was statistically significant for one of the two lines (Fig. 7.4C). In a second experiment, the *miox4-2* single mutant (SALK_027238) and *miox1/2/4/5* quadruple mutant (Endres and Tenhaken 2011) were tested. Both mutants were unaltered in basal resistance, but showed marginally reduced levels of BABA-IR against *Hpa* in comparison to Col-0 wild-type plants. However, this reduction in BABA-IR was not statistically significant (Fig. 7.4D). Together, these results suggest that *MIOX4* only marginally contributes to BABA-IR, and is not critical for the augmented defence expression during IBI1-dependent BABA-IR against *Hpa*.

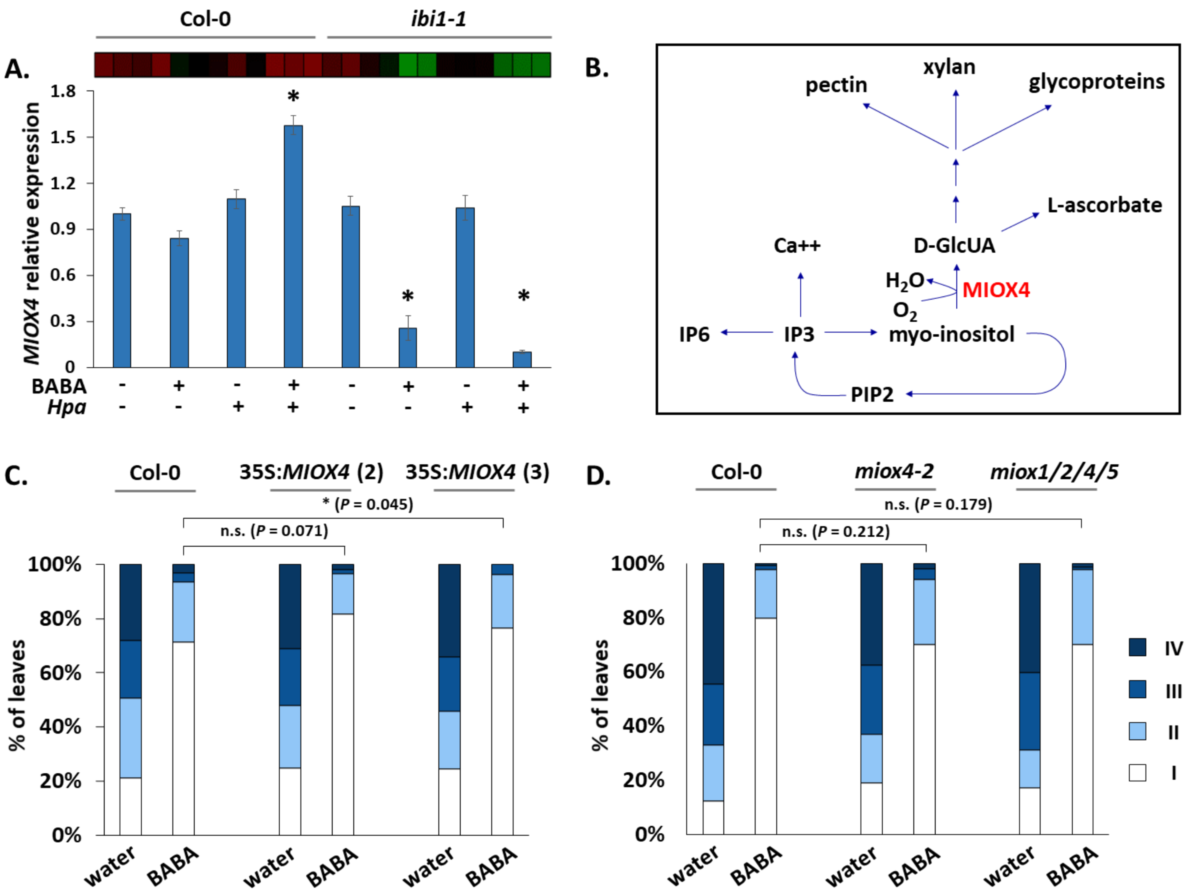

Figure 7.4:* MYO-INOSITOL OXYGENASE 4 (*MIOX4*) *contributes marginally to BABA-IR. A. *BABA primes* MIOX4 *for augmented expression after* Hpa *inoculation. The transcriptional profile of* MIOX4 *observed in the microarray (top inset) was confirmed by RT-qPCR. Data represent average gene expression values (± SEM) relative to water-treated, mock-inoculated Col-0 WT plants. Asterisks indicate statistically significant differences compared to water-treated, mock-inoculated samples of the same genotype (ANOVA with Sidak adjustment, p<0.05).* ***B.*** *MIOX4 regulates the synthesis of cell wall precursors, such as glucuronic acid (D-GlcUA), pectin and xylan, glycoproteins, as well as the antioxidant molecule L-ascorbic acid and the signalling molecules phosphatidyl-inositol-4,5,-bisphosphate (PIP2) and inositol-3-phosphate (IP3).* ***C.*** *BABA-IR against* Hpa *in two independent* MIOX4*-overexpressing lines (35S:*MIOX4*).* ***D.*** *BABA-IR against* Hpa *in two mutants deficient in* MIOX4 (miox4-2) *and four* MIOX *homologues* (miox1/2/4/5)*. Pathogen colonisation was assessed at 5 dpi by assigning trypan blue-stained leaves to different* Hpa *colonisation classes (see Chapter 2.4). Asterisk indicates statistically significant differences in distribution of pathogen colonisation classes (Fisher's exact test; *: p<0.05; n.s.: not significant; n=130–320).*

Metabolome analysis reveals distinct accumulation of compounds during BABA-primed defence. The low number of regulatory genes whose transcription correlates positively with IBI1-dependent expression of BABA-IR suggests a dominant role for post-transcriptional mechanisms regulating the augmented defence response against *Hpa*. Cellular signalling events are largely mediated by chemical conversions of metabolites, and result in a change of the abundance of these metabolites. The metabolome captures physiological changes that are controlled at the post-transcriptional level, and is therefore more proximal to the plant's phenotype than the transcriptome. In order to gain an understanding of metabolic processes associated with IBI1-dependent BABA-IR, the same samples harvested for the transcriptome were subjected to metabolome analysis by MALDI-MS. Metabolites were extracted from the freeze-dried samples, using methanol:water:formic acid (95:4.9:0.1 v/v/v) to obtain both polar and non-polar compounds, and subjected to MALDI-MS analysis. 32,698 ion peaks were obtained in positive mode, and 25,275 in negative mode, which is sufficient to obtain a broad overview of metabolic changes in the samples. Peaks were corrected for total ion current and dry weight of the sample. Six technical replicates of each biological sample were averaged. After pre-processing of raw data (centering, pareto scaling and log-transformation) as described in Chapter 2, PCA did not separate samples according to genotype-treatment combinations (data not shown). This is not unexpected for metabolome data of complex multi-tissue samples, since many metabolites show time-dependent fluctuations within and between tissues, causing relatively high levels of within-group variation. Therefore, to identify metabolic patterns that are related to differences between genotypes and treatments, Orthogonal Partial Least Square Discriminant Analysis (OPLS-DA) was used. Using this supervised multivariate analysis, *ibi1-1* plants displayed more between-treatment variation than Col-0 plants, which was apparent in both positive and negative ionisation mode. This pattern suggests that the metabolic responses to BABA and/or *Hpa* treatment have more profound impacts on *ibi1-1* plants than Col-0 plants. Within the Col-0 group, however, samples from BABA-treated, *Hpa*-inoculated plants clustered further apart than the other treatment combinations, indicating an enhanced metabolic response to *Hpa* in BABA-primed Col-0 plants (Fig. 7.5A).

To identify ions (m/z values) that differ significantly in abundance between genotype-treatment combinations, one-way ANOVA ($p \leq 0.01$) was used. Since MALDI-MS data are inherently noisy, and in order to maximise the exploratory potential of this analysis, this first statistical filter did not involve a false discovery rate (FDR) correction. The ANOVA revealed 460 and 500 ions showing statistically significant differences in negative and positive mode, respectively (Fig. 7.5B). To identify ions that correlate positively with augmented defence expression during IBI1-dependent BABA-IR (i.e. BABA-*Hpa*-Col), the set of significant ions from the ANOVA were subjected to average linkage clustering

(Pearson correlation). This analysis revealed two clusters whose ion intensities were exclusively up-regulated during the expression of IBI1-dependent BABA-IR against *Hpa* (BABA-*Hpa*-Col), containing 43 and 31 ions in positive and negative ionisation mode, respectively (Fig. 7.5B).

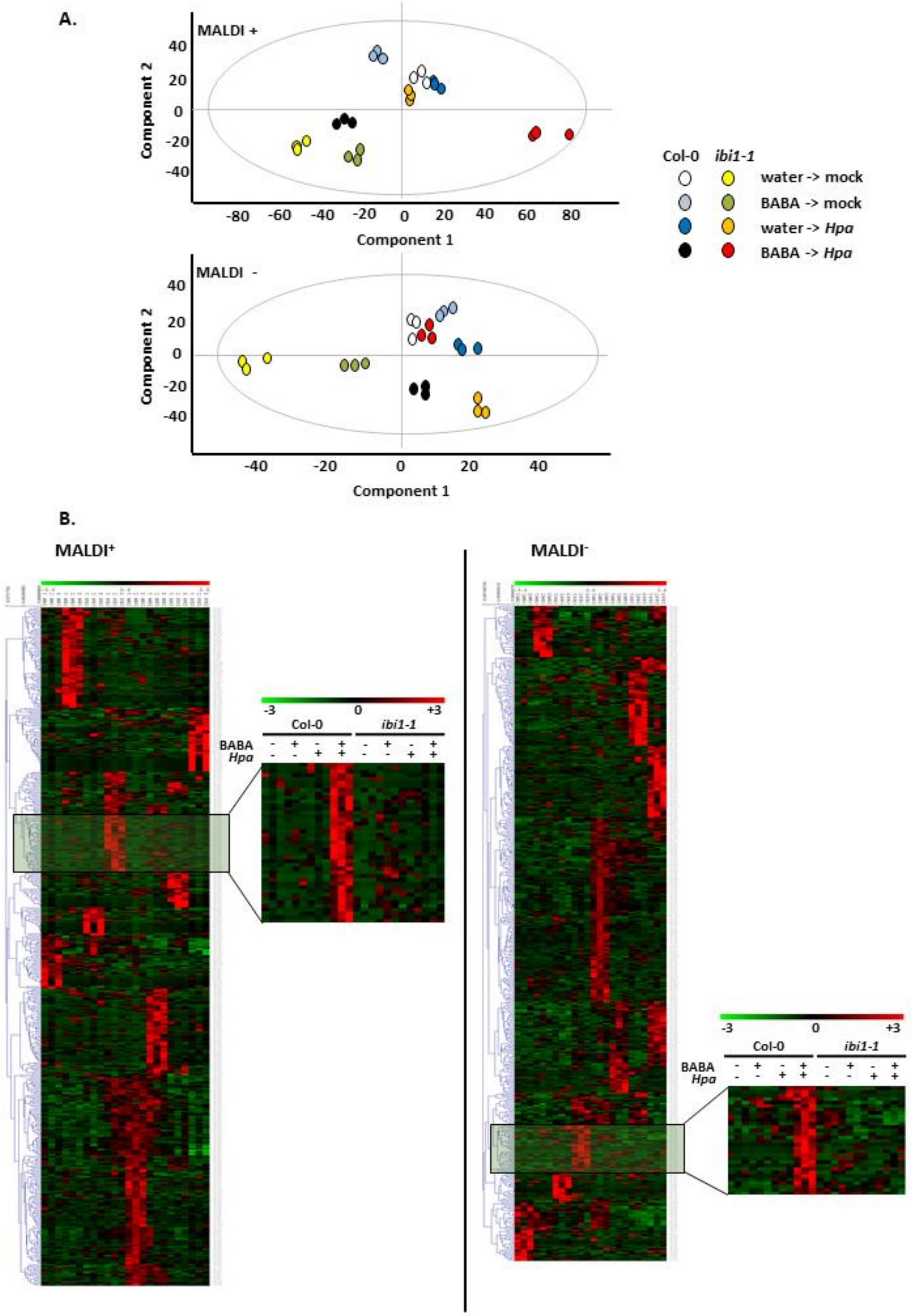
A.
MALDI +
Component 2
40
20
0
-20
-40
-80
-60
-40
-20
0
20
40
60
80
Component 1
MALDI -
Component 2
40
20
0
-20
-40
-40
-20
0
20
40
Component 1
Col-0
ibi1-1
water -> mock
BABA -> mock
water -> Hpa
BABA -> Hpa
B.
MALDI+
-3
0
+3
Col-0
ibi1-1
BABA
Hpa
- + - + - + - +
- - + + - - + +
MALDI-
-3
0
+3
Col-0
ibi1-1
BABA
Hpa
- + - + - + - +
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***Figure 7.5: Global impacts of IBI1 on the Arabidopsis metabolome.** The same shoot samples collected for transcriptome analysis were analysed for metabolic profiles by MALDI-MS. See legends to Figure 7.1 and 7.2 for experimental details. **A.** Orthogonal partial least square discriminant analysis (OPLS-DA) of m/z values obtained by MALDI-Q-TOF analysis in positive (top; $R^2X=0.67$; $Q^2=0.06$) or negative (bottom; $R^2X=0.61$; $Q^2=0.16$) ionisation mode. **B.** ANOVA (One-way ANOVA; $p<0.01$) identified 500 and 460 ions, whose abundance differed significantly between all genotype-treatment combinations in positive and negative mode, respectively (large heatmaps). Subsequent hierarchical clustering (Pearson correlation coefficient) revealed 43 and 31 ions in positive and negative mode, respectively (smaller heatmaps), that accumulate exclusively during augmented defence expression of IBI1-dependent BABA-IR against* Hpa *(BABA-*Hpa*-Col). These 74 ions were used for subsequent pathway analysis. Shown are the differences of (pre-processed) ion counts from the mean after row- and column-normalisation (see chapter 2.11).*

Ions accumulating during IBI1-mediated BABA-induced defence map to pathways of secondary metabolism. To identify the metabolic pathways contributing to augmented defence expression during IBI1-dependent BABA-IR, the 74 IBI1-dependent ions were mapped onto plant metabolic pathways. The MALDI-MS method applied did not involve separation of chemicals by chromatographic methods prior to ionisation and mass spectrometry assessment. Since multiple compounds can have the same m/z (mass/charge ratio) after ionisation, the obtained ion peaks can only be assigned putatively to specific metabolites. In order to do so, isotope and adduct correction for the 74 ions were performed using the software package MarVis (Kaever et al. 2009, 2015). The resulting predicted masses (‘markers’) were assigned to compounds in the KEGG and BioCyc databases (m/z tolerance = 50 ppm). Only 16 of the 74 IBI1-dependent ions (22%) could be assigned to putative metabolites. Of these, 4 markers could be annotated to the carotenoid biosynthesis pathway and 1 to ascorbate biosynthesis (Table 7.2). Together, these markers map onto a pathway cluster that converges at the xanthophyll cycle (Fig. 7.6A). Two additional markers could be assigned to the biosynthesis of isoquinoline alkaloids (Fig. 7.6B), which are secondary metabolites commonly associated with plant responses to biotic and abiotic stress (Iriti and Faoro 2009). This automatic annotation should be regarded with some scepticism, because the existence of complex alkaloids in Arabidopsis is doubtful (Facchini, Bird, and St-Pierre 2004). Finally, 4 markers corresponded to metabolites in chlorophyll biosynthesis and/or breakdown pathways (Fig. 7.6C), including two independent ions that mapped to the chlorophyll precursor protoporphyrin IX. However, the putative identification of phycocyanobilin is unlikely considering that this is a chromophore derived from cyanobacteria and chloroplasts of red algae, glaucophytes and cryptomonads (Cole, Chapman, and Siegelman 1967). On the other hand, while urobilin is more commonly found in mammals, the isolation of a urobilinogenoidic chlorophyll catabolite from barley (*Hordeum vulgare*) has been reported (Losey and Engel 2001).

Table 7.2: Pathway mapping of ions that accumulate under conditions of IBI1-mediated BABA-IR

Compound	Accurate m/z value	Adduct / n ^{13}C	Predicted Mass[1]	Difference (Da)[2]	Pathway[3]
L-Gulonate	197.0263	m+H / 0	196.0190	-0.0393	Ascorbate metabolism
3,4-Dehydro-lycopene	535.4330	m+H / 0	534.4257	0.0032	Carotenoid biosynthesis
Okenone	577.3969	m-H / 0	578.4042	-0.0082	Carotenoid biosynthesis
Antheraxanthin	585.4431	m+H / 0	584.4358	0.0129	Carotenoid biosynthesis
Presqualene diphosphate	585.2725	m-H / 0	586.2798	-0.0390	Carotenoid biosynthesis
Protoporphyrin IX	561.2010	m-H / 0	562.2083	-0.0497	Porphyrin/chlorophyll metabolism
Protoporphyrin IX	562.2112	m-H / 1	562.2151	-0.0429	Porphyrin/chlorophyll metabolism
(3Z)-Phycocyanobilin	585.2725	m-H / 0	586.2798	0.0007	Porphyrin/chlorophyll metabolism
D-Urobilin	587.2621	m-H / 0	588.2694	-0.0254	Porphyrin/chlorophyll metabolism
Norcoclaurine/Crinine/ N-Demethylnarwedine	272.1060	m+H / 0	271.0987	-0.0221	Isoquinoline alkaloid biosynthesis
S-Cheilanthifoline	326.1151	m+H / 0	325.1078	-0.0236	Isoquinoline alkaloid biosynthesis

[1] m/z values were corrected for adducts and/or ^{13}C carbon isotopes using MarVis software (m/z tolerance = 50 ppm). [2] Difference between predicted mass (=adduct/carbon isotope-corrected detected mass) and exact weight of the respective compound, as reported in the KEGG database. [3] Predicted masses were cross-referenced against the KEGG database.

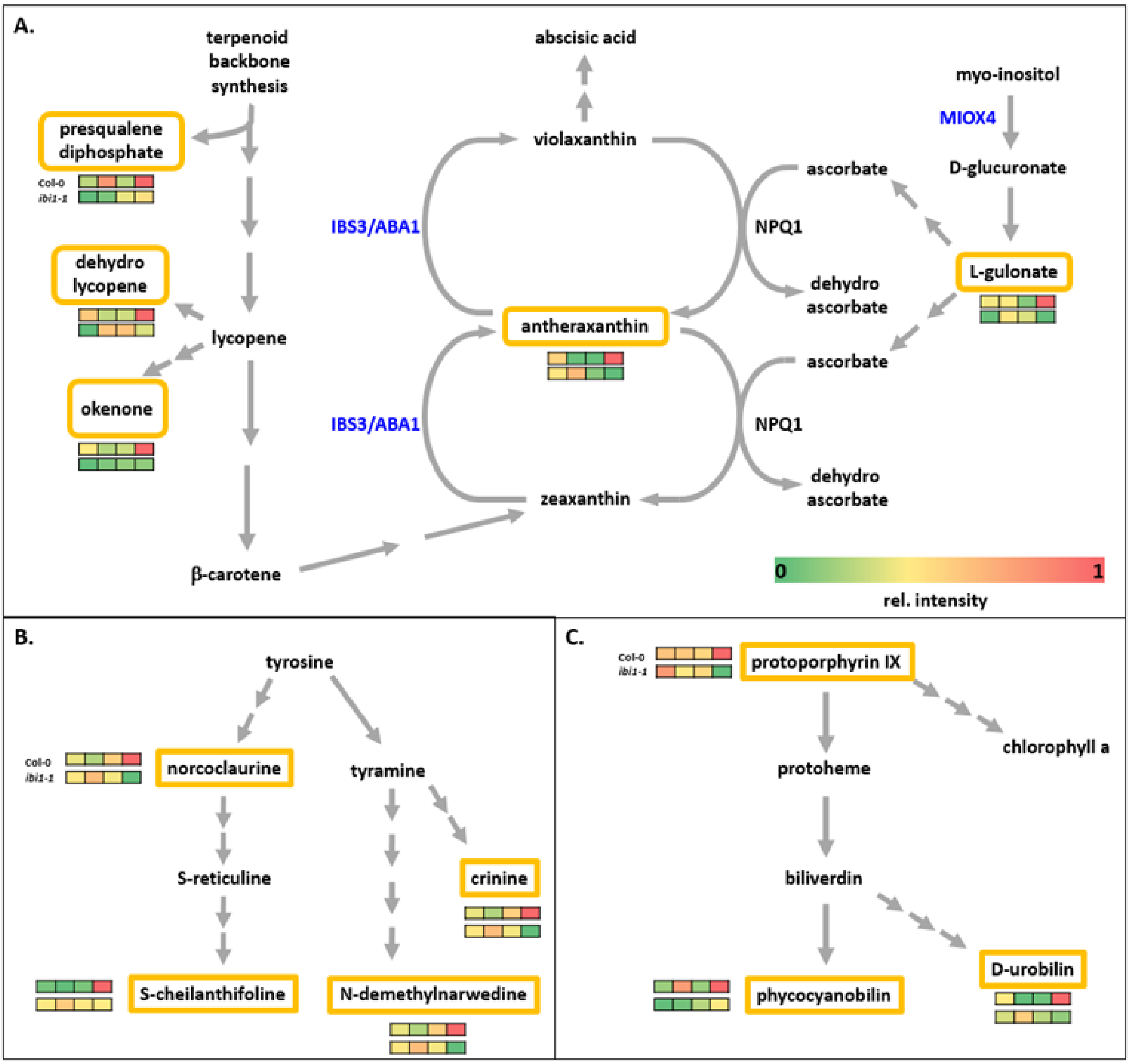

Figure 7.6: Putative metabolite markers and associated pathways displaying enhanced activity during the expression of IBI1-dependent BABA-IR against* Hpa.** *Out of 74 ions (m/z values) showing enhanced accumulation in BABA-primed Col-0 after* Hpa *inoculation, 16 could be assigned to plant-derived metabolites after correction for C isotopes and ionisation adducts, using KEGG and BioCyc databases (m/z tolerance = 50 ppm). Three pathways were identified to which more than one marker could be assigned.* ***A. *xanthophyll metabolism.* ***B.*** *isoquinoline alkaloid metabolism.* ***C.*** *chlorophyll metabolism. Insets below the named metabolites show relative intensities of the associated ions in Col-0 (top) and ibi1-1 (bottom) upon water - mock, BABA - mock, water –* Hpa *and BABA –* Hpa *treatments (left to right, respectively). Shown in blue are enzymes previously identified to be involved in BABA-IR, including MIOX4.*

Chlorophyll fluorescence measurements reveal IBI1-dependent changes in capacity for non-photochemical quenching during BABA-induced resistance. Two of the three pathways showing enhanced activity during the expression of IBI1-dependent BABA-IR can be linked to photosynthesis. The xanthophyll cycle, which involves the reversible conversion of violaxanthin to zeaxanthin (via the intermediate antheraxanthin), is essential for non-photochemical quenching (NPQ), a process that allows plants to safely dissipate excessive excitation energy (Niyogi, Grossman, and Björkman 1998). Interestingly, reduced NPQ has been associated with the enhanced expression of PAMP-triggered immunity, presumably via enhanced accumulation of photosynthesis-derived reactive oxygen species (Göhre et al. 2012). Reduced NPQ would damage the photosynthetic machinery, which is consistent with the observation that expression of IBI1-dependent BABA-IR coincides with changes in chlorophyll metabolism (Fig 7.6C). Further support for a possible role of NPQ in BABA-IR comes from the previous finding that a regulatory mutant in the zeaxanthin epoxidase gene *ABA1*/*NPQ2* develops reduced levels of BABA-IR against *Hpa* and salt stress (Ton et al. 2005). Accordingly, it is plausible that BABA treatment modulates NPQ in an IBI1-dependent manner. To test this hypothesis, NPQ was measured via chlorophyll fluorescence measurements in water or BABA (10 mg/L) pre-treated Col-0 and *ibi1-1* plants for 2 days after inoculation with *Hpa* or mock (water). In a first pilot experiment, BABA treatment alone had no effect on NPQ in Col-0 plants, but it caused a statistically significant repression of NPQ upon subsequent inoculation with *Hpa* (Fig. 7.7A). Notably, this effect was not apparent in the *ibi1-1* mutant. While the results of this pilot experiment are promising, it must be noted that the experimental setup did not control for all external effects on NPQ. Specifically, the intensity of NPQ is influenced by the circadian rhythm (García-Plazaola et al. 2017). Since samples were measured sequentially, rather than simultaneously, and each measurement takes approx. 25 minutes, samples were measured at considerably different times after the start of the photoperiod. To exclude the potential confounding effect of circadian rhythm on NPQ due to sequential measurements, levels of NPQ during expression of BABA-IR were measured in a split-pot assay, where plants of both genotypes (Col-0 and *ibi1-1*) were grown in the same pot. This enabled simultaneous NPQ measurements between BABA-treated Col-0 and *ibi1-1* plants after *Hpa* inoculation. Although the difference in average NPQ levels between Col and *ibi1-1* was only borderline statistically significant ($p = 0.052$; Fig. 7.7B), this second experiment confirmed reduced NPQ in Col-0 compared to *ibi1-1* when expressing IBI1-dependent BABA-IR.

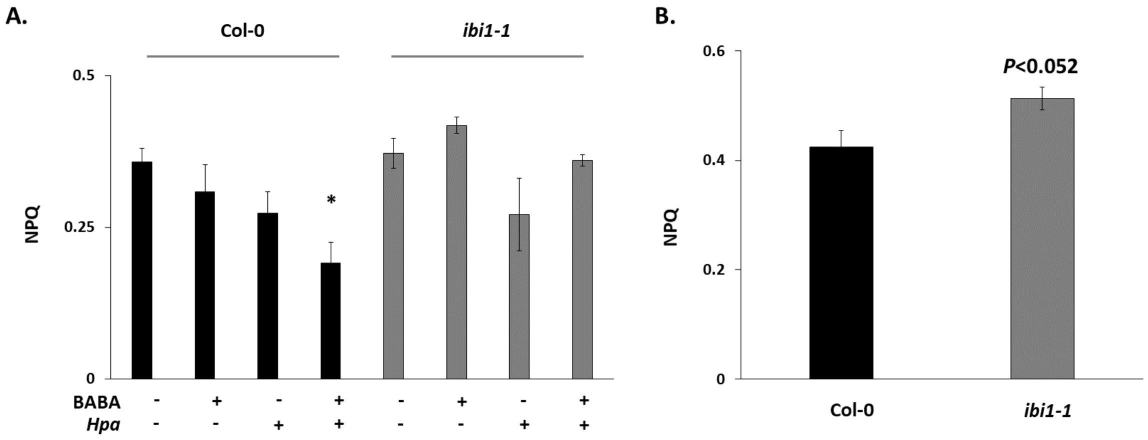

Figure 7.7: Expression of IBI1-dependent BABA-IR against* Hpa *is associated with a reduction in non-photochemical quenching (NPQ). A. *Quantification of NPQ in Col-0 and* ibi1-1 *plants (2.5-week old) after soil drench treatment with water or 10 mg/L BABA and subsequent challenge with* Hpa. *Plants were soil-drenched 2 days before inoculation with* Hpa, *and NPQ measurements were performed 2 days after inoculation. To measure the capacity for NPQ, plants were exposed to relatively high light intensity during the measurements. Shown are average steady-state NPQ levels (± SEM) at 400 µmol/m^2/s. Asterisk denotes a statistically significant difference (one-way ANOVA with Bonferroni's multiple comparisons test, p<0.01, n=4).* ***B.*** *Simultaneous quantification of NPQ in BABA-treated Col-0 and* ibi1-1 *after* Hpa *inoculation (2 dpi). To avoid uncontrolled effects on NPQ (e.g. circadian rhythms, relative humidity etc.), Col-0 and* ibi1-1 *plants were grown in the same pot, circumventing differences in timing of chlorophyll fluorescence measurements between genotypes. Shown are average steady-state NPQ levels (± SEM) at 400 µmol/m^2/s. The p-value (0.052) indicates the borderline statistical difference between genotypes (Student's t-test, n=4).*

7.4 Discussion

BABA is a non-proteinogenic amino acid that enhances plant disease resistance against a multitude of pathogens by simultaneously priming a variety of defence pathways. While BABA-IR against *Pseudomonas syringae* pv *tomato* DC3000 relies on SA- and NPR1-dependent signalling and is associated with transcriptional priming of SA-responsive genes, BABA-IR against *Hpa* is not affected by mutations in the SA pathway and is associated with enhanced deposition of callose-rich papillae (Zimmerli et al. 2000; Ton et al. 2005; Van der Ent et al. 2009). The SA-independent augmentation of callose by BABA has been linked to ABA signalling, which can mimic the positive effect of BABA on resistance to necrotrophic pathogens (Ton and Mauch-Mani 2004). The importance of ABA signalling for callose-mediated BABA-IR against *Hpa* has been hinted at by the identification of the *impaired in BABA-induced sterility3* (*ibs3*) mutant (Ton et al. 2005). This mutant was selected in a screen for reduced female sterility after treatment with high concentrations of BABA and is affected in the regulation of the zeaxanthin epoxidase gene *ABA1*/*NPQ2*. The *ibs3* mutant is partially affected in BABA-IR and shows reduced callose deposition in BABA-primed plants after *Hpa* inoculation (Ton et al. 2005), which suggests that ABA plays a positive regulatory role in *Hpa* resistance. In support of this, Hok et al. recently showed that the *IMPAIRED OOMYCETE SUSCEPTIBILITY1 (IOS1)* gene, a receptor-like kinase required for *Hpa* susceptibility (Hok et al. 2011), represses ABA signalling, suggesting that *Hpa* employs IOS1 in the host plant to repress unknown ABA-dependent defences (Hok et al. 2014). However, the role of ABA in *Hpa* resistance remains controversial. There is ample evidence that ABA represses SA-dependent defence (Audenaert, De Meyer, and Höfte 2002; Mohr and Cahill 2003; Ton, Flors, and Mauch-Mani 2009), which is effective against *Hpa* (Delaney et al. 1994). More specifically, Fan et al. showed that the dominant *cds2-1D* mutant, which accumulates higher ABA levels due to constitutively enhanced transcription of the *NCED* gene, was more susceptible to *Hpa,* whereas the ABA-deficient *aba3* mutant was more resistant to *Hpa* (Fan et al. 2009), again pointing to a repressive role of ABA in *Hpa* resistance. The transcriptome study in this chapter suggests an overall negative correlation between expression of IBI1-dependent BABA-IR and transcription of ABA/abiotic stress-responsive genes. This analysis was based on 139 genes that differed in transcription between Col-0 and *ibi1-1* after BABA treatment and subsequent *Hpa* inoculation, but not after BABA treatment alone (Fig. 7.3C), in order to enrich for genes that contribute to BABA-augmented defence, rather than BABA-induced stress. Hierarchical clustering and GO term analysis of these genes revealed that expression of IBI1-dependent BABA-IR against *Hpa* correlates with repressed transcript levels of ABA-/abiotic stress-responsive genes (Fig. 7.3E). However, most of these ABA-related genes displayed similarly low transcript levels in susceptible water-treated Col-0 after *Hpa* inoculation. Hence, repression of ABA signalling may have a contribution to BABA-IR, but by itself is not sufficient to mediate primed defence against *Hpa*. In light of the discovery that VOZ transcription factors mediate enhanced defence efficiency of callose during BABA-IR (Chapter 5), it is tempting to speculate that the previously reported repression of ABA response genes by VOZ transcription factors (Nakai, Nakahira, et al. 2013) is responsible for the repression of ABA-related genes during BABA-IR against *Hpa*.

The transcriptome analysis failed to identify large clusters of coordinated gene transcription that are exclusively up-regulated during expression of IBI1-dependent BABA-IR against *Hpa*. However, the microarray analysis revealed one gene, *MIOX4* (At4g26260), whose transcription was enhanced in BABA-treated Col-0 during *Hpa* attack and not in *ibi1-1* (Fig. 7.4A). MIOX4 is a myo-inositol oxygenase, which catalyses the conversion of myo-inositol into D-glucuronic acid (Fig 7.4B). Several pathways link MIOX4 to the regulation of stress resistance. One product of MIOX4 activity is D-glucuronic acid (D-GlcUA). Interestingly, a downstream product in the biosynthesis of pectin, D-galacturonic acid, induces resistance against the necrotrophic fungus *Plectosphaerella cucumerina*, while pre-treatment with BABA primes *P. cucumerina*-induced accumulation of D-galacturonic acid (Gamir et al. 2014). D-GlcUA and D-galacturonic acid are precursors of cell wall components, such as pectin polymers and hemicelluloses (Loewus, Kelly, and Neufeld 1962). However, the accumulation of these uronic acids into cell walls was repressed after defence priming and during expression of BABA-IR against *P. cucumerina* (Gamir et al. 2014). Accordingly, Gamir et al. (2014) suggested that the resistance-inducing effect of D-galacturonic acid is not related to cell wall-mediated defence. The substrate of MIOX4, myo-inositol, can also be converted to phosphatidyl-inositol-4,5,-bisphosphate (PIP2), an essential component of the calcium signalling pathway (Rudd and Franklin-Tong 1999). Interestingly, a mutation in the poly-phospho-inositide phosphatase *ibs2* leads to reduced levels in BABA-IR, suggesting that the phosphoinositide/Ca^{2+} pathway could be targeted by BABA (Ton et al. 2005). However, other data presented in this chapter support a third potential mechanism by which *MIOX4* can contribute to BABA-IR. Ascorbate (vitamin C) is the major anti-oxidant in plants (Smirnoff and Wheeler 2000; Caverzan et al. 2012). Animals synthesise ascorbate via reduction of D-GlcUA. However, the main biosynthetic pathway to ascorbate in plants proceeds from D-Glc-6P via D-Man-1P and L-galactono-1,4-lactone, which is then oxidised into L-ascorbate (Wheeler, Jones, and Smirnoff 1998; Smirnoff 2001). The existence of the animal pathway for ascorbate synthesis in plants remains controversial. Overexpression of L-gulono-1,4-lactone oxidase, the final enzyme of the animal pathway, increased vitamin C content in Arabidopsis, and rescued ascorbic acid content in *vtc* mutants, in which the D-Man-1P pathway is disrupted (Radzio et al. 2003). Furthermore, overexpression of *MIOX4* leads to a 2- to 3-fold increase in ascorbate in transformed Col-0 (Lorence et al. 2004). However, increased ascorbic acid content in MIOX4 overexpression plants could not be reproduced in a subsequent study (Endres and Tenhaken 2009). It should be noted that while the two independent 35S:*MIOX4* overexpressor lines used in the later study showed clear accumulation of MIOX4 mRNA, they had similar myo-inositol contents as untransformed control plants, indicating that post-transcriptional regulation of enzyme activity could confound the contribution of MIOX4 to ascorbate synthesis. The bioassays presented in this chapter revealed a small increase in the level of BABA-IR of 35S:*MIOX4* overexpression lines (Fig. 7.4C). It is plausible that the increased ascorbic acid content in the *MIOX4* overexpression lines impacts on the defence signalling pathways that are active during expression of BABA-IR. For instance, enhanced MIOX4 could modulate ABA biosynthesis through increased conversion of violaxanthin into zeaxanthin, or it could dampen H_2O_2-mediated ABA signalling. Conversely, in the absence of any MIOX isoforms, ascorbic acid synthesis from the oxidation of L-galactono-1,4-lactone would still be sufficient to avoid negative impacts on induced resistance. This redundancy in ascorbate biosynthesis may explain why the *miox* mutants were unaffected in BABA-IR (Fig. 7.4D).

Apart from *MIOX4*, the microarray analysis did not identify genes whose expression is exclusively altered during expression of IBI1-dependent BABA-IR against *Hpa*. This is in marked contrast to an earlier microarray study of BABA-IR against the bacterial pathogen *P. syringae*, which showed direct upregulation, as well as priming of SA-responsive genes by BABA (Tsai et al. 2011). This discrepancy confirms that the augmented defence response in BABA-primed plants depends on the nature of the challenging stress. Augmented defence against *Hpa* in BABA-primed plants has predominantly been associated with increased efficiency of callose deposition (Zimmerli et al. 2000; Ton et al. 2005; Van der Ent et al. 2009). This form of cell wall defence is based not only on callose synthesis at the sites of attack, but also on the deposition of antimicrobial compounds in the callose matrix. Callose-related defence is relatively quick and can take place independently of gene expression and *de novo* protein synthesis (Ellinger and Voigt 2014), which could explain the lack of DEGs associated with IBI1-dependent expression of BABA-IR against *Hpa*.

In addition to transcriptional changes, primed resistance has also been associated with changes in plant metabolites (Gamir et al. 2014; Pastor et al. 2014; Mhlongo et al. 2016; Llorens et al. 2016). Pastor et al. specifically studied the metabolomic changes related to priming by BABA (Pastor et al. 2014). This study revealed that BABA induces accumulation of compounds related to the tricarboxylic acid (TCA) cycle, as well as the defence hormones SA and JA. BABA also had an effect on amino acids, leading to increased accumulation in cysteine, methionine and serine and a decrease in arginine and threonine at 48 hours after BABA treatment. However, the study by Pastor et al. (2014) did not include post-challenge samples nor a mutant impaired in BABA-IR. As a consequence, this study could not differentiate between metabolic responses associated with BABA-induced stress and BABA-IR. The study presented in this chapter included the *ibi1-1* mutant and samples collected after challenge inoculation with *Hpa*. Interestingly, MALDI analysis of metabolite extracts revealed that the *ibi1-1* mutation had a much bigger impact on the metabolome than on the transcriptome, even in un-primed and mock-inoculated plants (Fig. 7.5A). Overall, 960 ions (500 in positive ionisation mode, 460 in negative mode) were detected whose abundance changed significantly in at least one condition. In contrast to the transcriptome, a subset of 74 ions (43 in positive, 31 in negative mode) showed increased accumulation exclusively during expression of IBI1-dependent BABA-IR in Col-0 (Fig. 7.5B), suggesting that they may have a contribution to the expression of IBI1-dependent BABA-IR.

The identity of ion markers obtained by MALDI-MS remains putative as multiple metabolites can give similar m/z values after ionisation. To increase the reliability of the analysis, subsequent analysis only considered putative metabolites if multiple ions could be mapped to one biochemical pathway. This identified three pathways showing increased activity during expression of IBI1-dependent BABA-IR against *Hpa*. Five markers mapped to metabolic pathways that converge at the xanthophyll cycle (Fig. 7.6A), which consists of the interconversion of zeaxanthin and violaxanthin (via the intermediate antheraxanthin), and plays a key role in NPQ to release excess excitation energy under high light conditions (Niyogi, Grossman, and Björkman 1998; Pogson and Rissler 2000). Interestingly, mutations in the zeaxanthin epoxidase gene *ABA1/NPQ2*, which catalyses the conversion of zeaxanthin to violaxanthin, affect BABA-IR against *Hpa* (Ton et al. 2005). Furthermore, treatment with the bacterial PAMP flg22 leads to rapid downregulation of NPQ in Arabidopsis, and mutants impaired in NPQ exhibit constitutive priming of early defence responses (Göhre et al. 2012). Accordingly, it has been proposed that regulation of NPQ is an intrinsic component of the plant immune system (Göhre et al. 2012). Reduction of NPQ can lead to increased production of singlet oxygen ($^1O_2$), a highly reactive oxygen species, via the triplet state of chlorophyll ($^3Chl^*$). $^1O_2$ is known to regulate gene expression, specifically of genes involved in the defence against photo-oxidative stress. While $^1O_2$ itself is too short-lived to act as a signalling molecule, it has been suggested that signal transduction responses to singlet oxygen involve degradation products of D1 protein and chlorophyll (Krieger-Liszkay 2005). This idea is supported by the finding that chlorophyll precursors, such as Mg-protoporphyrin IX, can act as signalling molecules during retrograde signalling from chloroplasts to the nucleus (Strand et al. 2003). Interestingly, apart from the putative involvement of the xanthophyll cycle, the metabolome analysis also detected enhanced accumulation of putative chlorophyll precursors during expression of IBI1-dependent BABA-IR, including protoporphyrin IX (Fig. 7.6C). The increased abundance of chlorophyll precursors indicates a compensatory mechanism to maintain chlorophyll levels during accelerated chlorophyll degradation by photo-oxidative damage. Based on this evidence, subsequent quantification of NPQ by chlorophyll fluorescence indeed confirmed an IBI1-dependent reduction in NPQ during expression of BABA-IR against *Hpa* (Figs. 7.7A & 7.7B). Controlled reduction of NPQ capacity during expression of BABA-IR might serve to modulate the cellular redox state and increase the activity of defence regulatory ROS. This response might also explain the enhanced expression of the *MIOX4* gene, which has been implicated in ascorbate biosynthesis (Lorence et al. 2004; Lisko et al. 2013), to contain the ROS burst. Alternatively, the modulation of the xanthophyll cycle may be a regulatory mechanism for altered ABA biosynthesis, because violaxanthin is a direct precursor of ABA. Further experiments are required to study the intrinsic interplay between ABA signalling, the xanthophyll cycle and homeostasis of defence-regulatory ROS during IBI1-dependent BABA-IR.

Chapter 8

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General Discussion

8.1 The biological relevance of β-aminobutyric acid: rhizosphere signal, defence hormone or bio-active xenobiotic compound?

β-aminobutyric acid-induced resistance (BABA-IR) has been described in more than 40 plant species, including both wild species and important crop plants (Cohen, Vaknin, and Mauch-Mani 2016). The wide-spread occurrence of BABA-IR, as well as the enantiomer-specific activity of R-BABA, has long puzzled scientists. For years, BABA has been considered a xenobiotic compound that is not naturally present in plants. The question of the biological relevance of BABA was further spurred by the discovery of the BABA receptor IBI1 (Luna et al., 2014). Why have plants evolved a specific receptor protein to a xenobiotic chemical? Does the molecule mimic a natural ligand, or does it induce a physiological state that is indicative of pathogen attack?

One theory proposes that BABA is produced by root-colonising microbes in the rhizosphere, from where it could be taken up by the plant and distributed systemically (Luna et al. 2014). This hypothesis is supported by the fact that BABA is effectively taken up by the root systems of plants after soil-drench treatment. It is relatively stable in soil, making it a suitable rhizosphere signal. Moreover, Luna et al. (2014) showed that the BABA receptor IBI1 is also required for priming of cell wall-mediated defence and induced systemic resistance (ISR) against *Hyaloperonospora arabidopsidis* (*Hpa*) after root colonisation by the plant growth-promoting rhizobacterium *Pseudomonas fluorescens* WCS417r (Luna et al. 2014). However, while BABA-IR and ISR share some similarities, they activate distinct transcriptional responses (Van der Ent et al. 2009).

Based on the molecular characterisation of BABA perception by IBI1 (Luna et al., 2014), a second theory was proposed about the biological relevance of this aspartyl-tRNA synthetase (AspRS) in plant defence (Luna et al. 2014). The fact that *IBI1* transcription is increased in response to pathogen attack and that transgenic overexpression of *IBI1* enhances basal disease resistance, suggests a native function of IBI1 in plant defence. Furthermore, binding of R-BABA to IBI1 leads to accumulation of uncharged $tRNA^{Asp}$, thus mimicking the effect of L-aspartic acid (L-Asp) deficiency. Based on these results, it was hypothesised that IBI1 contributes to basal resistance as a sensor of cellular L-Asp (Fig. 8.1; Schwarzenbacher, Luna, and Ton 2014). A sudden decline in cellular L-Asp concentrations could indicate parasitisation by a biotrophic pathogen and would reduce canonical AspRS activity by IBI1. It was suggested that this deprivation of canonical AspRS activity primes the alternative defence function of IBI1. This situation is mimicked by R-BABA, which blocks L-Asp binding to IBI1, tricking the protein into sensing low L-Asp levels (Fig. 8.1). However, a secondary stress signal is required upon pathogen attack to fully activate the defence function of IBI1, resulting in enhanced *IBI1* transcription, subcellular translocation of IBI1 from the endoplasmic reticulum (ER) to the cytoplasm, and augmented defence induction. Since BABA has been reported to prime PAMP-triggered immunity (PTI; Singh et al., 2012), the logical conclusion is that this secondary signal comes from pathogen-associated molecular patterns (PAMPs). Hence, IBI1 primes PAMP-triggered immunity by means of 'depleted self-recognition'. This mechanism provides plants with an improved capability to recognise biotrophic pathogens, thereby counteracting their specialist ability to suppress ‘non self-recognition’ of PAMPs (Dodds and Rathjen, 2010). Interestingly, one study found that aspartic acid was the only amino acid that decreased in abundance after infection with *Pseudomonas syringae* pv *tomato* DC3000 (Ward et al. 2010), lending further credibility to this hypothesis. However, chapter 3 in this thesis provided evidence that impairing canonical AspRS activity of IBI1 by site-directed mutagenesis of the L-Asp-binding domain did not enhance basal resistance against *Hpa* (Fig. 3.1). Thus, apart from substrate deprivation, priming of the defence function by IBI1 requires an additional mechanism, such as a change in tertiary protein structure. As discussed in Chapter 3, it is possible that such structural modifications are only triggered by specific changes in the stoichiometry of the AspRS reaction that do not occur by mutagenesis of the L-Asp-binding domain. Alternatively, the point mutation in the L-Asp-binding domain (GLN308ALA) could have had additional impacts on the folding of the protein, affecting its non-canoncial defence function. Whether cellular deprivation of L-Asp and/or IBI1-overexpression have similar impacts on IBI1 structure as binding of R-BABA requires further investigation.

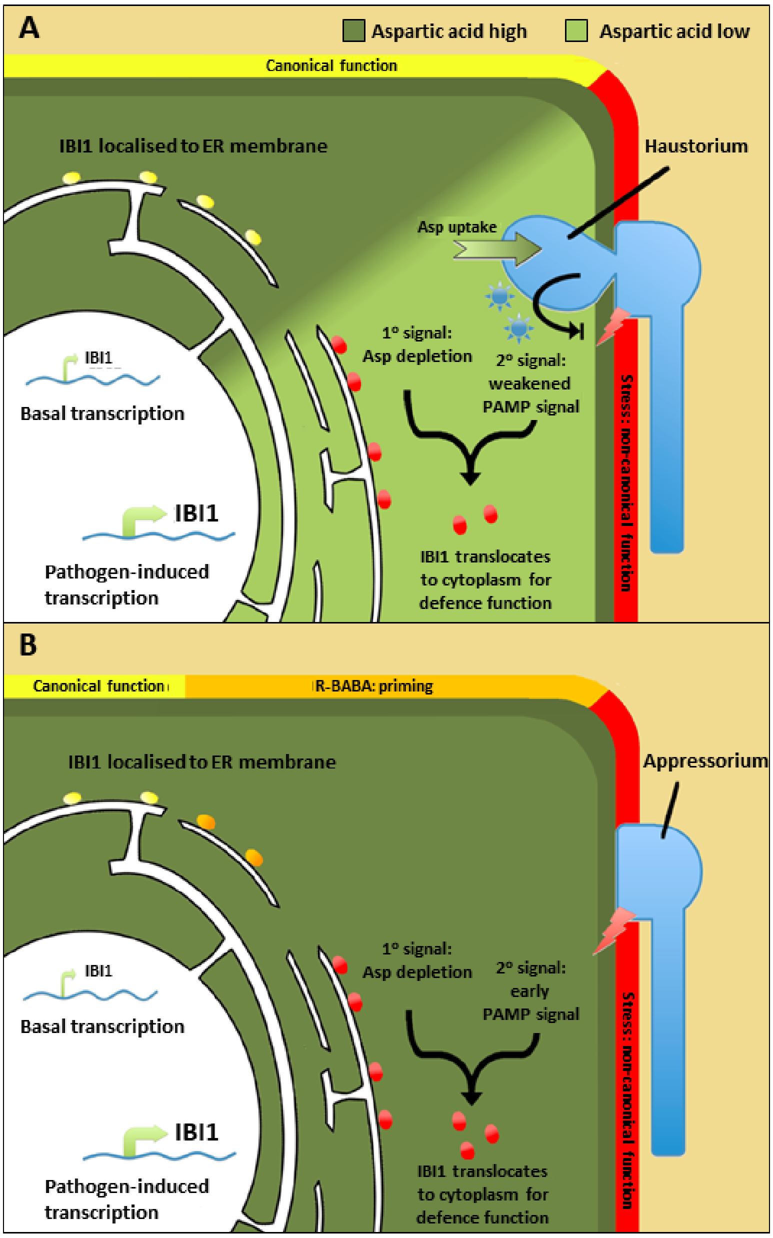

Figure 8.1: Model of IBI1 as a 'depleted-self sensor' in basal resistance (A) and as a regulator of BABA-induced resistance (B). ***A:*** *Role of IBI1 in basal resistance. Successful pathogen infection leads to suppression of PAMP recognition through the action of pathogen-derived effectors (blue asterisks). Ongoing parasitisation and amino acid uptake by the pathogen lowers cellular L-Asp levels, depriving IBI1 (yellow circles) from its canonical aspartyl-tRNA synthetase (AspRS) activity. This, together with effector-weakened PAMP perception, boosts IBI1 transcription and elicits translocation of the protein from the ER to the cytosol, where it activates broad-spectrum defences (red circles). Hence, IBI1 acts as a 'depleted-self' sensor to counteract effector-triggered suppression of 'non self-recognition'.* ***B:*** *Role of IBI1 in BABA-induced resistance (BABA-IR). Binding of R-BABA to ER-localised IBI1 deprives the protein of canonical AspRS activity, which 'primes' the protein's non-canonical defence activity (orange circles). Detection of pathogen-associated molecular patterns (PAMPs) during the early stages of pathogen infection boosts IBI1 gene transcription and triggers translocation of IBI1 to the cytosol, where it activates defence activity through interaction with immune-regulatory proteins (red circles). Figure taken from Schwarzenbacher, Luna, and Ton 2014.*

A third theory about the biological relevance of BABA and IBI1 comes from reports that BABA can naturally occur in plant tissues (Cohen, Vaknin, and Mauch-Mani 2016). However, it remained unclear from these reports whether the detected signal came from BABA, or from one of its isomers AABA (α-aminobutyric acid) and GABA (γ-aminobutyric acid). Only recently, Thevenet et al. (2017) developed a robust UPLC-MS/MS method to distinguish between AABA, BABA and GABA in plant tissue extracts (Thevenet et al. 2017). Using this method, they detected BABA in several plant species, including Arabidopsis, maize and the moss *Physcomitrella patens*. Furthermore, they showed that BABA accumulates in Arabidopsis over the course of several hours to days following exposure to various biotic and abiotic stresses. BABA thus behaves similar to other known defence hormones. One caveat of this study were the very low concentrations of BABA detected in the shoot tissues, even after stress inoculation. The highest concentrations of BABA, measured at 48 hours after soil-drenching with 200 mM NaCl, were around 120 ng/g fresh weight. In comparison, upon inoculation of cucumber with *P. syringae*, the plant defence hormone salicylic acid (SA) accumulated to concentrations of more than 40 mg/g in petiole exudates of systemic leaves at 24 hours after local inoculation (Rasmussen, Hammerschmidt, and Zook 1991). The concentration of SA during defence is thus orders of magnitude higher than that of BABA. Application of 2.5 mg/L BABA as a soil-drench induces partial resistance against downy mildew in Arabidopsis, whereas BABA fails to induce resistance when applied at 0.5 mg/L in the soil (R. Schwarzenbacher & E. Guest, unpublished). In studies with radioactively labelled BABA, 31% of ^{14}C-BABA could be recovered from tomato plants 24 hours after root application, with around 8% of extractable radioactivity found in leaves (Cohen and Gisi 1994). Assuming similar uptake and distribution of BABA in Arabidopsis, it may be possible that the *in planta* concentrations of BABA reported by Thevenet et al. (2017) are sufficient to induce resistance in stressed plants, particularly if specific cells or tissue types in stressed plants accumulate higher amounts than surrounding cells and/or tissues. On the other hand, the same research group reported earlier that BABA traces of up to 2,200 ng/g dry weight in seedlings and mature progeny from BABA-treated parental plants are below the concentration threshold for resistance induction (Slaughter et al. 2012). This directly contradicts the assumptions by Thevenet et al. (2017), even when taking into account the differences between dry weight and fresh weight (Arabidopis leaves have a relative water content of 70 – 90%; Gigon et al. 2004). All things considered, the assertion that BABA is an endogenous, stress-inducible phytohormone would be significantly strengthened by the discovery of a biosynthetic pathway for this unusual non-proteinogenic amino acid.

The above theories are not mutually exclusive. It is possible that aspartyl-tRNA synthetases initially acquired a secondary function in plants in monitoring amino acid status, enabling the recognition of a 'depleted-self' status. The co-opting of aminoacyl-tRNA synthetases for non-canonical functions beyond protein translation has been described earlier, and many examples of such activity have been demonstrated (Chapters 1 and 3, and references therein). Subsequently, the evolution of a stress-inducible pathway for BABA biosynthesis would have allowed plants to more actively control and modulate the defence responses in relation to amino acid homeostasis through priming of the non-canonical defence function of AspRS enzymes. L-Asp seems to be especially suited for monitoring of perturbations of amino acid homeostasis, because Asp-derived branched chain amino acids and other compounds play an important role in plant immunity (van Damme et al. 2009; Stuttmann et al. 2011; Zeier 2013). Future research into the IBI1 domains and molecular interactions necessary for priming and activation of its non-canonical defence function are needed to fully understand the potential role of aspartyl-tRNA synthetases as tuneable monitors of metabolic defence homeostasis.

8.2 The multifaceted role of ABA signalling in BABA-IR

The phytohormone abscisic acid (ABA) has diverse roles in seed dormancy, germination, root architecture and leaf senescence (Sah, Reddy, and Li 2016). In addition, ABA plays a positive regulatory role in tolerance to abiotic stresses, such as drought, cold and osmotic stress (Sah, Reddy, and Li 2016). The effect of ABA on pathogen resistance, on the other hand, is a much debated subject (Asselbergh, De Vleesschauwer, and Höfte 2008; Ton, Flors, and Mauch-Mani 2009). While a decrease in ABA synthesis or responsiveness generally leads to increased pathogen resistance in tomato (Asselbergh, De Vleesschauwer, and Höfte 2008), the effects of ABA on resistance in Arabidopsis appear to be more complex and to depend on the stage of infection and specific plant-pathogen interaction. For example, ABA plays an important role in stomatal closure during early, pre-invasive defence against *P. syringae* pv. *tomato* and *Sclerotinia sclerotiorum* (Guimarães and Stotz 2004; de Torres-Zabala et al. 2007). At the same time, ABA has mostly negative effects on post-invasive defence responses, particularly those that are controlled by salicylic acid (SA). Effectors of *Pst* DC3000 have been shown to induce ABA response genes to induce disease susceptibility in Arabidopsis (de Torres-Zabala et al. 2007). Similarly, *Dickeya dadantii* infection triggers accumulation of ABA in Arabidopsis, which correlates with increased production of bacterial virulence factors and enhanced susceptibility (Van Gijsegem et al. 2017). With regards to necrotrophic fungal pathogens, both positive (Ton and Mauch-Mani 2004) and negative (Anderson et al. 2004) outcomes of ABA have been reported. Several pathways have been proposed to explain the varied effect of ABA on disease resistance, including suppression of phenylpropanoid biosynthesis and ROS production, modulation of the negative cross-talk between SA signalling and callose deposition, and a general shift towards abiotic stress responses (Asselbergh, De Vleesschauwer, and Höfte 2008).

The role of ABA signalling has also been studied in the context of BABA-IR. The first indication for involvement of ABA in BABA-IR came from observations that augmented deposition of callose during expression of BABA-IR against the necrotrophic pathogens *Plectosphaerella cucumerina* and *Alternaria brassicicola* is affected in ABA signalling mutants (Ton and Mauch-Mani 2004). Priming treatment with BABA has also been shown to result in augmented accumulation of ABA after exposure to salt stress (Jakab et al. 2005) or inoculation with the hemi-biotrophic fungus *Leptosphaeria maculans* (Kaliff et al. 2007). In tomato, exposure to salt stress, which is commonly associated with ABA signalling, had a synergistic effect on BABA-IR against *Pst* (Baysal et al. 2007). A study on BABA-induced thermotolerance revealed a more complex role of ABA (Zimmerli et al. 2008), showing that

BABA induced a mild, but statistically significant, induction of the ABA-responsive genes *ABI1, ABI2, ABI3* and *ABI5*. However, while BABA-primed acquired thermotolerance was impaired in the ABA-insensitive mutant *abi1-1,* the *abi2-1* mutation had no or even a positive effect on BABA-primed induced tolerance to short term and prolonged heat exposure, respectively. Interestingly, BABA-induced thermotolerance was not impaired in the ABA biosynthesis mutant *aba2-1*, indicating that BABA modulates ABA signalling independently of cellular ABA levels during exposure to heat stress.

Multiple lines of evidence from the work presented in this thesis confirm that ABA plays a role in IBI1-dependent BABA-IR. In Chapter 7, genome-wide transcriptome analysis of BABA-IR in Col-0 and *ibi1-1* revealed that expression of IBI1-dependent BABA-IR against *Hpa* is associated with suppression of ABA-inducible genes (Fig. 7.3E). Whether this repression of ABA-dependent gene expression is caused by a global feedback mechanism, or whether this marks a direct signal, remains to be demonstrated. Subsequent metabolome analysis of the same samples suggested an increase in the ABA precursor antheraxanthin, as well as increased accumulation of compounds that contribute to biosynthesis of xanthophylls, which are direct precursors of ABA (Chapter 7, Fig. 7.6). Finally, chlorophyll fluorescence measurements revealed that Col-0 plants develop lower maximal levels of non-photochemical quenching (NPQ) during expression of IBI1-dependent BABA-IR compared to *ibi1-1* plants (Chapter 7; Fig. 7.7). This is similar to findings from Hurry et al. (1997), who reported that the ABA biosynthesis mutants *aba1-3* and *aba1-4* develop lower levels of NPQ upon prolonged exposure to high irradiance (Hurry et al. 1997). Together, the data presented in Chapter 7 confirm and expand our current knowledge about the role of ABA signalling in (BABA-induced) resistance, but, by themselves, do not clarify the exact role of ABA in BABA-IR.

Further evidence for a regulatory role of ABA in BABA-IR came from the yeast two-hybrid (Y2H) screen presented in Chapter 4, which identified the transcription factors (TFs) VASCULAR PLANT ONE ZINC-FINGER 1 (VOZ1) and VOZ2 as protein interactors of IBI1. Homologues of these proteins have been found in a range of distantly related plant species, including monocots, dicots, gymnosperms and even the moss *Physcomitrella patens,* showing that VOZ transcription factors are evolutionarily ancient proteins (Mitsuda et al. 2004). VOZ1 and VOZ2 act redundantly in Arabidopsis, and current evidence implies a role of VOZ proteins in plant responses to both biotic and abiotic stresses, as well as the control of flowering and root growth (Yasui et al. 2012; Nakai, Fujiwara, et al. 2013). Overexpression of VOZ2 increases resistance against *Colletotrichum higginsianum,* but severely reduced cold acclimation (Nakai, Fujiwara, et al. 2013). By contrast, the *voz1-2/voz2-2 (voz1/2-2)* double mutant is more resistant to cold and drought-stress, which is associated with increased expression of the ABA-inducible cold-response genes *CBF4/DREB1d, ZAT12* and *RD29B* (Nakai, Nakahira, et al. 2013). On the other hand, *voz1/voz2-2* mutant plants are significantly more susceptible to bacterial (*P. syringae)* and fungal (*C. higginsianum)* pathogens (Nakai, Nakahira, et al. 2013). Furthermore, while wild-type plants only close their stomata upon addition of exogenous ABA, the stomata of *voz1/2-2* always remained closed, despite unchanged levels of endogenous ABA in the double mutant (Nakai, Nakahira, et al. 2013). Hence, VOZ proteins act as negative regulators of ABA signalling. Based on these opposing (a)biotic stress phenotypes of the VOZ2 overexpression line and the *voz1/2-2* mutant, it has been proposed that VOZ1 and VOZ2 regulate the balance between abiotic and biotic stress signalling by mediating negative feedback on ABA-dependent defence genes (Nakai, Nakahira, et al. 2013). Interestingly, repression of ABA-dependent genes is conspicuously evident from the IBI1-dependent transcriptome during expression of BABA-IR against *Hpa* (Chapter 7, Fig. 7.3).

Chapter 5 showed that the VOZ TFs are required for both basal callose-mediated defence against *Hpa*, as well as the augmented callose-mediated defence during expression of IBI1-dependent BABA-IR (Chapter 5, Fig. 5.2B). As mentioned above, callose-mediated BABA-IR against the necrotrophic pathogens *P. cucumerina* and *A. brassicicola* depends on ABA signalling (Ton and Mauch-Mani 2004), suggesting a positive regulatory role of ABA in callose-dependent BABA-IR. By contrast, Chapter 7 revealed that expression of augmented callose-mediated defence during IBI1-dependent BABA-IR against *Hpa* coincides with global repression of ABA-responsive genes. Expression analysis by RT-qPCR and Western blotting has suggested that the nuclear activity of VOZ proteins is under post-translational control through ubiquitination and degradation by the 26S proteasome, while the cytoplasmic levels of VOZ protein are regulated transcriptionally (Nakai, Nakahira, et al. 2013). Indeed, while *VOZ2* transcription is not altered by ABA, *VOZ1* is upregulated by more than 2-fold within 3 hours after application of 10 µM ABA (Winter et al. 2007, AtGenExpress Consortium), suggesting that ABA increases the total pool of cytoplasmic VOZ protein.

Based on the combined results from Chapters 5 and 7, the following model emerges about the role of ABA in plant defence and BABA-IR (Fig. 8.2): During early defence signalling in response to PAMP recognition, a short transient increase in ABA induces VOZ activity, possibly by increasing *VOZ1* transcription. A transient increase in cellular ABA has been reported for various plant-pathogen interactions (Whenham et al. 1986; Iriti and Faoro 2008; Ton, Flors, and Mauch-Mani 2009). A driving mechanism behind PAMP-induced ABA induction could be endoplasmic reticulum (ER) stress, which is known to occur during early PTI (Eichmann and Schäfer 2012), and has been reported to lead to ABA-dependent gene expression responses (H. Zhang et al. 2008; L. Liu et al. 2011). Therefore, it is tempting to speculate that the reduction of AspRS activity in BABA-bound IBI1 aggravates PAMP-induced ER stress during PTI, which may explain the strongly augmented translocation of IBI1 from the ER to the cytoplasm during pathogen attack in BABA-treated plants (Luna et al. 2014). The subsequent interaction of VOZ proteins with IBI1 in the cytoplasm enhances the efficiency of callose-mediated defence via nuclear activity of these TFs (Chapter 5, Fig. 5.3). At the same time, VOZ proteins down-regulate ABA-dependent genes, which would otherwise repress SA-dependent defence responses and potentially contribute to pathogen susceptibility. BABA treatment primes both ABA synthesis and IBI1 translocation to the cytoplasm, thereby reinforcing the interaction between VOZ2 and IBI1, resulting in augmented callose defence and global ABA-dependent gene repression. This model would also explain recent findings by Wilkinson et al. (2017) regarding the interaction between BABA and ABA in defence against the hemi-necrotrophic fungus *Botrytis cinerea* in tomato (Wilkinson et al. 2017). This study reported that increased resistance against *B. cinerea* in tomato fruit from BABA-treated plants was associated with elevated levels of ABA. Interestingly, however, ABA treatment alone had a negative effect on pathogen resistance, whereas combined treatment with BABA + ABA increased resistance to even higher levels than BABA alone. These results are in agreement with the model presented in Fig. 8.2, since co-application of BABA might have increased (through priming of IBI1 translocation) VOZ-dependent callose defence against *B. cinerea*, while at the same time antagonising ABA-dependent repression of defence genes.

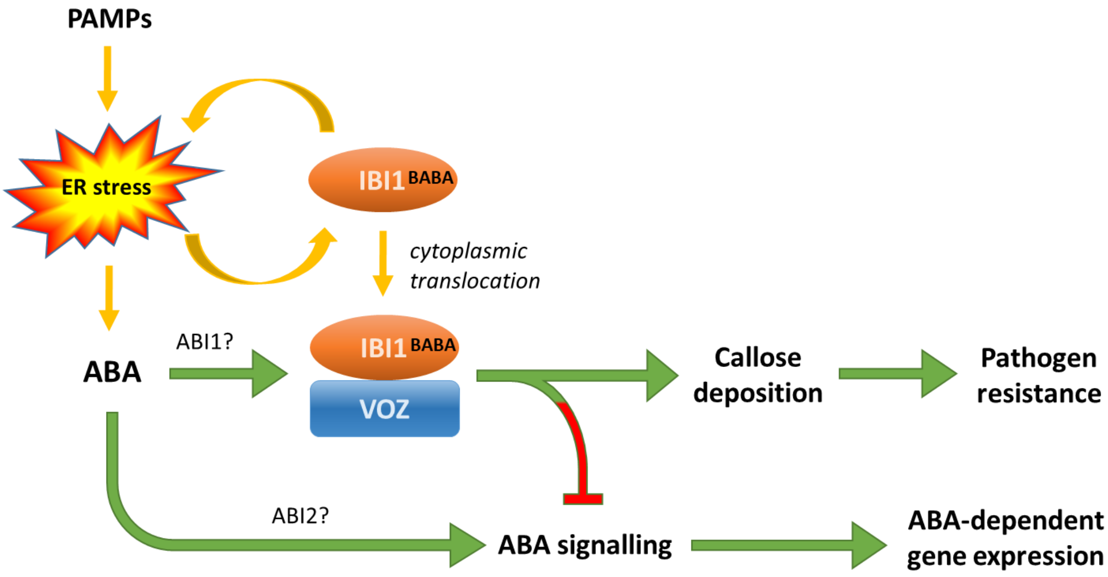

Figure 8.2: Model explaining the role of ABA in BABA-induced resistance. *PAMP recognition during the early stages of pathogen attack triggers ER stress, which is aggravated by binding of BABA to IBI1. This enhanced ER stress triggers ABA synthesis and increased translocation of IBI1 to the cytoplasm, where it interacts with the transcription factors VOZ1 and VOZ2. ABA-induced expression of* VOZ1 *further promotes this interaction. Upon interaction with IBI1, VOZ proteins augment pathogen resistance by enhancing callose deposition during basal and induced defence. At the same time, VOZ TFs act as repressors of ABA-dependent gene expression, which would otherwise interfere with biotic stress resistance. BABA regulates ABA-mediated stress responses by priming both ABA accumulation and the interaction between IBI1 and VOZ proteins.*

The suggested model may also explain the complex role of ABA signalling in BABA-induced tolerance to heat stress. As mentioned above, the *abi1-1* mutation had a negative effect on basal and BABA-primed heat acclimation (Zimmerli et al. 2008). By contrast, improved heat stress acclimation upon BABA treatment was even further augmented in the *abi2-1* mutant. Notably, the *voz1/2-2* double mutant shows increased heat sensitivity, presumably due to its constitutively closed stomata, which interfere with heat dissipation (Nakai, Nakahira, et al. 2013). Thus, it seems possible that ABI1-regulated signalling results in increased interaction between IBI1 and VOZ2 and callose deposition, whereas ABI2 controls a separate branch of ABA signalling that leads to stomatal closure, drought and cold resistance. Divergent roles for the largely redundant proteins ABI1 and ABI2 in callose deposition have been described before (Kaliff et al. 2007).

While the model provides an attractive mechanism by which ABA and ER stress control augmented callose deposition during BABA-IR, there are still unanswered questions about the role of ABA in other BABA responses. For example, BABA primes resistance against an exceptionally wide range of stresses, including drought and salt stress. Interestingly, BABA primes expression of the ABA-inducible cold- and drought-response gene *RAB18* upon exposure to salt stress (Jakab et al. 2005), but not upon inoculation with *A. brassicicola* or *P. cucumerina* (Ton and Mauch-Mani 2004), while Chapter 7 showed that ABA-inducible gene expression is repressed during expression of BABA-IR against *Hpa*. The model proposed above does not explain how stressor-specific regulation of ABA-dependent gene responses is achieved in BABA-treated Arabidopsis. Analysis of BABA-IR in tomato mutants or RNAi lines affected in *VOZ* homologues could clarify whether the mechanism proposed above is conserved across plant species. Furthermore, given the effect of phytochrome-dependent perception of red/far-red light on disease resistance (Cerrudo et al. 2012) and the interaction between VOZ2 and phyB (Yasui et al. 2012), it is tempting to speculate that light signalling plays a role in the interaction between IBI1 and VOZ2 and BABA-IR.

8.3 The role of light signalling in BABA-IR

Light is of fundamental importance to plants. As primary producers, plants use sunlight as an energy source to drive the production of sugars from atmospheric CO_2. Sufficient light is thus essential for plant growth and development. At the same time, excessive light energy can damage plants through various mechanisms (Foyer, Lelandais, and Kunert 1994). Accordingly, perception of light quantity and quality has a big impact on many aspects of the plant's physiology. It is therefore unsurprising that light has emerged as an important modulator of BABA-IR in this thesis. Three aspects of light perception and signalling in particular have been investigated: phytochrome signalling, photorespiration and non-photochemical quenching.

Chapter 5 showed that the VOZ TFs are essential regulators of basal and BABA-primed callose defence. A previous report has demonstrated that VOZ2 directly interacts with the P_r form of the red/far-red light receptor phytochrome B (phyB), and proposed that translocation of VOZ proteins from the cytoplasm to the nucleus mediates the early steps of phyB signalling (Yasui et al. 2012). Modulation of BABA-IR by phyB is further suggested by the finding that high sowing densities (which reduce the red/far-red light ratio and lead to increased conversion of P_{fr} to P_r) weaken BABA-IR against *Hpa* (MBioSci thesis Emily Guest, 2017). This hypothesis became more unlikely by the finding that the *phyA/phyB* double mutant is not impaired in BABA-IR against *Hpa* (Chapter 5; Fig. 5.4C), suggesting that phyB signalling is not critical for BABA-IR. However, the severe pleiotropic effects of this double mutation makes it difficult to quantitatively compare levels of BABA-IR to the wild-type, and it cannot be excluded that the *phyA/phyB* double mutant is reduced in effectiveness of BABA-IR. Nevertheless, far-red light supplementation did not significantly alter levels of BABA-IR against *Hpa* (Chapter 5, Fig. 5.4B), although this could be due to differences in the physiological effect of horizontal versus vertical far-red light (C. L. Ballaré, Scopel, and Sánchez 1990). Interestingly, transcription factor binding motif enrichment analysis on the promoter regions of the 139 genes whose expression is specifically altered during expression of IBI1-dependent BABA-IR (Chapter 7, Fig. 7.3), 9 out of the 10 most significantly enriched TFs were direct or indirect interactors of phyB (Appendix 6), suggesting that phyB may still be an important modulator of BABA-IR.

Photorespiration, the light-dependent consumption of O_2 and production of CO_2 in plants, necessitates a complex cellular pathway to remove toxic intermediates and regenerate C3 skeletons for CO_2 assimilation. Using mutants impaired in a glycolate oxidase (GOX), a key H_2O_2-generating enzyme of the photorespiratory cycle, Chapter 6 demonstrates that photorespiration is required for full expression of BABA-IR. Since photorespiration is a major source of intracellular ROS (Noctor et al. 2002) and ROS are important second messengers of plant immunity (Sharma et al. 2012; Victoria Pastor et al. 2013), it is plausible that the role of photorespiration in BABA-IR is based on GOX-derived ROS.

Non-photochemical quenching (NPQ) is based on the interconversion of zeaxanthin, antheraxanthin and violaxanthin, and allows safe dissipation of excess light energy in the form of heat (Müller, Li, and Niyogi 2001). Chapter 7 revealed that NPQ is reduced during expression of IBI1-dependent BABA-IR against *Hpa* (Fig. 7.7). NPQ is directly linked to cellular redox regulation. Apart from the fact that insufficient NPQ under high-light conditions increases the production of singlet oxygen, conversion of violaxanthin to zeaxanthin is coupled to the oxidation of ascorbate to dehydro-ascorbate. In addition, the xanthophylls involved in NPQ are also direct precursors of the plant hormone abscisic acid (ABA), and regulation of the xanthophyll pool could thus contribute to BABA-IR by influencing light-dependent redox balance, ABA signalling, or both.

Plants generally prioritise shade avoidance and optimal light harvesting over pathogen defence (T. Griebel and Zeier 2008; Cerrudo et al. 2012). To mediate this trade-off, plants require mechanisms to link their light-dependent energy status to defence and modulate immune reactions accordingly. Photorespiration and NPQ are more prevalent under abundant light. Thus, it would make sense if plants have recruited these mechanisms to mediate stronger defence responses against attacking pathogens. The VOZ2-interacting P_r form of phytochrome B, on the other hand, is activated by conditions associated with low light and shading (Yasui et al. 2012; Casal 2013), and could be expected to dampen induced resistance responses. Interactions between phyB and VOZ2 have only been demonstrated to occur in the cytoplasm (Yasui et al. 2012), whereas VOZ2 needs to translocate to the nucleus in order to contribute to basal and BABA-induced resistance against downy mildew (Chapter 5, Fig. 5.3). Accordingly, it is possible that P_r retains VOZ2 in the cytoplasm, thereby suppressing immune reactions under shading conditions. Further research is needed to elucidate the molecular mechanisms that allow fine-tuning of primed defence to light availability. For example, it might be of interest to test whether overexpression of VOZ2-NLS reduces the negative effect of high seedling densities on BABA-IR, which would indicate that cytoplasmic retention of VOZ2 is indeed a regulatory mechanism to suppress disease resistance under shading conditions.

8.4 Translational value of BABA-IR for improving food security

Considering that BABA induces effective resistance in commercially important crops against economically damaging diseases, BABA-IR remains of interests for agricultural exploitation as a priming agent. Previous research into the molecular details of BABA perception has already demonstrated that the beneficial resistance response to BABA can be separated from the undesirable stress response that becomes particularly prevalent at high concentrations of the chemical (Luna et al. 2014). However, this study has raised a major concern with respect to commercial exploitation of BABA, by demonstrating that the chemical interferes with the activity of an aspartyl-tRNA synthetase (AspRS). In support of the earlier findings by Luna et al. (2014), Chapter 3 provides further evidence that BABA binds to the highly conserved L-Asp binding domain of AspRS enzymes, thus affecting their primary activity in protein synthesis. Aminoacyl tRNA synthetases are essential and evolutionarily ancient proteins, which are highly conserved between all extant species (O'Donoghue and Luthey-Schulten 2003). The possibility that BABA binds to proteins homologous to IBI1 is also confirmed in this thesis, since overexpression of At4g26870, which shares 74% amino acid identity with IBI1, can complement BABA-IR in the *ibi1-1* mutant (Chapter 3). Accordingly, it is very plausible that BABA will also bind to human AspRS, and cause metabolic disruption by activation of the GCN2 pathway. This concern is exacerbated by the slow metabolic turnover of BABA (Jakab et al. 2001), which may lead to chemical residues in BABA-treated plants. Alarmingly, Wilkinson et al. (2017) recently confirmed this concern in tomatoes from BABA-treated plants.

Chemical analogues of BABA that are less stable and/or faster metabolised in plant tissues might provide a safer route towards agricultural exploitation of IBI1-dependent defence. Alternatively, genetic modifications (both naturally-occurring and via genetic engineering) could lead to constitutively primed plants, abolishing the need for chemical treatments altogether. For example, overexpression of *IBI1* leads to augmentation of both basal and induced resistance (Chapter 3, Fig. 3.1; Luna et al. 2014). As discussed above, this could be a consequence of an altered ratio between IBI1 and L-Asp (Schwarzenbacher, Luna, and Ton 2014), but could also sensitise the plant for the extremely low levels of BABA accumulating during (a)biotic stress (Thevenet et al. 2017). Compared to other forms of induced resistance (e.g. SAR), BABA-IR protects plants against an exceptionally wide spectrum of stresses. Consequently, constitutively primed plants would not necessarily suffer from negative cross-talk between immune pathways, because the plants would retain their ability to gear defence responses against specific stressors. Manipulation of IBI1-interacting proteins and other components of BABA-IR downstream of IBI1 could thus produce crop varieties with improved resistance to biotic and abiotic stresses. Detailed knowledge about the role of light perception in stress resistance could allow for the development of optimised light regimes for plants grown in controlled environments. While further research is needed before these strategies can be optimised for commercial exploitation, the work presented in this thesis provides novel information for further development of plant immune priming as a reliable and safe strategy of crop protection.

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Appendix 1

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Sequences of IBI1^{SDM} and IBI1 – At4g26870 chimeric constructs

(Chapter 3)

IBI1^{SDM}

A point mutation resulting in **GLN308ALA** is predicted to disrupt the binding of IBI1 with the amino group of both Asp and R-BABA, according to modelling by the software package Discovery Studio.

The mutated IBI1 construct was generated by PCR from the plasmid pENTR-IBI1, using a Forward primer incorporating the mismatched nucleotides (CAG in wild-type *IBI1*, GCT in *IBI1*^{SDM}, shown in red below), and a Reverse primer without mismatches.

ATGTCGTCGGAATCTGAAATCCCGCCGTTGTCGTCATCAACCGCCGCAGCGGAGGAATCTGGAGAGAAGACC
AGCAAGAAAGCGGCTAAGAAGGAAGCTGCCAAGCTAGAGAAGTTAAGACGTCGTCAAGAACAAGAGGAAG
CAACGCGTCGAACAGCTTCGATCTCTCTGGAAGAGAATGACGAGTTTTCCAATAACTACGGCGACGTGACTCT
TACCGAGTTGCAATCGTCGGCGGATCCGAAAGCCGGGAAGTGGATAGAGGCTGTTGAGGGAAAGGAGTGG
ACCGATGTGAGCGATTTGGTGGAAGAGATGTTGGAATCAGAGGTTCTGATCAGAGGCCGAGTGCACACGAA
TCGTCCAACGTCTAACAAATTGGGGTTTGTGGTCTTGAGGGAGAGCGGATCAACTGTTCAGTGCGTGGTTAG
CCAATCAGAGAAGACCAAAGTAGGTGCCAACATGGTTAAATACCTCAAGCAGCTGAGTCGCGAATCCTTTGT
CGATGTTATCGGTGTCGTCACTCTCCCCAAGGAGCCGCTGACGGGAACTACGCAGCAGGTTGAAATTCAAGT
GAGAAAAGTGTACTGCATCAACAAATCCTTGGCCAAATTACCACTTAGTGTGGAGGATGCTGCTCGGAGTGA
AGCAGATATCGAAGCATCTCTTCAGACTCCATCTCCAGCTGCTCGTGTCAATCAGGATACACGTTTGAACTATA
GGGTGCTCGACCTCAGAACACCGGCTAATCAAGCCATCTTCCAGCTTCAGTACGAAGTCGAATATGCCTTCAG
AGAAAAATTACGATTTAAGAATTTTGTTGGAATCCACACACCAAAACTGATGGCTGGTAGTAGTGAAGGAGG
TTCTGCTGTATTTAGGTTGGAATACAAAGGGCAACCTGCTTGTCTAGCTCAGTCTCCTGCTCTCCACAAGCAAA
TGGCAATATGTGGTGACTTGCGACGTGTCTTTGAGGTAGGTCCTGTTTTCAGGGCCGAAGACTCCTTCACTCA
TAGACACCTGTGTGAATTCGTTGGTCTTGATGTGGAGATGGAGATTCGGAAACACTATTCTGAGATAATGGAT
CTTGTGGACGAGTTATTTGTGTTTATATTCACTAGTTTGAATGAGAGGTGCAAAAAAGAACTGCAAGCTGTTG
GAAAGCAATACCCATTTGAACCTTTGAAGTTTCTACCAAAAACATTGAGGTTAACGTTTGAAGAAGGGGTTCA
AATGCTTAAGGAAGCTGGTGTGGAGGTTGATCCTCTTGGCGATCTAAACACTGAATCTGAGAGAAAACTAGG
CCAGCTTGTATTGGAAAAGTACAACACTGAGTTCTATATTCTGCATCGGTATCCTAAGGCAGTTAGGCCTTTCT
ACACAATGACTTGTGCCGATAATCCTCTTTACAGCAACTCTTTTGATGTCTTCATCAGAGGTGAGGAGATCATA
TCAGGAGCTCAACGTGTCCATATCCCAGAAGTCTTGGAGCAACGTGCAGGAGAATGTGGCATTGATGTCAAG
ACAATATCCACATACATTGATTCATTCAGGTACGGTGCGCCTCTACACGGTGGATTTGGAGTGGGGCTGGAG
CGAGTGGTCATGCTTTTCTGTGCACTGAACAACATTCGGAAAACATCCCTCTTCCCTCGTGACCCTCAAAGGCT
TTCACCCTAA

Primers IBI1^{SDM}

FwSDM1: CTCCTGCTCTCCACAAGCAAATGGC

RvSDM1: ACTGAGCTAGACAAGCAGGTTG

IBI1-HM1 chimeras: Sequences derived from IBI1 are in plain text, sequences derived from At4g26870 are underlined

IBI1 CDS

Nucleotide sequence:

ATGTCGTCGGAATCTGAAATCCCGCCGTTGTCGTCATCAACCGCCGCAGCGGAGGAATCTGGAGAGAAGACC
AGCAAGAAAGCGGCTAAGAAGGAAGCTGCCAAGCTAGAGAAGTTAAGACGTCGTCAAGAACAAGAGGAAG
CAACGCGTCGAACAGCTTCGATCTCTCTGGAAGAGAATGACGAGTTTTCCAATAACTACGGCGACGTGACTCT
TACCGAGTTGCAATCGTCGGCGGATCCGAAAGCCGGGAAGTGGATAGAGGCTGTTGAGGGAAAGGAGTGG
ACCGATGTGAGCGATTTGGTGGAAGAGATGTTGGAATCAGAGGTTCTGATCAGAGGCCGAGTGCACACGAA
TCGTCCAACGTCTAACAAATTGGGGTTTGTGGTCTTGAGGGAGAGCGGATCAACTGTTCAGTGCGTGGTTAG
CCAATCAGAGAAGACCAAAGTAGGTGCCAACATGGTTAAATACCTCAAGCAGCTGAGTCGCGAATCCTTTGT
CGATGTTATCGGTGTCGTCACTCTCCCCAAGGAGCCGCTGACGGGAACTACGCAGCAGGTTGAAATTCAAGT
GAGAAAAGTGTACTGCATCAACAAATCCTTGGCCAAATTACCACTTAGTGTGGAGGATGCTGCTCGGAGTGA
AGCAGATATCGAAGCATCTCTTCAGACTCCATCTCCAGCTGCTCGTGTCAATCAGGATACACGTTTGAACTATA
GGGTGCTCGACCTCAGAACACCGGCTAATCAAGCCATCTTCCAGCTTCAGTACGAAGTCGAATATGCCTTCAG
AGAAAAATTACGATTTAAGAATTTTGTTGGAATCCACACACCAAAACTGATGGCTGGTAGTAGTGAAGGAGG
TTCTGCTGTATTTAGGTTGGAATACAAAGGGCAACCTGCTTGTCTAGCTCAGTCTCCTCAGCTCCACAAGCAAA
TGGCAATATGTGGTGACTTGCGACGTGTCTTTGAGGTAGGTCCTGTTTTCAGGGCCGAAGACTCCTTCACTCA
TAGACACCTGTGTGAATTCGTTGGTCTTGATGTGGAGATGGAGATTCGGAAACACTATTCTGAGATAATGGAT
CTTGTGGACGAGTTATTTGTGTTTATATTCACTAGTTTGAATGAGAGGTGCAAAAAAGAACTGCAAGCTGTTG
GAAAGCAATACCCATTTGAACCTTTGAAGTTTCTACCAAAAACATTGAGGTTAACGTTTGAAGAAGGGGTTCA
AATGCTTAAGGAAGCTGGTGTGGAGGTTGATCCTCTTGGCGATCTAAACACTGAATCTGAGAGAAAACTAGG
CCAGCTTGTATTGGAAAAGTACAACACTGAGTTCTATATTCTGCATCGGTATCCTAAGGCAGTTAGGCCTTTCT
ACACAATGACTTGTGCCGATAATCCTCTTTACAGCAACTCTTTTGATGTCTTCATCAGAGGTGAGGAGATCATA
TCAGGAGCTCAACGTGTCCATATCCCAGAAGTCTTGGAGCAACGTGCAGGAGAATGTGGCATTGATGTCAAG
ACAATATCCACATACATTGATTCATTCAGGTACGGTGCGCCTCTACACGGTGGATTTGGAGTGGGGCTGGAG
CGAGTGGTCATGCTTTTCTGTGCACTGAACAACATTCGGAAAACATCCCTCTTCCCTCGTGACCCTCAAAGGCT
TTCACCC

IBI1 fragment amplification:

FW = M13 FW: 5'-GTTGTAAAACGACGGCCAGTC-3', Tm = 61.3˚C

REV = M13 Rev: 5'- CCAGGAAACAGCTATGACCATG-3', Tm = 62.1˚C

HM1 CDS

Nucleotide sequence:

ATGGTGGGATCAGAGGTTTTGGAGGAATGTGGAGAAAAAATAAGCAAGAAAGAGTCAAAGAAGCGAGCCGCTAAACTGGAGAAGTTGCTCCGTAAACAGGAACGAGAAGAAGCCACATCATCATCTCTTTCTCTGGAGGAGGAGGATGAATCGTGTTCCAGCAACTACGGTGACGTGACTACTAACGAGTTGCAGTCGGCTGTTGAGGGAAAAGAGCTCACTGATGTGAGCAACTTGGTTGAAGAGATTGTGGGGTCAGAGGTTTCGATCAGAGGTCGACTGCACAAGAATCGCTTAGTCGGTACCAAATTGTTTGTGATCTTGAGGGAAAGTGGATTCACGGTTCAATGCGTGGTGGAGGAGACCAGAGTTGGTGCAAACATGATTAAATTTGTCAAGCAGCTGAGTCGTGAATCTGTTGTTGAGCTTATCGGTGTCGTCTCTCACCCTAAGAAGCCTCTCACAGGAACCACCCAGCAGGTTGAAATACATGTCAGAAAAATGTACTGCCTCAGCAGATCCTTGCCAAATTTACCACTTGTTGTGGAGGATGCTGCTCGTAGTGAATCAGATATTGAAAAATCTGGCAAGGATGGCAAACAAGCTGCTCGTGTCCTTCAGGACACACGTTTGAATAATAGGGTTCTTGACATCAGAACACCGGCTAATCAAGCCATCTTCCGTATTCAGTGCCAAGTCCAAATTGCGTTCAGAGAATACTTACAATCCAAGGGGTTTCTTGAAATCCACACACCGAAATTGATCGCTGGCAGTAGTGAAGGAGGTTCTGCTGTGTTTAGGTTGGACTACAAAGGGCAGCCTGCTTGTCTGGCTCAGTCTCCTCAGCTTCATAAGCAGATGGCGATATGTGGTGACATGCGACGCGTCTTTGAGGTTGGTCCTGTTTTCAGAGCTGAAGACTCCTTCACTCATAGACACCTGTGTGAATTCGTTGGTCTTGATGTGGAGATGGAGATTCGAATGCACTACTCTGAGATAATGGATCTTGTGGGGGAGTTGTTTCCGTTCATATTCACAAAAATAGAAGAAAGGTGCCCAAAGGAACTTGAATCTGTCAGAAAGCAATACCCTTTTCAATCTTTGAAGTTTCTTCCGCAAACATTGAGGCTAACCTTTGCAGAAGGGATTCAAATGCTTAAGGAAGCTGGCGAGGAGGTTGATCCTCTTGGTGATCTAAATACAGAATCTGAGAGGAAACTTGGCCAGCTTGTTCTGGAAAAGTACAAGACGGAGTTCTACATGCTGCATCGCTATCCATCGGCTGTCAGACCGTTCTACACTATGCCCTATGAAAATGATTCTAACTACAGCAACTCTTTCGATGTCTTCATCAGAGGAGAGGAGATCATGTCAGGAGCTCAACGTATCCATGACCCAGAACTCTTGGAGAAGCGCGCAAGAGAATGCGGCATTGATGTCAAGACAATATCCACGTACATTGATGCATTCAGGTACGGTGCACCACCTCACGGTGGATTCGGAGTGGGGCTGGAGCGTGTGGTAATGCTCTTATGTGCCCTCAATAACATCCGCAAAACTTCGCTATTCCCTCGTGACTCTCAAAGGCTCACTCCC

HM1 fragment amplification:

FW = M13 FW: 5'-GTTGTAAAACGACGGCCAGTC-3', Tm = 61.3˚C

REV = M13 Rev: 5'- CCAGGAAACAGCTATGACCATG-3', Tm = 62.1˚C

Chimera 1: 28% IBI1 - 72% HM1

Nucleotide sequence:

ATGTCGTCGGAATCTGAAATCCCGCCGTTGTCGTCATCAACCGCCGCAGCGGAGGAATCTGGAGAGAAGACCAGCAAGAAAGCGGCTAAGAAGGAAGCTGCCAAGCTAGAGAAGTTAAGACGTCGTCAAGAACAAGAGGAAGCAACGCGTCGAACAGCTTCGATCTCTCTGGAAGAGAATGACGAGTTTTCCAATAACTACGGCGACGTGACTCTTACCGAGTTGCAATCGTCGGCGGATCCGAAAGCCGGGAAGTGGATAGAGGCTGTTGAGGGAAAGGAGTGGACCGATGTGAGCGATTTGGTGGAAGAGATGTTGGAATCAGAGGTTCTGATCAGAGGCCGAGTGCACACGAATCGTCCAACGTCTAACAAATTGGGGTTTGTGGTCTTGAGGGAGAGCGGATCAACTGTTCAGTGCGTGGTTAGCCAATCAGAGAAGACCAAAGTAGGTGCCAACATGGTTAAATACCTCAAGCAGCTGAGTCGTGAATCTGTTGTTGAGCTTATCGGTGTCGTCTCTCACCCTAAGAAGCCTCTCACAGGAACCACCCAGCAGGTTGAAATACATGTCAGAAAAATGTACTGCCTCAGCAGATCCTTGCCAAATTTACCACTTGTTGTGGAGGATGCTGCTCGTAGTGAATCAGATATTGAAAAATCTGGCAAGGATGGCAAACAAGCTGCTCGTGTCCTTCAGGACACACGTTTGAATAATAGGGTTCTTGACATCAGAACACCGGCTAATCAAGCCATCTTCCGTATTCAGTGCCAAGTCCAAATTGCGTTCAGAGAATACTTACAATCCAAGGGGTTTCTTGAAATCCACACACCGAAATTGATCGCTGGCAGTAGTGAAGGAGGTTCTGCTGTGTTTAGGTTGGACTACAAAGGGCAGCCTGCTTGTCTGGCTCAGTCTCCTCAGCTTCATAAGCAGATGGCGATATGTGGTGACATGCGACGCGTCTTTGAGGTTGGTCCTGTTTTCAGAGCTGAAGACTCCTTCACTCATAGACACCTGTGTGAATTCGTTGGTCTTGATGTGGAGATGGAGATTCGAATGCACTACTCTGAGATAATGGATCTTGTGGGGGAGTTGTTTCCGTTCATATTCACAAAAATAGAAGAAAGGTGCCCAAAGGAACTTGAATCTGTCAGAAAGCAATACCCTTTTCAATCTTTGAAGTTTCTTCCGCAAACATTGAGGCTAACCTTTGCAGAAGGGATTCAAATGCTTAAGGAAGCTGGCGAGGAGGTTGATCCTCTTGGTGATCTAAATACAGAATCTGAGAGGAAACTTGGCCAGCTTGTTCTGGAAAAGTACAAGACGGAGTTCTACATGCTGCATCGCTATCCATCGGCTGTCAGACCGTTCTACACTATGCCCTATGAAAATGATTCTAACTACAGCAACTCTTTCGATGTCTTCATCAGAGGAGAGGAGATCATGTCAGGAGCTCAACGTATCCATGACCCAGAACTCTTGGAGAAGCGCGCAAGAGAATGCGGCATTGATGTCAAGACAATATCCACGTACATTGATGCATTCAGGTACGGTGCACCACCTCACGGTGGATTCGGAGTGGGGCTGGAGCGTGTGGTAATGCTCTTATGTGCCCTCAATAACATCCGCAAAACTTCGCTATTCCCTCGTGACTCTCAAAGGCTCACTCCC

IBI1 fragment amplification:

FW = M13 FW: 5'-GTTGTAAAACGACGGCCAGTC-3', Tm = 61.3˚C

REV = IBI-25N Rev: 5'-GATTCACGACTCAGCTGCTTG-3' ; 1 mismatch (A should be G)

HM1 fragment amplification:

FW = HM1-75C Fw: 5'- CAAGCAGCTGAGTCGTGAATC-3'; Tm = 61.3˚C

REV = M13 Rev: 5'- CCAGGAAACAGCTATGACCATG-3', Tm = 62.1˚C

Chimera 2: 50% IBI1 - 50% HM1

Nucleotide sequence:

ATGTCGTCGGAATCTGAAATCCCGCCGTTGTCGTCATCAACCGCCGCAGCGGAGGAATCTGGAGAGAAGACC
AGCAAGAAAGCGGCTAAGAAGGAAGCTGCCAAGCTAGAGAAGTTAAGACGTCGTCAAGAACAAGAGGAAG
CAACGCGTCGAACAGCTTCGATCTCTCTGGAAGAGAATGACGAGTTTTCCAATAACTACGGCGACGTGACTCT
TACCGAGTTGCAATCGTCGGCGGATCCGAAAGCCGGGAAGTGGATAGAGGCTGTTGAGGGAAAGGAGTGG
ACCGATGTGAGCGATTTGGTGGAAGAGATGTTGGAATCAGAGGTTCTGATCAGAGGCCGAGTGCACACGAA
TCGTCCAACGTCTAACAAATTGGGGTTTGTGGTCTTGAGGGAGAGCGGATCAACTGTTCAGTGCGTGGTTAG
CCAATCAGAGAAGACCAAAGTAGGTGCCAACATGGTTAAATACCTCAAGCAGCTGAGTCGCGAATCCTTTGT
CGATGTTATCGGTGTCGTCACTCTCCCCAAGGAGCCGCTGACGGGAACTACGCAGCAGGTTGAAATTCAAGT
GAGAAAAGTGTACTGCATCAACAAATCCTTGGCCAAATTACCACTTAGTGTGGAGGATGCTGCTCGGAGTGA
AGCAGATATCGAAGCATCTCTTCAGACTCCATCTCCAGCTGCTCGTGTCAATCAGGATACACGTTTGAACTATA
GGGTGCTCGACCTCAGAACACCGGCTAATCAAGCCATCTTCCAGCTTCAGTACGAAGTCGAATATGCCTTCAG
AGAAAAATTACGATTTAAGAATTTTGTTGGAATCCACACACCAAAACTGATGGCTGGTAGTAGTGAAGGAGG
TTCTGCTGTGTTTAGGTTGGACTACAAAGGGCAGCCTGCTTGTCTGGCTCAGTCTCCTCAGCTTCATAAGCAG
ATGGCGATATGTGGTGACATGCGACGCGTCTTTGAGGTTGGTCCTGTTTTCAGAGCTGAAGACTCCTTCACTC
ATAGACACCTGTGTGAATTCGTTGGTCTTGATGTGGAGATGGAGATTCGAATGCACTACTCTGAGATAATGG
ATCTTGTGGGGGAGTTGTTTCCGTTCATATTCACAAAAATAGAAGAAAGGTGCCCAAAGGAACTTGAATCTGT
CAGAAAGCAATACCCTTTTCAATCTTTGAAGTTTCTTCCGCAAACATTGAGGCTAACCTTTGCAGAAGGGATTC
AAATGCTTAAGGAAGCTGGCGAGGAGGTTGATCCTCTTGGTGATCTAAATACAGAATCTGAGAGGAAACTTG
GCCAGCTTGTTCTGGAAAAGTACAAGACGGAGTTCTACATGCTGCATCGCTATCCATCGGCTGTCAGACCGTT
CTACACTATGCCCTATGAAAATGATTCTAACTACAGCAACTCTTTCGATGTCTTCATCAGAGGAGAGGAGATC
ATGTCAGGAGCTCAACGTATCCATGACCCAGAACTCTTGGAGAAGCGCGCAAGAGAATGCGGCATTGATGTC
AAGACAATATCCACGTACATTGATGCATTCAGGTACGGTGCACCACCTCACGGTGGATTCGGAGTGGGGCTG
GAGCGTGTGGTAATGCTCTTATGTGCCCTCAATAACATCCGCAAAACTTCGCTATTCCCTCGTGACTCTCAAAG
GCTCACTCCC

IBI1 fragment amplification:

FW = M13 Fw: 5'-GTTGTAAAACGACGGCCAGTC-3', Tm = 61.3˚C

REV = IBI1-50N Rev: 5'-CAGCAGAACCTCCTTCACTAC-3' Tm = 61.3˚C

HM1 fragment amplification:

FW = HM-50C-Fw: 5'-GTAGTGAAGGAGGTTCTGCTG-3', Tm = 61.3˚C

REV = M13 Rev: 5'- CCAGGAAACAGCTATGACCATG-3', Tm = 62.1˚C

Chimera 3: 73% IBI1 - 27% HM1

Nucleotide sequence:

ATGTCGTCGGAATCTGAAATCCCGCCGTTGTCGTCATCAACCGCCGCAGCGGAGGAATCTGGAGAGAAGACC
AGCAAGAAAGCGGCTAAGAAGGAAGCTGCCAAGCTAGAGAAGTTAAGACGTCGTCAAGAACAAGAGGAAG
CAACGCGTCGAACAGCTTCGATCTCTCTGGAAGAGAATGACGAGTTTTCCAATAACTACGGCGACGTGACTCT
TACCGAGTTGCAATCGTCGGCGGATCCGAAAGCCGGGAAGTGGATAGAGGCTGTTGAGGGAAAGGAGTGG
ACCGATGTGAGCGATTTGGTGGAAGAGATGTTGGAATCAGAGGTTCTGATCAGAGGCCGAGTGCACACGAA
TCGTCCAACGTCTAACAAATTGGGGTTTGTGGTCTTGAGGGAGAGCGGATCAACTGTTCAGTGCGTGGTTAG
CCAATCAGAGAAGACCAAAGTAGGTGCCAACATGGTTAAATACCTCAAGCAGCTGAGTCGCGAATCCTTTGT
CGATGTTATCGGTGTCGTCACTCTCCCCAAGGAGCCGCTGACGGGAACTACGCAGCAGGTTGAAATTCAAGT
GAGAAAAGTGTACTGCATCAACAAATCCTTGGCCAAATTACCACTTAGTGTGGAGGATGCTGCTCGGAGTGA
AGCAGATATCGAAGCATCTCTTCAGACTCCATCTCCAGCTGCTCGTGTCAATCAGGATACACGTTTGAACTATA
GGGTGCTCGACCTCAGAACACCGGCTAATCAAGCCATCTTCCAGCTTCAGTACGAAGTCGAATATGCCTTCAG
AGAAAAATTACGATTTAAGAATTTTGTTGGAATCCACACACCAAAACTGATGGCTGGTAGTAGTGAAGGAGG
TTCTGCTGTATTTAGGTTGGAATACAAAGGGCAACCTGCTTGTCTAGCTCAGTCTCCTCAGCTCCACAAGCAAA
TGGCAATATGTGGTGACTTGCGACGTGTCTTTGAGGTAGGTCCTGTTTTCAGGGCCGAAGACTCCTTCACTCA
TAGACACCTGTGTGAATTCGTTGGTCTTGATGTGGAGATGGAGATTCGGAAACACTATTCTGAGATAATGGAT
CTTGTGGACGAGTTATTTGTGTTTATATTCACTAGTTTGAATGAGAGGTGCAAAAAAGAACTGCAAGCTGTTG
GAAAGCAATACCCATTTGAACCTTTGAAGTTTCTACCAAAAACATTGAGGTTAACGTTTGAAGAAGGGGTTCA
AATGCTTAAGGAAGCTGGCGAGGAGGTTGATCCTCTTGGTGATCTAAATACAGAATCTGAGAGGAAACTTGG
CCAGCTTGTTCTGGAAAAGTACAAGACGGAGTTCTACATGCTGCATCGCTATCCATCGGCTGTCAGACCGTTC
TACACTATGCCCTATGAAAATGATTCTAACTACAGCAACTCTTTCGATGTCTTCATCAGAGGAGAGGAGATCAT
GTCAGGAGCTCAACGTATCCATGACCCAGAACTCTTGGAGAAGCGCGCAAGAGAATGCGGCATTGATGTCAA
GACAATATCCACGTACATTGATGCATTCAGGTACGGTGCACCACCTCACGGTGGATTCGGAGTGGGGCTGGA
GCGTGTGGTAATGCTCTTATGTGCCCTCAATAACATCCGCAAAACTTCGCTATTCCCTCGTGACTCTCAAAGGC
TCACTCCC

IBI1 fragment amplification:

FW = M13 Fw: 5'-GTTGTAAAACGACGGCCAGTC-3', Tm = 61.3˚C

REV = IBI1-75N Rev: 5'- CTTCCTTAAGCATTTGAACCCCTTC -3', Tm = 64.1˚C

HM1 fragment amplification:

FW = HM1-25C Fw: 5'- GAAGGGGTTCAAATGCTTAAGGAAG-3', Tm= 64.1˚C (1 mismatch, G should be A)

REV = M13 Rev: 5'- CCAGGAAACAGCTATGACCATG-3', Tm = 62.1˚C

Chimera 4: 73%HM1 - 27% IBI1

Nucleotide sequence:

ATGGTGGGATCAGAGGTTTTGGAGGAATGTGGAGAAAAAATAAGCAAGAAAGAGTCAAAGAAGCGAGCCG
CTAAACTGGAGAAGTTGCTCCGTAAACAGGAACGAGAAGAAGCCACATCATCATCTCTTTCTCTGGAGGAGG
AGGATGAATCGTGTTCCAGCAACTACGGTGACGTGACTACTAACGAGTTGCAGTCGGCTGTTGAGGGAAAAG
AGCTCACTGATGTGAGCAACTTGGTTGAAGAGATTGTGGGGTCAGAGGTTTCGATCAGAGGTCGACTGCACA
AGAATCGCTTAGTCGGTACCAAATTGTTTGTGATCTTGAGGGAAAGTGGATTCACGGTTCAATGCGTGGTGG
AGGAGACCAGAGTTGGTGCAAACATGATTAAATTTGTCAAGCAGCTGAGTCGTGAATCTGTTGTTGAGCTTAT
CGGTGTCGTCTCTCACCCTAAGAAGCCTCTCACAGGAACCACCCAGCAGGTTGAAATACATGTCAGAAAAATG
TACTGCCTCAGCAGATCCTTGCCAAATTTACCACTTGTTGTGGAGGATGCTGCTCGTAGTGAATCAGATATTG
AAAAATCTGGCAAGGATGGCAAACAAGCTGCTCGTGTCCTTCAGGACACACGTTTGAATAATAGGGTTCTTG
ACATCAGAACACCGGCTAATCAAGCCATCTTCCGTATTCAGTGCCAAGTCCAAATTGCGTTCAGAGAATACTT
ACAATCCAAGGGGTTTCTTGAAATCCACACACCGAAATTGATCGCTGGCAGTAGTGAAGGAGGTTCTGCTGT
GTTTAGGTTGGACTACAAAGGGCAGCCTGCTTGTCTGGCTCAGTCTCCTCAGCTTCATAAGCAGATGGCGATA
TGTGGTGACATGCGACGCGTCTTTGAGGTTGGTCCTGTTTTCAGAGCTGAAGACTCCTTCACTCATAGACACC
TGTGTGAATTCGTTGGTCTTGATGTGGAGATGGAGATTCGAATGCACTACTCTGAGATAATGGATCTTGTGGG
GGAGTTGTTTCCGTTCATATTCACAAAAATAGAAGAAAGGTGCCCAAAGGAACTTGAATCTGTCAGAAAGCA
ATACCCTTTTCAATCTTTGAAGTTTCTTCCGCAAACATTGAGGCTAACCTTTGCAGAAGGGGTTCAAATGCTTA
AGGAAGCTGGTGTGGAGGTTGATCCTCTTGGCGATCTAAACACTGAATCTGAGAGAAAACTAGGCCAGCTTG
TATTGGAAAAGTACAACACTGAGTTCTATATTCTGCATCGGTATCCTAAGGCAGTTAGGCCTTTCTACACAATG
ACTTGTGCCGATAATCCTCTTTACAGCAACTCTTTTGATGTCTTCATCAGAGGTGAGGAGATCATATCAGGAGC
TCAACGTGTCCATATCCCAGAAGTCTTGGAGCAACGTGCAGGAGAATGTGGCATTGATGTCAAGACAATATC
CACATACATTGATTCATTCAGGTACGGTGCGCCTCTACACGGTGGATTTGGAGTGGGGCTGGAGCGAGTGGT
CATGCTTTTCTGTGCACTGAACAACATTCGGAAAACATCCCTCTTCCCTCGTGACCCTCAAAGGCTTTCACCC

HM1 fragment amplification:

FW = M13 Fw: 5'-GTTGTAAAACGACGGCCAGTC-3', Tm = 61.3˚C

REV = HM1-75N-Rev: 5'-CTTCCTTAAGCATTTGAACCCCTTC-3', (1 mismatch, C should be T)

IBI1 fragment amplification:

FW = IBI1-25C Fw: 5'- GAAGGGGTTCAAATGCTTAAGGAAG-3', Tm= 64.1˚C

REV = M13 Rev: 5'-CCAGGAAACAGCTATGACCATG-3', Tm = 62.1˚C

Chimera 5: 50% HM1 - 50% IBI1

Nucleotide sequence:

ATGGTGGGATCAGAGGTTTTGGAGGAATGTGGAGAAAAAATAAGCAAGAAAGAGTCAAAGAAGCGAGCCG
CTAAACTGGAGAAGTTGCTCCGTAAACAGGAACGAGAAGAAGCCACATCATCATCTCTTTCTCTGGAGGAGG
AGGATGAATCGTGTTCCAGCAACTACGGTGACGTGACTACTAACGAGTTGCAGTCGGCTGTTGAGGGAAAAG
AGCTCACTGATGTGAGCAACTTGGTTGAAGAGATTGTGGGGTCAGAGGTTTCGATCAGAGGTCGACTGCACA
AGAATCGCTTAGTCGGTACCAAATTGTTTGTGATCTTGAGGGAAAGTGGATTCACGGTTCAATGCGTGGTGG
AGGAGACCAGAGTTGGTGCAAACATGATTAAATTTGTCAAGCAGCTGAGTCGTGAATCTGTTGTTGAGCTTAT
CGGTGTCGTCTCTCACCCTAAGAAGCCTCTCACAGGAACCACCCAGCAGGTTGAAATACATGTCAGAAAAATG
TACTGCCTCAGCAGATCCTTGCCAAATTTACCACTTGTTGTGGAGGATGCTGCTCGTAGTGAATCAGATATTG
AAAAATCTGGCAAGGATGGCAAACAAGCTGCTCGTGTCCTTCAGGACACACGTTTGAATAATAGGGTTCTTG
ACATCAGAACACCGGCTAATCAAGCCATCTTCCGTATTCAGTGCCAAGTCCAAATTGCGTTCAGAGAATACTT
ACAATCCAAGGGGTTTCTTGAAATCCACACACCGAAATTGATCGCTGGCAGTAGTGAAGGAGGTTCTGCTGT
ATTTAGGTTGGAATACAAAGGGCAACCTGCTTGTCTAGCTCAGTCTCCTCAGCTCCACAAGCAAATGGCAATA
TGTGGTGACTTGCGACGTGTCTTTGAGGTAGGTCCTGTTTTCAGGGCCGAAGACTCCTTCACTCATAGACACC
TGTGTGAATTCGTTGGTCTTGATGTGGAGATGGAGATTCGGAAACACTATTCTGAGATAATGGATCTTGTGGA
CGAGTTATTTGTGTTTATATTCACTAGTTTGAATGAGAGGTGCAAAAAAGAACTGCAAGCTGTTGGAAAGCAA
TACCCATTTGAACCTTTGAAGTTTCTACCAAAAACATTGAGGTTAACGTTTGAAGAAGGGGTTCAAATGCTTA
AGGAAGCTGGTGTGGAGGTTGATCCTCTTGGCGATCTAAACACTGAATCTGAGAGAAAACTAGGCCAGCTTG
TATTGGAAAAGTACAACACTGAGTTCTATATTCTGCATCGGTATCCTAAGGCAGTTAGGCCTTTCTACACAATG
ACTTGTGCCGATAATCCTCTTTACAGCAACTCTTTTGATGTCTTCATCAGAGGTGAGGAGATCATATCAGGAGC
TCAACGTGTCCATATCCCAGAAGTCTTGGAGCAACGTGCAGGAGAATGTGGCATTGATGTCAAGACAATATC
CACATACATTGATTCATTCAGGTACGGTGCGCCTCTACACGGTGGATTTGGAGTGGGGCTGGAGCGAGTGGT
CATGCTTTTCTGTGCACTGAACAACATTCGGAAAACATCCCTCTTCCCTCGTGACCCTCAAAGGCTTTCACCC

HM1 fragment amplification:

FW = M13 Fw: 5'-GTTGTAAAACGACGGCCAGTC-3', Tm = 61.3˚C

REV = HM1-50N-Rev: 5'-CAGCAGAACCTCCTTCACTAC-3', Tm = 61.3˚C

IBI1 fragment amplification:

FW = IBI1-50C-Fw: 5'-GTAGTGAAGGAGGTTCTGCTG-3', Tm = 61.3˚C

REV = M13 Rev: 5'-CCAGGAAACAGCTATGACCATG-3', Tm = 62.1˚C

Chimera 6: 28%HM1 - 72% IBI1

Nucleotide sequence:

ATGGTGGGATCAGAGGTTTTGGAGGAATGTGGAGAAAAAATAAGCAAGAAAGAGTCAAAGAAGCGAGCCG
CTAAACTGGAGAAGTTGCTCCGTAAACAGGAACGAGAAGAAGCCACATCATCATCTCTTTCTCTGGAGGAGG
AGGATGAATCGTGTTCCAGCAACTACGGTGACGTGACTACTAACGAGTTGCAGTCGGCTGTTGAGGGAAAAG
AGCTCACTGATGTGAGCAACTTGGTTGAAGAGATTGTGGGGTCAGAGGTTTCGATCAGAGGTCGACTGCACA
AGAATCGCTTAGTCGGTACCAAATTGTTTGTGATCTTGAGGGAAAGTGGATTCACGGTTCAATGCGTGGTGG
AGGAGACCAGAGTTGGTGCAAACATGATTAAATTTGTCAAGCAGCTGAGTCGCGAATCCTTTGTCGATGTTAT
CGGTGTCGTCACTCTCCCCAAGGAGCCGCTGACGGGAACTACGCAGCAGGTTGAAATTCAAGTGAGAAAAGT
GTACTGCATCAACAAATCCTTGGCCAAATTACCACTTAGTGTGGAGGATGCTGCTCGGAGTGAAGCAGATATC
GAAGCATCTCTTCAGACTCCATCTCCAGCTGCTCGTGTCAATCAGGATACACGTTTGAACTATAGGGTGCTCG
ACCTCAGAACACCGGCTAATCAAGCCATCTTCCAGCTTCAGTACGAAGTCGAATATGCCTTCAGAGAAAAATT
ACGATTTAAGAATTTTGTTGGAATCCACACACCAAAACTGATGGCTGGTAGTAGTGAAGGAGGTTCTGCTGTA
TTTAGGTTGGAATACAAAGGGCAACCTGCTTGTCTAGCTCAGTCTCCTCAGCTCCACAAGCAAATGGCAATAT
GTGGTGACTTGCGACGTGTCTTTGAGGTAGGTCCTGTTTTCAGGGCCGAAGACTCCTTCACTCATAGACACCT
GTGTGAATTCGTTGGTCTTGATGTGGAGATGGAGATTCGGAAACACTATTCTGAGATAATGGATCTTGTGGA
CGAGTTATTTGTGTTTATATTCACTAGTTTGAATGAGAGGTGCAAAAAAGAACTGCAAGCTGTTGGAAAGCAA
TACCCATTTGAACCTTTGAAGTTTCTACCAAAAACATTGAGGTTAACGTTTGAAGAAGGGGTTCAAATGCTTA
AGGAAGCTGGTGTGGAGGTTGATCCTCTTGGCGATCTAAACACTGAATCTGAGAGAAAACTAGGCCAGCTTG
TATTGGAAAAGTACAACACTGAGTTCTATATTCTGCATCGGTATCCTAAGGCAGTTAGGCCTTTCTACACAATG
ACTTGTGCCGATAATCCTCTTTACAGCAACTCTTTTGATGTCTTCATCAGAGGTGAGGAGATCATATCAGGAGC
TCAACGTGTCCATATCCCAGAAGTCTTGGAGCAACGTGCAGGAGAATGTGGCATTGATGTCAAGACAATATC
CACATACATTGATTCATTCAGGTACGGTGCGCCTCTACACGGTGGATTTGGAGTGGGGCTGGAGCGAGTGGT
CATGCTTTTCTGTGCACTGAACAACATTCGGAAAACATCCCTCTTCCCTCGTGACCCTCAAAGGCTTTCACCC

HM1 fragment amplification:

FW = M13 Fw: 5'-GTTGTAAAACGACGGCCAGTC-3', Tm = 61.3˚C

REV = HM1-25N Rev: 5'-GATTCGCGACTCAGCTGCTTG-3' (1 mismatch, G should be A)

IBI1 fragment amplification:

FW = IBI1-75C Fw: 5'-CAAGCAGCTGAGTCGCGAATC-3', Tm = 63.3˚C

REV = M13 Rev: 5'- CCAGGAAACAGCTATGACCATG-3', Tm = 62.1˚C

Appendix 2

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List of *Hpa*-responsive genes

(Chapter 7)

The following list contains the genes that showed significant differences in expression values between (water-pretreated) mock- and Hpa-inoculated Col-0 on the microarrays (Welch's t-test; p ≤ 0.05; fold change ≥ ±2).

Genes upregulated in response to *Hyaloperonospora arabidopsidis*

	CHM1	CHM2	CHM3	CHP1	CHP2	CHP3
PCC1	4.2	4.8	6.0	11.3	11.1	10.1
ACD6	3.2	3.1	3.5	8.2	7.6	7.1
PCR1	3.1	3.7	4.0	8.3	7.6	7.4
TAT3	4.3	3.4	4.2	8.6	7.7	7.3
AT3G22235	3.3	4.1	4.0	7.9	7.5	6.6
WRKY70	6.1	5.8	6.3	10.0	9.6	9.1
AT1G73805	3.2	3.5	3.8	7.5	7.0	6.5
FAMT	3.9	3.5	2.8	7.6	6.8	6.1
AIG1	4.8	4.4	5.0	8.3	8.2	8.0
WAK1	2.3	2.4	2.4	6.3	5.9	5.1
GSTF7	4.7	4.5	5.1	8.2	8.3	7.8
AT1G24145	4.5	4.1	5.3	8.3	8.1	7.5
AT5G39670	3.2	3.4	3.7	7.0	6.8	6.4
AT1G51920	4.3	4.2	4.0	7.7	7.6	6.9
AT3G18250	4.4	4.2	4.7	8.0	7.6	7.5
XBAT34	4.1	4.7	4.6	8.0	7.8	7.3
AT5G03350	3.9	4.0	4.6	7.7	7.5	6.9
LURP1	3.6	1.9	2.8	6.7	5.6	5.4
AT1G09932	3.6	3.7	4.0	7.3	7.0	6.3
CRK7	2.8	3.1	1.9	6.2	5.9	5.0
AT1G76960	3.6	3.4	4.4	7.4	6.8	6.4
AT2G25510	7.7	7.2	8.4	11.0	10.8	10.5
AT5G42530	9.4	9.1	9.8	12.6	12.6	12.1
PUB54	3.0	2.5	3.1	5.9	6.6	5.0
AT1G57630	3.1	3.7	4.2	6.9	6.4	6.5
AT4G18250	5.0	5.0	4.8	8.1	8.1	7.4
PBS3	3.6	3.1	3.6	6.6	6.5	5.9
ALD1	3.1	3.7	3.0	6.4	6.6	5.5
NAC004	2.5	3.4	3.5	6.6	6.5	5.0

	CHM1	CHM2	CHM3	CHP1	CHP2	CHP3
AT5G39580	4.2	4.3	4.1	6.2	5.6	6.7
AT5G25250	3.5	3.5	4.1	6.1	5.6	5.2
ECS1	9.7	9.8	9.9	11.9	11.8	11.6
PNP-A	3.0	2.6	3.0	5.5	4.8	4.1
AT2G23680	4.5	5.2	4.6	6.8	7.0	6.2
RLP52	3.4	3.3	3.7	5.5	5.4	5.1
BG3	2.5	2.4	2.0	4.4	4.3	3.8
AT1G10340	3.6	3.8	3.7	5.9	5.6	5.3
PAD4	2.0	2.3	2.1	4.0	4.6	3.4
RTFL12	3.4	3.5	3.4	5.7	5.5	4.9
AT1G05675	1.6	2.5	2.2	4.2	4.0	3.8
AT5G05300	3.8	3.3	3.2	5.5	5.1	5.4
CYP71B23	3.1	2.8	3.5	5.3	5.1	4.7
FRK1	3.4	3.5	3.4	5.5	5.2	5.1
AT5G41740	4.7	5.0	5.5	7.2	7.1	6.5
CRK14	5.3	5.2	5.6	7.6	7.2	6.8
AT5G10760	3.2	3.3	3.7	5.6	4.9	5.1
RLP34	3.4	3.7	3.4	5.8	5.1	5.2
CNGC3	2.9	2.4	2.4	4.6	4.7	4.0
NAC042	3.7	3.7	3.4	5.9	5.5	4.9
AT4G32870	4.7	5.7	4.5	7.2	6.8	6.4
AT1G66880	5.8	6.3	6.1	8.3	8.0	7.4
AT3G47480	1.7	2.0	2.0	3.8	4.1	3.4
AT5G22520	2.4	2.2	2.0	4.2	3.8	4.0
AT2G28400	4.4	4.1	4.3	6.3	5.8	6.2
AT1G19020	2.9	3.0	2.7	5.0	4.9	4.2
AT3G57710	2.6	2.5	3.5	5.2	4.2	4.7
AT3G63380	2.9	2.5	3.5	5.1	4.8	4.5
RLK1	2.8	3.3	2.6	5.2	4.6	4.4

GSTF6	5.0	5.1	5.3	8.3	8.0	7.7
CYP79B2	4.0	3.6	3.1	6.9	6.3	6.1
AT1G35710	3.9	3.8	4.1	7.3	6.8	6.2
AT4G12490	4.2	4.9	6.2	7.8	7.6	8.4
AT5G19240	2.9	2.7	3.5	6.2	5.8	5.5
PDF1.2b	4.1	3.2	4.1	7.0	6.9	5.8
AT5G24200	2.4	2.2	3.0	5.2	5.4	5.2
GPT2	3.2	3.8	3.2	6.3	6.4	5.6
LHT7	3.0	2.3	2.9	5.6	5.4	5.4
NIT2	3.4	2.3	4.2	5.9	5.2	6.8
YLS9	3.6	4.0	3.4	6.5	6.2	6.3
MDHAR	4.3	4.8	4.9	7.9	7.4	6.8
AT1G44130	4.0	3.8	3.8	6.6	6.4	6.6
WRKY54	3.3	3.2	3.3	6.4	6.0	5.4
STZ	5.4	5.6	5.8	8.6	8.2	8.0
AT5G08760	2.6	2.3	2.3	5.5	5.1	4.4
AT2G22880	3.0	2.7	3.2	5.8	5.7	5.2
CCOAMT	2.7	2.5	2.4	5.4	4.9	5.0
PLA2A	7.0	6.9	7.3	9.8	9.9	9.2
scpl16	1.9	3.6	3.3	6.2	5.8	4.3
AT5G52750	1.5	1.6	2.3	4.7	4.4	3.9
AACT1	3.5	4.0	3.1	6.2	6.1	5.8
RLP41	2.8	3.8	2.8	6.4	5.4	5.0
ATAF2	5.3	5.6	5.4	8.2	7.9	7.5
EDA39	4.0	3.4	3.5	6.3	6.0	6.0
PR1	1.5	1.4	1.4	4.5	3.4	3.8
MYB51	5.6	5.7	5.9	8.3	8.3	8.0
AT4G11890	3.9	3.6	4.0	6.5	6.4	5.9
HR4	7.9	8.0	8.1	10.6	10.6	9.9
AT2G41090	6.0	5.8	6.1	8.8	8.7	7.6
NUDT6	3.6	4.1	4.4	6.5	6.7	6.0
AT3G16530	4.5	4.1	4.1	7.0	6.5	6.2
AT2G20142	3.3	3.5	3.2	6.0	5.7	5.4
AT1G59865	2.8	3.0	2.9	5.6	5.4	4.9
AT5G57510	3.5	3.8	3.0	6.2	5.4	5.8
AT5G44568	4.8	4.7	4.6	7.3	7.3	6.5
AT1G72920	3.5	3.7	4.0	6.6	6.1	5.5
AT2G18690	4.5	4.8	4.9	7.4	7.1	6.7
YLS5	4.0	4.0	4.3	6.6	6.3	6.3
GSTF2	7.6	7.9	8.1	10.4	10.3	9.9
PDR12	3.4	3.2	2.8	6.1	5.4	4.8
AT1G80160	5.0	4.8	5.0	7.4	7.0	7.3
GSTF3	2.6	3.2	3.5	5.6	5.5	5.2
MSRB8	1.7	1.8	2.3	4.6	4.6	3.4
AT5G48657	3.9	4.4	3.7	6.8	6.3	5.8
AT2G47130	3.5	4.3	4.1	6.7	6.3	5.6
NIT4	5.6	5.6	5.8	7.7	7.7	7.0
AT4G11000	3.6	4.1	3.1	5.7	5.8	4.8
AT1G72070	4.8	4.7	4.9	6.9	6.7	6.1
AT2G24600	4.0	4.0	4.3	6.1	6.0	5.6
ERF6	3.5	4.5	4.6	6.2	6.2	5.5
NAC036	2.8	2.7	2.9	4.8	4.6	4.3
AT4G15610	7.7	7.6	7.8	9.6	9.3	9.6
AT5G41750	3.8	2.8	2.9	5.3	4.8	4.7
AT1G72900	2.8	2.3	2.5	4.8	3.7	4.4
AT4G25070	4.5	4.5	4.2	6.5	6.4	5.6
WRKY45	4.5	4.7	4.9	6.4	6.7	6.3
PR4	7.7	7.4	7.5	9.4	9.4	9.1
AT4G00315	2.2	2.1	2.8	4.2	4.6	3.6
AT3G01290	7.9	7.9	7.7	9.8	9.6	9.2
CRK13	2.9	3.1	2.9	4.9	4.9	4.2
CZF1	2.6	2.5	2.7	4.7	4.2	4.1
CRK36	3.3	2.5	2.9	4.9	4.9	4.2
AT4G08770	4.8	4.3	4.3	6.4	6.4	5.8
NRT2.6	2.6	2.7	3.0	4.8	4.2	4.4
BGLU11	4.5	4.8	5.3	6.5	6.5	6.8
AT5G67340	4.0	4.4	4.1	6.1	5.8	5.7
AT1G74440	4.8	4.6	4.2	6.5	6.5	5.7
ERF2	5.7	5.6	5.6	7.7	7.6	6.8
AT5G59670	4.2	3.7	3.7	6.4	5.4	4.9
AT1G36622	3.0	3.1	3.1	5.3	4.3	4.7
NAC3	3.0	3.1	3.2	5.1	4.5	4.7
AT2G30140	6.8	6.7	7.0	8.7	8.4	8.5
AT4G16260	4.5	4.6	4.8	6.5	6.3	6.1
AT4G12290	4.9	4.6	5.1	6.4	6.8	6.5
AT1G15790	4.1	4.4	4.1	6.0	6.0	5.5
AT3G23550	4.4	4.2	4.1	6.0	6.1	5.6
PAD3	1.2	1.3	1.2	3.4	2.7	2.7
WAKL10	2.8	3.2	3.3	5.4	4.6	4.2
AT1G74360	3.1	3.4	3.5	5.3	5.2	4.4
AT5G05460	4.9	5.1	4.7	6.8	6.8	6.1
CYP89A9	5.6	5.5	6.0	7.3	7.0	7.8
AT4G12500	4.9	3.9	5.1	6.2	6.0	6.6
PCR2	2.4	3.6	3.6	4.7	5.2	4.6
AT2G23321	2.3	2.8	2.8	4.5	4.2	4.0
NUDT7	6.1	6.2	6.0	7.9	7.8	7.4
AT5G26690	1.8	1.5	2.0	4.0	3.2	2.9
AT4G01700	5.0	5.0	5.3	7.0	6.8	6.2
AT3G25610	3.9	3.9	3.8	5.9	5.6	4.9
RLP46	3.9	3.9	4.4	5.8	5.6	5.5
SYP122	5.6	5.7	5.5	7.5	7.3	6.7
EXO70E2	4.8	4.9	5.0	6.6	6.5	6.2

RLP38	2.8	3.7	3.4	5.9	5.6	5.1	**AT1G73810**	3.2	3.9	4.0	5.4	5.4	4.9
AT1G23840	2.2	3.1	2.6	5.5	4.8	4.3	**BCB**	10.3	10.3	10.5	11.9	11.8	12.0
AT1G78410	3.7	2.6	3.0	5.8	5.1	5.1	**PDF1.4**	6.4	6.0	6.7	7.9	7.5	8.3
DMR6	7.7	7.6	7.8	10.3	9.9	9.6	**CRK37**	2.6	2.5	2.5	4.4	3.9	3.8
AT5G51190	3.1	3.5	3.9	5.7	5.9	5.4	**AKN2**	5.1	5.0	5.2	6.8	6.7	6.3
EDS5	4.9	4.7	5.0	7.2	7.6	6.3	**AT4G22305**	3.8	4.1	3.9	5.9	5.6	4.9
AT2G32160	2.9	3.0	2.5	5.6	5.0	4.2	**RK3**	3.3	3.6	4.5	5.9	5.0	5.1
WRKY33	8.4	8.5	8.5	10.8	10.7	10.3	**TCH3**	7.9	8.4	8.2	9.9	9.8	9.2
CHI	3.1	3.0	2.7	5.5	4.6	5.2	**AT1G06020**	3.0	2.4	2.6	4.3	4.2	4.0
AT5G42830	4.9	5.1	4.9	7.4	7.1	6.7	**WRKY40**	6.3	6.5	6.6	8.2	8.1	7.7
AT1G72060	5.9	6.0	6.4	8.5	8.3	8.0	**AT2G43620**	4.4	3.4	4.0	5.3	5.5	5.5
RLP50	4.0	4.4	4.2	6.7	6.5	5.7	**AT2G46600**	4.8	5.2	4.8	6.3	6.6	6.4
WRKY53	3.3	3.2	3.6	5.8	5.7	5.0	**AT3G19010**	4.5	4.4	4.9	6.4	6.0	5.8
MC2	5.3	4.7	4.5	7.5	7.1	6.1	**NIMIN-2**	4.4	4.6	3.9	6.2	5.9	5.3
CYP72A8	3.9	4.1	4.7	6.4	6.4	6.2	**AT1G51890**	4.2	4.3	3.3	5.6	5.6	5.1
WRKY38	3.5	3.6	3.6	6.2	5.7	5.0	**AT1G13750**	4.5	4.5	4.6	6.4	6.0	5.7
FMO1	2.3	2.3	2.6	4.5	5.0	3.8	**AT5G09290**	3.4	3.6	2.6	4.8	4.6	4.6
SIB1	3.5	3.1	3.8	5.9	5.6	5.0	**CNGC13**	5.9	5.6	6.1	7.4	7.4	7.4
AT2G15042	4.0	4.1	4.6	6.6	6.4	5.8	**HLECRK**	5.2	5.5	5.9	7.1	6.9	7.0
CYP81F2	5.0	4.6	4.5	7.0	6.9	6.4	**AT1G19960**	4.1	4.5	4.1	5.8	6.0	5.4
PUB24	5.6	5.4	5.4	7.8	7.5	7.2	**MEK1**	5.6	6.3	6.3	7.7	7.6	7.3
CYP83B1	7.6	7.4	7.6	9.8	9.7	9.2	**UCP5**	4.4	4.2	4.5	5.9	5.8	5.9
COBL5	5.0	5.1	5.2	7.4	7.3	6.7	**TRX5**	6.1	6.4	6.2	8.0	7.7	7.5
MEKK3	5.1	5.1	5.1	7.3	7.3	6.8	**AT5G44575**	3.4	2.9	3.3	4.7	4.5	4.7
CBP60G	5.0	4.9	4.8	7.2	7.0	6.7	**AT5G52760**	1.2	1.1	1.4	3.3	2.3	2.5
FUT4	2.1	1.8	2.2	4.6	4.0	3.6	**AT5G64120**	6.8	7.1	7.4	8.6	8.5	8.5
EXT4	6.2	6.1	7.3	8.7	8.0	9.0	**CRK11**	5.1	5.0	4.6	6.7	6.3	6.0
AT5G24210	6.2	6.4	6.8	8.8	8.4	8.2	**AT2G23200**	4.4	4.3	4.9	6.3	6.1	5.7
RLP36	2.9	3.1	3.5	5.7	5.1	4.7	**pEARLI 1**	7.6	7.4	8.3	9.0	8.8	9.9
AT2G32200	3.4	4.3	3.2	5.4	5.6	5.8	**PLDGAMMA1**	5.3	5.2	5.6	7.2	6.7	6.5
AT3G57460	3.5	3.2	3.0	5.6	5.7	4.4	**CNGC10**	5.1	5.5	4.9	6.7	6.9	6.2
AT5G18470	5.2	5.2	5.3	7.5	7.3	6.8	**BIP3**	2.6	2.6	2.5	4.0	4.4	3.6
AT3G61280	2.9	3.3	3.0	5.5	5.0	4.6	**AT2G45220**	4.1	4.0	4.1	5.5	5.4	5.5
AT3G11340	4.1	3.7	3.6	5.8	5.8	5.7	**APK2A**	4.4	4.7	4.9	6.4	6.3	5.7
AT1G72520	4.5	4.6	4.4	6.7	6.4	6.3	**DIN11**	3.1	2.8	3.4	4.6	4.7	4.3
AT5G44585	5.6	5.9	5.1	7.9	7.6	7.0	**AT1G35210**	1.9	2.2	2.1	3.9	3.5	2.9
degLHT1	5.8	6.0	5.6	7.4	7.2	7.0	**AT1G30730**	4.2	4.1	3.9	5.5	4.8	5.4
AT1G66090	3.9	3.4	4.1	5.5	5.1	5.1	**AT5G39050**	4.7	4.7	5.6	6.2	5.9	6.4
CYP706A2	4.3	3.3	3.8	4.9	5.3	5.5	**AT1G49050**	5.5	5.8	5.6	7.0	6.8	6.6
AT3G47090	3.8	4.1	4.2	5.8	5.3	5.2	**AT5G25930**	2.8	2.9	3.0	4.2	4.0	4.0
AT4G23610	2.1	1.6	2.1	3.3	3.7	3.1	**AT4G19370**	3.4	3.2	3.6	4.8	4.9	4.0
PRB1	4.4	4.1	3.9	5.9	5.9	4.9	**PAP17**	3.8	3.5	3.4	4.8	5.0	4.4
AT4G37530	7.1	7.2	7.4	8.7	8.7	8.5	**DIC2**	3.6	3.9	3.4	5.3	4.7	4.3
AT2G42360	4.2	4.1	4.6	5.8	5.6	5.7	**AT3G59880**	3.8	4.1	3.9	5.1	5.0	5.1
MLO3	5.0	4.4	5.2	6.1	6.6	6.1	**AZI1**	8.3	8.1	8.6	9.4	9.4	9.7
LBD1	3.8	3.8	4.2	5.8	5.3	4.9	**AT5G42050**	8.8	9.1	9.2	10.2	10.2	10.2

AT3G15356	6.1	6.0	6.6	7.9	7.8	7.1	**AT5G39020**	6.6	6.6	6.2	8.1	7.7	7.2
PRXCB	5.2	5.1	5.5	6.7	6.5	6.7	**AT5G12340**	2.9	2.6	2.6	4.3	3.8	3.4
RLP23	4.3	3.6	3.7	5.5	5.4	4.8	**VSR7**	4.8	4.4	4.5	6.0	5.5	5.6
NAC6	5.4	5.1	5.4	6.3	6.8	6.9	**AT1G48320**	4.5	4.6	4.7	6.0	5.8	5.5
SOBIR1	5.5	5.8	5.9	7.3	7.1	6.9	**AT1G33950**	3.4	3.2	2.9	4.3	4.5	4.1
WRKY18	4.9	5.1	5.0	6.4	6.5	6.1	**AT1G55450**	6.6	6.6	6.9	8.1	8.0	7.5
MYB50	2.8	2.6	3.5	4.6	4.8	3.7	**WRKY62**	2.3	2.6	2.2	4.0	3.4	3.1
AT1G05575	2.8	2.9	3.4	4.2	4.7	4.3	**AT1G16260**	5.7	5.2	5.7	7.2	6.7	6.3
AT3G22060	7.6	7.5	7.7	9.1	9.0	8.8	**CNGC11**	3.3	3.6	3.3	4.8	4.6	4.2
YLS2	5.8	6.1	6.4	7.7	7.4	7.2	**AT1G66920**	4.2	4.1	4.6	5.4	5.3	5.5
AT3G29000	1.3	1.5	1.6	3.4	2.6	2.5	**MLO12**	6.0	6.7	6.1	7.7	7.4	7.1
AT1G56060	4.7	4.3	4.2	5.7	6.0	5.6	**BGAL4**	9.4	9.4	9.6	10.6	10.5	10.6
AT5G64510	5.1	4.7	5.2	6.9	6.2	6.0	**ACA11**	6.9	6.6	6.7	8.1	7.9	7.6
AT5G44570	3.4	3.4	3.0	4.5	4.9	4.4	**AT4G27460**	4.7	5.0	5.0	6.0	6.2	6.0
WAK2	6.2	6.3	6.4	8.0	7.6	7.5	**CAMBP25**	5.8	6.2	6.1	7.4	6.9	7.2
AT3G09405	3.4	4.1	3.6	5.6	5.0	4.6	**PTR3**	5.2	5.7	5.1	6.4	6.2	6.8
AT2G04400	5.8	5.7	5.7	7.4	7.1	6.7	**RHL41**	2.3	2.0	1.5	3.2	3.0	3.0
AT3G09020	2.4	2.5	2.8	3.9	3.6	4.3	**RLP21**	4.8	5.0	4.9	5.9	6.1	6.1
NAP	5.0	4.8	4.8	6.5	6.3	5.9	**IVD**	9.5	9.8	9.8	10.9	10.8	10.8
AT4G22470	4.9	4.9	5.8	6.3	6.0	7.3	**AT3G21080**	3.3	2.4	2.9	4.4	3.9	3.7
AMT1;1	6.2	6.4	6.3	7.9	7.7	7.3	**WRKY26**	6.5	6.8	6.7	7.7	7.9	7.8
EP1	3.5	3.3	2.8	4.6	4.5	4.6	**ADR1-L2**	6.4	6.3	6.7	7.8	7.6	7.4
RLP7	3.9	3.7	4.1	5.2	5.0	5.5	**SERK4**	7.1	7.3	6.9	8.4	8.2	8.0
NDR1	4.5	3.6	4.5	5.7	5.6	5.3	**CRK12**	4.7	5.3	5.4	6.3	6.5	6.0
RLP42	6.6	7.1	7.4	8.6	8.7	7.8	**AT3G16565**	4.5	4.1	4.4	5.6	5.5	5.2
AT2G39210	6.6	6.7	6.8	8.5	8.0	7.6	**LACS3**	5.3	5.5	6.0	6.9	7.0	6.3
AT5G35735	7.0	7.1	7.4	8.7	8.4	8.5	**AT2G37980**	4.0	3.9	4.0	5.4	4.8	5.0
AT4G11521	2.7	2.1	2.6	3.6	4.1	3.8	**AT5G54860**	5.0	5.4	5.0	6.2	6.5	6.0
CMPG1	4.0	4.2	4.0	5.6	5.2	5.4	**AT5G61610**	4.1	4.0	3.9	5.1	5.2	5.0
SOT16	7.4	7.2	7.0	8.6	8.6	8.3	**AT3G62950**	6.6	6.7	7.0	8.0	7.5	8.2
AT2G39518	4.1	4.0	4.1	5.8	5.0	5.4	**WAKL6**	5.5	5.3	5.4	6.8	6.3	6.4
AT1G08050	4.3	4.6	4.5	6.1	6.0	5.3	**PPDK**	7.0	7.3	7.2	8.5	8.4	8.0
CRK39	1.8	1.9	2.7	3.6	3.4	3.4	**AT1G48210**	6.0	6.7	6.6	7.9	7.3	7.4
ERF5	3.3	3.5	3.3	4.5	4.9	4.7	**ZCF37**	3.1	3.3	3.3	4.6	4.3	4.1
WRKY50	3.9	4.0	3.4	5.4	5.3	4.6	**EXO70B2**	4.2	4.1	3.8	5.3	5.5	4.6
AT1G13990	4.7	4.9	5.3	6.4	6.3	6.2	**AT1G31540**	4.3	4.3	4.4	5.8	5.2	5.3
AT3G48080	4.2	3.8	4.0	5.7	5.6	4.6	**CHX17**	3.1	3.2	3.2	4.3	4.6	3.9
ATBFRUCT1	6.9	7.1	7.4	8.7	8.3	8.4	**AT4G38540**	5.1	5.0	5.4	6.5	6.3	5.9
WRKY30	3.4	3.6	3.6	5.2	4.6	4.6	**CNBT1**	6.1	6.3	6.2	7.6	7.3	7.0
AT1G15125	10.7	10.1	10.4	11.7	12.0	11.4	**AT3G22600**	4.9	5.4	5.5	6.5	6.1	6.3
AT1G74300	3.8	3.8	3.6	5.0	5.5	4.6	**AT1G34750**	3.5	3.7	3.6	5.1	4.8	4.2
AT5G01100	4.9	4.4	4.5	6.0	5.9	5.8	**MTN3**	4.9	4.6	4.8	6.0	5.8	5.8
AT4G33150	6.4	6.6	6.6	7.8	7.6	8.2	**ACS6**	5.4	5.1	5.3	6.4	6.5	6.2
AT1G17147	5.2	5.3	5.7	6.5	7.0	6.5	**RBOHD**	5.9	5.9	6.0	7.2	6.8	7.0
AT1G14540	3.5	3.6	3.6	5.3	4.3	5.0	**AT1G27020**	6.0	6.1	6.1	7.2	7.5	6.7
AT1G30755	5.5	5.4	5.4	6.9	6.9	6.4	**AT5G16170**	5.0	5.1	5.1	6.2	6.5	5.8

WRKY6	6.3	6.6	6.7	8.0	7.6	7.7	**TSA1**	8.1	8.2	7.9	9.5	9.2	8.8
PUB23	6.8	6.8	6.7	8.2	8.1	7.8	**AT3G04210**	7.4	7.5	8.0	9.0	8.7	8.4
PCK2	4.0	4.1	4.8	5.8	5.3	5.6	**AT3G51890**	7.2	7.4	7.3	8.6	8.3	8.2
AT3G15518	2.4	2.1	3.0	4.3	3.6	3.5	**AT1G10140**	6.2	6.3	6.3	7.4	7.1	7.5
AT5G48540	3.3	2.4	3.2	4.4	4.2	4.2	**WAK3**	3.7	3.2	2.9	4.6	4.1	4.3
AT5G38900	4.4	3.6	3.6	5.6	5.0	4.9	**AT5G15870**	5.4	6.0	5.9	7.1	6.8	6.6
AT5G44990	2.6	2.4	1.8	3.7	3.7	3.2	**AT5G51830**	4.4	4.6	4.6	5.7	5.7	5.3
ACA1	5.3	5.4	5.4	6.6	7.0	6.3	**ANK**	3.4	3.7	3.6	5.1	4.7	4.2
ZAT6	2.0	1.8	2.0	3.5	3.2	3.0	**CNI1**	2.3	1.7	2.0	3.1	2.8	3.2
AT1G28190	5.3	5.7	5.1	7.0	6.5	6.5	**GSTL1**	2.3	2.5	2.1	3.8	3.2	3.1
AT1G21326	6.1	6.2	6.7	7.8	7.6	7.3	**CYP704A2**	5.2	5.6	6.0	6.8	6.6	6.7
ATL2	3.8	3.9	4.1	5.3	5.3	4.9	**SZF1**	5.0	5.0	4.8	6.2	6.0	5.9
NDA2	3.3	3.7	3.2	5.1	4.6	4.3	**AT4G26060**	5.2	5.9	6.2	7.1	6.7	6.7
AT1G07135	4.0	3.9	4.2	5.4	5.6	5.0	**SD1-29**	5.5	5.7	5.9	7.0	6.5	6.7
AT3G54150	2.4	2.8	2.7	4.4	3.5	3.8	**AT3G51440**	4.8	4.7	4.2	5.9	5.4	5.5
AT1G33030	3.4	3.5	2.9	5.0	4.0	4.6	**AT5G60280**	4.9	5.7	4.9	6.3	6.6	5.8
AT2G44290	3.5	3.6	3.6	5.2	4.7	4.5	**AT3G26840**	4.1	4.3	4.3	5.2	5.5	5.1
ASA1	7.7	7.8	7.8	9.4	9.2	8.5	**AT1G30720**	6.6	6.6	6.9	8.0	7.4	7.8
EDS16	4.9	5.2	5.6	6.5	6.6	6.4	**AT4G20000**	2.1	2.0	1.9	3.3	3.2	2.7
AT1G34420	4.3	3.7	3.7	5.3	5.1	5.1	**RLP37**	5.2	5.4	5.6	6.7	6.5	6.2
AT5G25440	6.5	6.6	6.5	8.1	7.9	7.3	**AT4G38560**	3.6	3.3	3.6	4.8	4.6	4.3
AT5G54710	5.5	5.2	5.5	6.7	6.8	6.4	**GDH1**	7.5	7.7	7.4	8.6	8.7	8.4
AT1G19380	6.2	6.3	6.4	7.6	7.5	7.5	**AT2G17740**	3.9	3.7	3.7	5.0	4.9	4.5
ARO4	2.9	3.1	2.9	4.3	4.5	4.0	**PFK3**	6.0	6.3	5.9	7.4	7.1	6.8
AT3G60420	3.7	3.7	3.2	5.1	4.8	4.5	**ADR1-L1**	7.0	7.4	7.4	8.5	8.3	8.2
AT1G03290	4.9	5.1	5.2	6.5	6.2	6.2	**RLK**	4.6	4.7	4.7	5.8	5.9	5.5
AIG2	3.9	3.5	4.0	5.1	5.1	4.9	**OPCL1**	6.2	6.2	6.7	7.6	7.6	7.0
AT1G58602	7.1	7.1	7.7	8.7	8.7	8.3	**AT1G70810**	3.7	3.8	4.1	4.8	5.0	4.9
cwINV6	3.2	3.5	3.4	4.6	4.7	4.4	**PGIP1**	6.2	6.2	6.3	7.3	7.1	7.5
MRP7	3.6	3.8	3.1	5.0	5.0	4.2	**AT5G39030**	4.4	4.8	5.0	6.3	5.6	5.4
CRK20	4.0	3.3	3.5	4.9	5.3	4.4	**AT4G15233**	5.4	5.3	5.5	6.7	6.2	6.4
WRKY8	3.5	3.4	3.6	4.7	4.7	4.9	**AT5G44582**	4.0	4.2	3.9	5.5	4.9	4.8
AT4G27300	5.1	4.8	5.3	6.5	6.5	5.8	**NHL3**	6.2	6.4	7.0	7.8	7.3	7.7
WRKY25	5.8	5.7	6.6	7.4	7.2	7.2	**ERF4**	7.3	7.2	7.6	8.3	8.4	8.5
CRK5	2.0	2.0	2.2	3.3	3.1	3.3	**GSR 1**	8.1	8.0	7.8	9.0	9.0	9.0
AT1G65500	4.5	4.1	5.0	5.9	5.7	5.6	**AT5G59330**	4.7	4.9	4.4	5.6	6.0	5.4
AT2G26440	6.4	6.6	6.8	8.1	7.8	7.5	**AT3G07195**	5.1	5.0	5.0	6.3	6.2	5.6
AT5G57010	2.8	2.8	2.8	4.1	4.2	3.7	**MPK3**	8.2	8.1	8.3	9.4	9.2	9.1
AT4G29780	3.9	4.5	4.4	5.5	5.5	5.4	**AT2G40600**	8.5	8.6	8.4	9.6	9.6	9.3
GSTU10	2.1	2.2	2.1	3.2	3.7	3.0	**AT1G61340**	2.7	3.4	3.2	4.3	4.3	3.8
MYB15	2.0	1.7	2.0	3.5	2.6	3.2	**AT2G38790**	2.9	3.5	3.3	4.5	4.4	3.9
RLP39	3.3	3.8	4.2	5.5	4.9	4.5	**AT2G40095**	4.2	4.8	4.2	5.7	5.4	5.1
AR781	7.0	6.6	6.8	8.2	8.0	7.8	**AT2G26190**	6.4	6.4	6.7	7.8	7.5	7.3
NRT1.8	5.1	4.9	4.6	6.3	5.8	6.0	**AAT1**	3.7	3.4	3.9	5.0	4.5	4.5
AT3G11402	4.6	4.6	4.5	6.1	5.8	5.5	**AT4G11170**	2.6	2.8	2.3	3.5	3.9	3.3
CML41	2.5	2.5	2.4	4.0	3.7	3.1	**AT3G02800**	4.1	3.6	3.6	5.1	4.8	4.4

AT5G46500	3.7	3.0	2.8	4.5	4.4	4.2	**ATHAL3B**	4.7	4.3	4.8	6.1	5.4	5.3
AT1G15040	5.6	5.9	5.5	7.3	6.6	6.6	**AT3G01175**	3.1	3.5	3.3	4.5	4.0	4.5
ALDH7B4	7.9	7.9	8.2	9.1	9.2	9.3	**GAD4**	4.0	4.1	4.1	5.1	4.7	5.4
AT5G10695	3.7	3.3	3.8	5.0	4.6	4.8	**AT5G46871**	3.7	2.9	3.5	4.3	4.5	4.2
JAZ1	7.0	6.9	7.0	8.3	8.0	8.1	**THA1**	8.6	8.7	8.6	9.7	9.7	9.5
AT5G28160	2.4	1.8	1.8	3.4	2.8	3.4	**AT1G50740**	7.3	6.9	7.1	8.3	8.2	7.9
AT1G69730	3.4	3.8	3.5	5.1	4.8	4.4	**MYB122**	3.0	3.1	2.9	4.3	4.1	3.6
ZIP11	5.3	5.3	5.2	6.5	6.9	6.0	**AT5G49680**	3.2	3.1	3.5	4.6	4.3	4.1
MSS1	7.9	8.1	8.3	9.5	9.3	9.0	**AT2G30550**	4.2	3.9	4.0	5.4	4.9	4.8
AT5G36930	5.4	5.4	5.5	6.8	6.2	6.2	**AT1G75000**	4.7	4.9	4.8	5.8	5.9	5.8

Genes downregulated in response to *Hyaloperonospora arabidopsidis*

	CHM1	**CHM2**	**CHM3**	**CHP1**	**CHP2**	**CHP3**		**CHM1**	**CHM2**	**CHM3**	**CHP1**	**CHP2**	**CHP3**
PPC3	6.1	5.9	5.8	5.3	4.7	4.7	**GH9C2**	7.9	8.3	8.5	6.6	6.8	7.7
AK3	5.0	4.9	4.9	3.6	3.9	4.3	**MS2**	9.5	9.8	10.1	8.3	8.6	8.7
AT2G27402	7.0	6.8	7.1	5.8	6.1	6.0	**ZIP8**	3.6	3.5	3.4	2.2	2.4	2.2
AT3G55750	6.6	6.4	6.7	5.8	5.2	5.7	**FRO2**	4.9	5.2	4.4	3.3	4.0	3.5
AT1G55990	4.7	4.8	4.2	4.0	3.4	3.3	**TOM7-2**	6.1	6.1	7.0	5.6	4.6	5.1
AT4G15680	6.0	6.4	6.1	5.5	4.9	4.9	**AT2G13422**	3.7	2.9	2.8	2.4	1.9	1.3
GRP4	8.6	9.1	8.6	7.7	7.7	7.7	**CYP83A1**	8.7	8.9	9.1	7.2	7.3	8.0
AT1G77530	4.5	4.2	4.1	3.5	2.7	3.4	**GSTU20**	7.5	7.5	7.6	5.8	6.1	6.4
AT4G39470	4.5	4.9	4.8	3.3	3.9	3.8	**IMD1**	5.7	5.5	6.0	4.2	4.2	4.4
EXPB3	7.5	7.4	7.6	6.2	6.2	6.8	**AT1G71740**	4.9	5.2	5.3	3.6	4.0	3.4
IDL5	5.3	5.9	5.7	4.4	4.3	4.8	**IPMI2**	5.0	5.0	5.2	3.3	3.5	3.9
AT5G09520	6.4	6.9	6.9	5.2	6.1	5.5	**NIA1**	8.1	8.4	8.3	6.3	7.0	7.0
AT3G54830	7.6	7.6	7.8	6.4	6.6	6.4	**XTR8**	5.7	6.1	6.4	4.6	3.9	4.9
AT3G46880	4.9	5.0	4.5	3.0	4.2	3.6	**BCAT4**	6.0	6.2	6.3	4.1	4.3	5.1
FLN2	7.1	7.4	7.2	5.9	6.1	6.1	**MYB29**	5.5	5.6	5.9	4.0	3.5	4.2
CRK30	5.1	5.5	5.0	4.1	4.3	3.6							

Appendix 3

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List of genes differentially expressed between Col-0 and *ibi1-1*

(Chapter 7)

The following four lists contain the genes that showed significant genotypic differences in expression values between Col-0 and *ibi1-1* on the microarrays (Welch's t-test; $p \leq 0.05$; fold change $\geq \pm 2$). Pairwise comparisons of Col-0 vs *ibi1-1* samples were performed for each condition tested (water-mock; BABA-mock; water-*Hpa*; BABA-*Hpa*).

water-treated and mock-inoculated

	CHM 1	CHM 2	CHM 3	**CHM avg**	CHP 1	CHP 2	CHP 3	**CHP avg**	CBM 1	CBM 2	CBM 3	**CBM avg**	CBP 1	CBP 2	CBP 3	**CBP avg**	IHM 1	IHM 2	IHM 3	**IHM avg**	IHP1	IHP2	IHP3	**IHP avg**	IBM 1	IBM 2	IBM 3	**IBM avg**	IBP1	IBP2	IBP3	**IBP avg**
ACD6	3.2	3.1	3.5	**3.3**	8.2	7.6	7.1	**7.6**	5.9	6.4	5.4	**5.9**	8.2	8.7	8.3	**8.4**	5.0	4.3	4.0	**4.4**	8.2	8.0	8.1	**8.1**	6.1	5.4	5.7	**5.7**	7.1	8.7	8.6	**8.1**
AT1G06020	3.0	2.4	2.6	**2.7**	4.3	4.2	4.0	**4.2**	3.8	3.5	3.1	**3.5**	4.2	3.6	4.0	**4.0**	4.1	3.7	4.1	**4.0**	4.1	4.4	3.8	**4.1**	3.1	4.0	2.9	**3.3**	3.3	3.5	3.8	**3.5**
AT1G14250	7.3	7.6	7.5	**7.5**	6.7	6.9	6.9	**6.8**	7.0	6.6	6.5	**6.7**	7.2	6.7	6.8	**6.9**	6.1	6.3	6.1	**6.2**	6.3	6.4	6.5	**6.4**	6.3	6.8	6.3	**6.5**	7.0	6.7	6.6	**6.8**
AT1G58050	2.3	2.0	1.8	**2.1**	2.4	2.5	2.4	**2.4**	2.1	2.6	2.4	**2.4**	2.6	2.1	1.8	**2.2**	3.4	2.9	2.9	**3.1**	2.9	1.8	1.9	**2.2**	1.9	2.3	2.4	**2.2**	1.8	2.0	2.1	**2.0**
AT1G76960	3.6	3.4	4.4	**3.8**	7.4	6.8	6.4	**6.9**	6.0	6.5	5.9	**6.1**	7.4	7.5	7.1	**7.3**	6.2	5.0	4.9	**5.4**	7.3	7.4	7.6	**7.4**	7.2	7.2	6.6	**7.0**	6.6	8.2	8.2	**7.7**
AT4G02230	8.1	8.6	8.2	**8.3**	8.3	8.4	8.0	**8.3**	8.4	8.0	8.4	**8.2**	8.1	8.3	7.9	**8.1**	6.3	6.3	6.3	**6.3**	6.2	6.2	6.1	**6.1**	6.1	5.6	6.0	**5.9**	5.9	6.0	5.8	**5.9**
AT4G17470	6.9	6.4	6.3	**6.5**	5.3	5.7	6.4	**5.8**	6.1	5.6	5.7	**5.8**	6.4	6.0	6.3	**6.2**	5.5	5.5	5.2	**5.4**	5.3	6.0	5.9	**5.7**	5.8	6.2	5.6	**5.9**	6.3	5.5	5.9	**5.9**
AT4G31180	8.7	9.1	9.1	**8.9**	9.2	9.1	9.0	**9.1**	9.1	9.1	9.4	**9.2**	9.3	9.3	9.3	**9.3**	7.6	7.9	7.1	**7.5**	7.3	7.8	7.6	**7.6**	7.7	7.6	7.3	**7.5**	7.6	7.7	7.6	**7.6**
AT5G42530	9.4	9.1	9.8	**9.4**	12.6	12.6	12.1	**12.4**	12.0	12.0	11.8	**11.9**	12.6	12.9	12.7	**12.7**	11.4	10.5	10.4	**10.8**	12.6	12.7	12.8	**12.7**	11.8	11.4	11.5	**11.6**	11.8	12.8	12.7	**12.4**
ENODL7	3.9	3.4	3.6	**3.6**	2.8	2.9	2.8	**2.8**	2.7	3.2	3.2	**3.0**	2.9	3.1	2.8	**3.0**	2.6	2.2	2.8	**2.5**	2.7	3.1	3.1	**2.9**	3.1	3.0	2.3	**2.8**	3.0	2.9	2.9	**2.9**
MRD1	3.5	3.2	2.9	**3.2**	2.2	3.2	3.0	**2.8**	3.2	3.3	2.7	**3.1**	3.2	3.2	2.7	**3.0**	6.1	5.8	5.7	**5.9**	5.7	5.9	5.7	**5.8**	5.4	5.8	6.1	**5.8**	6.0	5.9	5.8	**5.9**
RLP34	3.4	3.7	3.4	**3.5**	5.8	5.1	5.2	**5.4**	5.1	4.7	4.3	**4.7**	5.8	6.3	5.8	**6.0**	4.7	4.5	4.5	**4.6**	6.4	6.0	6.1	**6.1**	4.7	4.7	4.5	**4.6**	5.0	6.4	5.9	**5.8**
RTFL18	2.8	2.5	3.1	**2.8**	2.2	1.6	2.3	**2.0**	3.7	3.7	2.4	**3.3**	2.0	2.9	1.7	**2.2**	4.0	3.3	4.3	**3.9**	2.5	3.2	2.4	**2.7**	3.8	3.5	3.2	**3.5**	1.9	2.8	2.7	**2.4**
SP1L2	3.2	2.8	2.4	**2.8**	2.6	2.4	2.6	**2.5**	2.1	2.0	1.7	**1.9**	3.4	2.4	3.1	**2.9**	1.4	2.1	1.9	**1.8**	1.9	2.5	2.1	**2.1**	2.2	1.9	2.4	**2.2**	1.7	2.2	2.2	**2.0**

BABA-treated and mock-inoculated

	CHM 1	CHM 2	CHM 3	**CHM avg**	CHP 1	CHP 2	CHP 3	**CHP avg**	CBM 1	CBM 2	CBM 3	**CBM avg**	CBP 1	CBP 2	CBP 3	**CBP avg**	IHM 1	IHM 2	IHM 3	**IHM avg**	IHP1	IHP2	IHP3	**IHP avg**	IBM 1	IBM 2	IBM 3	**IBM avg**	IBP1	IBP2	IBP3	**IBP avg**
ABCB4	5.4	5.8	5.6	**5.6**	6.0	5.7	6.0	**5.9**	5.9	5.8	5.8	**5.8**	6.1	6.2	5.7	**6.0**	5.8	5.7	5.9	**5.8**	6.0	6.1	6.2	**6.1**	7.5	8.3	7.5	**7.7**	8.6	7.1	6.6	**7.4**
AGL12	2.4	2.4	2.2	**2.3**	3.0	1.7	2.5	**2.4**	2.4	1.9	1.7	**2.0**	3.0	2.0	2.3	**2.4**	2.4	2.5	2.3	**2.4**	2.2	2.1	2.0	**2.1**	2.9	3.3	3.1	**3.1**	3.1	2.8	2.2	**2.7**
AGP2	3.4	4.0	3.6	**3.7**	3.7	3.3	5.1	**4.0**	3.8	3.0	3.6	**3.5**	4.0	3.8	3.9	**3.9**	3.5	3.0	3.2	**3.2**	3.7	3.5	3.5	**3.6**	4.8	4.6	4.2	**4.6**	5.5	4.4	4.2	**4.7**
ALD1	3.1	3.7	3.0	**3.3**	6.4	6.6	5.5	**6.2**	3.4	3.4	3.8	**3.5**	6.7	7.3	6.2	**6.7**	3.9	3.5	2.7	**3.4**	7.1	6.9	6.7	**6.9**	5.4	5.5	4.9	**5.3**	5.4	6.2	6.5	**6.0**
AOX1A	4.6	4.6	4.9	**4.7**	5.5	5.3	5.9	**5.5**	5.1	5.0	5.7	**5.3**	5.8	6.1	5.7	**5.9**	5.1	4.8	4.9	**4.9**	5.7	5.9	5.9	**5.8**	6.3	6.9	6.3	**6.5**	7.8	6.4	6.5	**6.9**
APR1	4.3	3.9	4.6	**4.3**	4.7	4.7	4.4	**4.6**	4.4	4.1	4.8	**4.5**	5.0	4.8	4.9	**4.9**	3.9	4.2	4.0	**4.0**	4.6	5.0	5.0	**4.9**	5.2	6.0	5.6	**5.6**	5.7	5.2	5.1	**5.3**
ARI12	2.7	3.6	2.8	**3.0**	3.4	4.0	3.7	**3.7**	2.9	3.2	3.9	**3.3**	3.7	3.9	3.7	**3.8**	3.3	3.5	3.1	**3.3**	3.5	4.4	3.8	**3.9**	4.3	5.1	4.1	**4.5**	4.5	3.8	4.4	**4.2**
ARR15	5.7	6.0	6.2	**6.0**	5.4	5.3	5.9	**5.6**	6.0	5.6	5.9	**5.8**	5.5	4.7	5.9	**5.4**	6.1	5.7	5.6	**5.8**	5.6	5.8	5.1	**5.5**	4.5	4.7	4.9	**4.7**	4.2	5.2	5.5	**4.9**
ArthMp003	9.8	10.5	10.7	**10.3**	10.6	10.6	10.6	**10.6**	10.9	10.8	10.7	**10.8**	10.5	9.5	10.7	**10.3**	10.7	10.8	7.6	**9.7**	9.0	9.0	9.5	**9.2**	8.1	8.4	8.7	**8.4**	9.1	9.7	9.1	**9.3**
ASL5	3.4	3.7	3.1	**3.4**	3.0	2.3	3.0	**2.8**	3.3	2.9	3.6	**3.3**	3.3	3.0	3.3	**3.2**	3.4	3.5	3.0	**3.3**	3.0	2.6	2.5	**2.7**	5.5	4.4	5.0	**5.0**	4.6	3.2	3.5	**3.8**
AT1G05060	6.3	6.1	6.1	**6.2**	6.5	6.3	6.6	**6.4**	6.3	6.1	6.3	**6.2**	6.4	6.3	6.2	**6.3**	6.1	5.9	6.4	**6.2**	6.3	6.4	6.6	**6.4**	7.1	7.2	7.4	**7.3**	7.3	6.9	6.9	**7.0**
AT1G07560	2.2	2.9	2.4	**2.5**	1.9	1.7	2.5	**2.0**	2.2	1.5	2.2	**2.0**	2.5	1.9	2.7	**2.4**	3.5	2.4	2.5	**2.8**	2.9	2.5	2.2	**2.5**	3.4	2.9	2.8	**3.0**	2.5	1.9	2.6	**2.3**
AT1G13480	3.4	3.9	3.3	**3.5**	3.5	3.9	4.0	**3.8**	3.9	3.3	3.9	**3.7**	4.0	4.3	4.3	**4.2**	3.3	3.8	3.8	**3.6**	4.1	4.0	4.5	**4.2**	5.0	5.2	4.3	**4.8**	4.8	3.9	4.4	**4.4**
AT1G13830	4.3	4.9	4.0	**4.4**	5.4	4.8	5.0	**5.1**	4.5	4.2	4.7	**4.4**	4.9	5.3	4.9	**5.1**	4.9	4.7	4.6	**4.7**	5.2	5.3	5.3	**5.2**	6.0	5.5	5.5	**5.7**	5.6	5.0	5.0	**5.2**
AT1G15040	5.6	5.9	5.5	**5.7**	7.3	6.6	6.6	**6.8**	5.9	5.7	6.5	**6.0**	7.0	6.7	6.9	**6.9**	5.5	5.5	5.7	**5.6**	7.1	7.1	7.3	**7.2**	4.5	3.9	4.4	**4.2**	4.7	3.8	5.6	**4.7**
AT1G21120	1.9	3.5	3.9	**3.1**	5.7	5.6	4.5	**5.3**	2.4	3.8	2.7	**3.0**	4.8	5.9	4.8	**5.2**	3.1	3.0	2.9	**3.0**	6.7	5.8	5.5	**6.0**	4.7	5.8	4.7	**5.1**	6.1	5.5	5.6	**5.7**
AT1G27030	6.5	6.5	6.6	**6.5**	5.5	5.8	5.9	**5.7**	6.0	6.1	6.4	**6.2**	6.1	5.9	6.0	**6.0**	7.1	6.6	6.7	**6.8**	5.9	5.9	6.0	**5.9**	7.3	7.0	7.3	**7.2**	6.5	6.6	6.8	**6.6**
AT1G29020	3.8	4.7	3.8	**4.1**	4.0	3.8	3.0	**3.6**	3.5	3.5	4.5	**3.8**	4.3	3.9	3.6	**3.9**	5.1	4.0	4.3	**4.5**	2.9	3.9	3.6	**3.5**	6.7	6.0	6.3	**6.3**	5.1	4.8	4.3	**4.7**
AT1G29195	3.6	3.2	3.4	**3.4**	3.1	3.6	3.3	**3.3**	2.8	3.5	3.2	**3.2**	2.6	3.1	3.3	**3.0**	2.5	3.2	2.9	**2.9**	3.4	3.1	3.3	**3.2**	4.3	4.2	4.1	**4.2**	5.0	4.3	3.9	**4.4**
AT1G30720	6.6	6.6	6.9	**6.7**	8.0	7.4	7.8	**7.7**	6.8	6.8	7.2	**6.9**	8.0	7.9	7.5	**7.8**	6.3	6.7	6.7	**6.6**	8.5	8.0	7.9	**8.1**	5.6	5.2	5.9	**5.6**	6.8	6.3	6.8	**6.6**
AT1G30730	4.2	4.1	3.9	**4.1**	5.5	4.8	5.4	**5.2**	4.5	4.5	5.0	**4.7**	5.6	5.2	5.4	**5.4**	4.2	4.0	4.1	**4.1**	6.0	5.4	5.7	**5.7**	3.8	3.5	3.8	**3.7**	5.0	4.5	4.7	**4.7**
AT1G33750	4.4	4.4	3.6	**4.1**	3.7	3.7	3.5	**3.6**	4.0	3.1	4.3	**3.8**	3.8	3.7	4.7	**4.0**	4.2	4.0	4.2	**4.2**	3.7	3.4	3.5	**3.5**	6.5	5.2	5.4	**5.7**	4.7	4.1	4.1	**4.3**
AT1G34050	3.3	3.7	3.2	**3.4**	3.7	3.7	3.5	**3.6**	3.7	3.3	3.7	**3.6**	3.4	2.8	3.6	**3.3**	3.9	3.7	3.8	**3.8**	3.2	3.4	3.5	**3.4**	6.0	6.4	5.4	**5.9**	5.8	4.3	4.2	**4.7**
AT1G43910	2.1	2.4	2.4	**2.3**	3.4	3.6	2.6	**3.2**	2.3	2.8	2.6	**2.6**	3.1	3.1	3.1	**3.1**	2.4	2.1	2.3	**2.3**	3.7	3.6	3.8	**3.7**	3.9	5.1	4.0	**4.3**	5.2	4.0	4.0	**4.4**
AT1G48670	4.6	4.6	4.3	**4.5**	3.9	3.5	4.0	**3.8**	3.7	3.6	4.2	**3.8**	3.7	4.2	4.2	**4.0**	4.3	4.3	4.2	**4.3**	4.0	3.9	3.7	**3.9**	5.4	4.5	5.1	**5.0**	4.8	4.6	4.7	**4.7**
AT1G50040	5.3	4.9	4.9	**5.1**	4.9	4.3	4.9	**4.7**	4.7	5.1	5.2	**5.0**	4.5	4.3	4.7	**4.5**	4.9	4.8	5.1	**4.9**	4.8	4.5	4.2	**4.5**	3.7	4.0	3.9	**3.8**	4.3	3.2	3.3	**3.6**
AT1G51380	3.5	4.0	3.5	**3.7**	3.5	3.2	3.0	**3.3**	3.7	3.6	3.4	**3.6**	3.5	3.0	3.5	**3.3**	3.9	3.9	3.7	**3.9**	3.3	2.2	2.7	**2.7**	4.8	4.5	4.9	**4.7**	4.8	3.8	3.1	**3.9**
AT1G52060	3.0	3.4	2.9	**3.1**	4.9	3.3	2.9	**3.7**	3.1	1.8	3.1	**2.7**	3.2	4.3	3.7	**3.7**	4.6	3.3	4.3	**4.1**	3.1	2.3	3.0	**2.8**	6.8	4.3	6.1	**5.7**	3.0	2.8	2.9	**2.9**
AT1G54890	2.1	1.5	2.0	**1.9**	2.4	1.7	2.1	**2.1**	1.8	2.1	1.9	**1.9**	1.8	2.4	1.8	**2.0**	2.4	1.6	2.2	**2.1**	2.0	2.3	1.8	**2.0**	3.4	2.8	2.7	**3.0**	2.5	1.7	2.2	**2.1**
AT1G54950	2.8	1.9	1.8	**2.2**	1.9	2.7	1.8	**2.1**	1.6	1.6	1.8	**1.7**	1.8	1.8	1.8	**1.8**	1.8	2.0	2.8	**2.2**	2.0	2.4	1.8	**2.1**	4.4	3.3	3.1	**3.6**	2.2	1.9	2.0	**2.1**
AT1G56320	2.7	3.3	3.0	**3.0**	2.8	2.6	3.1	**2.8**	2.6	2.7	2.6	**2.7**	3.2	2.8	2.7	**2.9**	3.6	3.0	2.8	**3.1**	2.9	3.5	2.8	**3.1**	3.3	3.9	3.8	**3.7**	4.0	3.0	3.3	**3.4**
AT1G64590	4.9	5.3	4.9	**5.1**	4.4	3.9	4.6	**4.3**	4.3	3.9	4.7	**4.3**	4.3	4.4	4.3	**4.3**	5.7	4.9	5.0	**5.2**	4.4	4.3	4.1	**4.2**	6.0	5.6	5.4	**5.7**	5.5	4.0	4.6	**4.7**
AT1G64710	6.8	7.0	7.3	**7.0**	7.3	7.5	7.4	**7.4**	7.0	7.1	7.2	**7.1**	7.8	8.0	7.6	**7.8**	7.3	7.1	7.4	**7.3**	7.6	7.6	7.7	**7.6**	8.0	8.5	7.9	**8.1**	8.3	8.3	8.1	**8.2**
AT1G65845	8.0	8.1	8.3	**8.1**	8.9	8.7	8.7	**8.8**	8.2	8.4	8.5	**8.4**	8.8	9.3	9.1	**9.1**	8.4	8.3	8.2	**8.3**	9.3	9.2	9.1	**9.2**	7.2	7.1	7.2	**7.2**	8.5	8.3	8.6	**8.5**
AT1G67148	2.7	3.1	2.6	**2.8**	2.9	2.2	2.6	**2.6**	2.6	2.5	3.2	**2.8**	2.6	2.7	3.4	**2.9**	3.3	2.6	3.1	**3.0**	2.3	2.5	2.2	**2.3**	3.5	4.2	4.2	**3.9**	3.4	2.5	2.5	**2.8**
AT1G70260	2.2	2.6	2.3	**2.4**	2.5	2.0	2.2	**2.2**	2.6	2.4	2.3	**2.4**	2.3	2.3	2.8	**2.4**	2.6	2.2	2.6	**2.5**	2.2	2.2	2.3	**2.2**	3.9	3.5	4.1	**3.9**	2.8	3.6	3.1	**3.2**
AT1G72060	5.9	6.0	6.4	**6.1**	8.5	8.3	8.0	**8.2**	6.6	7.4	6.9	**7.0**	8.3	8.5	8.5	**8.4**	5.9	6.2	6.2	**6.1**	8.6	8.6	8.8	**8.7**	6.0	5.9	5.7	**5.9**	7.4	8.0	7.9	**7.8**
AT1G80160	5.0	4.8	5.0	**5.0**	7.4	7.0	7.3	**7.3**	4.9	5.6	5.7	**5.4**	6.0	6.3	7.0	**6.4**	4.9	5.2	4.8	**5.0**	7.3	7.4	7.8	**7.5**	3.8	4.7	4.2	**4.2**	7.2	5.2	6.0	**6.1**
AT2G04050	2.4	2.7	2.3	**2.4**	1.6	2.7	1.8	**2.0**	2.5	2.2	2.6	**2.4**	2.5	2.1	2.1	**2.3**	1.6	1.4	2.4	**1.8**	1.7	1.8	1.8	**1.7**	4.4	5.5	4.3	**4.7**	5.7	4.6	3.7	**4.7**
AT2G04070	2.6	2.6	2.5	**2.5**	3.1	3.0	2.6	**2.9**	2.4	2.6	3.2	**2.7**	3.5	3.8	3.2	**3.5**	2.9	3.0	2.1	**2.7**	3.1	3.9	3.4	**3.5**	5.3	6.2	5.6	**5.7**	6.5	4.6	3.8	**5.0**
AT2G05540	10.1	10.3	10.2	**10.2**	9.9	10.0	10.2	**10.0**	10.0	10.1	10.0	**10.0**	9.5	9.7	9.9	**9.7**	10.0	10.0	10.0	**10.0**	9.9	9.9	9.9	**9.9**	8.7	9.1	9.1	**9.0**	9.6	9.4	9.3	**9.4**
AT2G15780	1.8	2.0	2.4	**2.1**	2.5	2.5	2.5	**2.5**	2.0	2.4	2.6	**2.4**	2.5	2.4	2.1	**2.4**	1.8	2.4	1.9	**2.0**	2.1	2.2	1.9	**2.1**	3.3	4.2	4.3	**3.9**	3.2	3.1	2.3	**2.9**
AT2G17080	3.0	4.0	3.2	**3.4**	3.3	3.1	3.2	**3.2**	3.4	2.5	3.1	**3.0**	3.9	3.2	3.3	**3.5**	3.5	3.5	3.5	**3.5**	3.1	2.7	2.5	**2.8**	4.7	3.8	4.3	**4.3**	3.3	3.3	2.9	**3.2**
AT2G18150	2.8	3.8	3.3	**3.3**	3.1	4.4	3.1	**3.5**	2.8	3.4	3.7	**3.3**	3.5	2.1	4.0	**3.2**	3.2	3.4	3.6	**3.4**	3.6	3.4	3.3	**3.5**	4.5	5.7	5.0	**5.1**	4.6	3.4	3.3	**3.8**
AT2G18193	2.2	1.3	1.5	**1.7**	1.3	1.7	2.2	**1.7**	2.4	1.4	2.1	**2.0**	1.7	1.8	1.6	**1.7**	1.9	1.8	2.1	**1.9**	1.7	2.5	2.2	**2.1**	4.3	5.6	4.6	**4.8**	6.1	4.2	3.3	**4.5**
AT2G18270	7.5	7.6	8.0	**7.7**	8.2	8.1	7.7	**8.0**	7.5	7.9	7.8	**7.7**	8.1	7.9	7.9	**7.9**	8.0	7.9	6.3	**7.4**	7.2	7.5	7.5	**7.4**	6.7	5.4	6.4	**6.2**	6.7	7.6	7.5	**7.3**
AT2G18980	4.5	5.0	4.4	**4.6**	4.9	4.4	4.9	**4.8**	4.6	3.9	5.1	**4.5**	5.6	4.6	5.4	**5.2**	5.0	4.3	5.0	**4.8**	4.8	4.3	3.7	**4.3**	6.7	5.5	5.8	**6.0**	4.8	4.9	4.4	**4.7**
AT2G21640	4.1	4.1	4.0	**4.1**	3.9	4.0	3.9	**3.9**	4.1	3.6	4.1	**3.9**	4.1	4.4	4.5	**4.3**	4.2	4.1	4.1	**4.1**	4.6	4.6	4.2	**4.5**	6.0	6.7	6.3	**6.3**	7.5	6.1	5.5	**6.3**
AT2G23830	2.9	2.9	2.8	**2.9**	3.3	3.9	3.2	**3.5**	2.3	2.4	3.1	**2.6**	3.0	3.4	3.5	**3.3**	3.9	3.0	2.7	**3.2**	3.8	4.3	4.5	**4.2**	4.0	4.8	4.3	**4.4**	5.1	4.7	4.7	**4.8**
AT2G23960	5.6	5.8	5.9	**5.7**	5.0	4.9	5.0	**5.0**	5.5	4.2	5.5	**5.1**	5.8	5.0	5.4	**5.4**	5.6	5.3	5.8	**5.6**	5.5	5.3	4.7	**5.2**	7.4	6.4	6.4	**6.7**	5.2	4.9	5.2	**5.1**
AT2G30766	4.4	4.2	4.1	**4.2**	4.1	4.3	4.1	**4.2**	4.4	4.8	4.1	**4.4**	4.1	5.1	4.1	**4.4**	4.6	4.5	4.3	**4.5**	4.5	4.5	4.1	**4.3**	6.2	6.6	5.7	**6.2**	5.2	6.9	6.2	**6.1**

AT2G31390	5.8	6.0	6.3	**6.0**	6.1	6.3	5.9	**6.1**	6.3	5.8	6.1	**6.1**	6.3	6.6	6.5	**6.5**	6.1	5.7	6.0	**5.9**	6.7	6.8	6.4	**6.6**	7.4	6.9	6.9	**7.1**	7.3	6.8	6.7	**6.9**
AT2G35380	3.7	4.2	3.7	**3.9**	3.7	3.6	3.5	**3.6**	3.8	3.1	4.0	**3.7**	3.8	3.6	3.7	**3.7**	4.5	4.1	4.0	**4.2**	4.2	4.2	3.5	**4.0**	4.8	5.1	5.4	**5.1**	5.0	3.6	4.2	**4.3**
AT2G37800	3.1	3.3	2.5	**3.0**	3.1	2.9	2.9	**2.9**	2.8	3.0	2.9	**2.9**	3.1	2.8	2.9	**2.9**	2.7	3.2	3.2	**3.0**	2.7	2.6	2.9	**2.7**	4.7	4.6	4.3	**4.6**	4.0	3.2	3.8	**3.6**
AT2G38823	2.8	3.0	2.5	**2.8**	2.7	2.4	2.3	**2.5**	2.4	3.1	2.5	**2.7**	2.5	2.4	2.6	**2.5**	2.8	2.5	3.0	**2.8**	2.7	2.4	2.5	**2.5**	4.3	5.2	4.3	**4.6**	4.9	3.4	3.1	**3.8**
AT2G39510	2.7	2.1	2.4	**2.4**	2.2	2.5	2.4	**2.4**	2.4	2.4	2.0	**2.3**	2.7	3.3	2.8	**2.9**	2.1	2.5	2.2	**2.3**	2.0	2.3	2.7	**2.3**	3.8	3.6	4.7	**4.0**	3.5	3.6	4.1	**3.7**
AT2G40113	3.1	3.9	3.9	**3.6**	2.6	3.1	3.1	**2.9**	3.4	3.2	3.6	**3.4**	3.5	2.9	3.2	**3.2**	3.8	3.3	3.6	**3.6**	3.1	3.2	3.4	**3.3**	4.8	4.5	4.6	**4.6**	4.5	3.2	3.5	**3.7**
AT2G42360	4.2	4.1	4.6	**4.3**	5.8	5.6	5.7	**5.7**	5.0	5.3	5.6	**5.3**	6.0	6.1	5.9	**6.0**	4.7	4.9	4.6	**4.8**	6.6	6.1	6.3	**6.3**	4.2	3.9	4.3	**4.1**	6.4	5.1	5.8	**5.8**
AT2G47540	3.7	3.3	3.3	**3.4**	3.5	3.2	3.1	**3.2**	2.8	2.8	3.5	**3.0**	3.2	2.9	3.0	**3.1**	3.6	3.3	3.5	**3.5**	2.9	3.3	2.8	**3.0**	4.7	4.0	4.3	**4.3**	3.6	3.0	2.9	**3.2**
AT2G48080	3.3	3.7	3.5	**3.5**	2.9	2.9	3.5	**3.1**	2.8	2.5	3.7	**3.0**	2.9	3.2	3.0	**3.0**	3.6	3.6	3.5	**3.6**	3.4	3.6	3.7	**3.5**	4.7	4.7	3.9	**4.4**	4.1	3.7	4.0	**3.9**
AT2G48130	5.0	5.6	4.8	**5.1**	4.8	4.4	5.0	**4.7**	4.9	4.6	5.3	**4.9**	4.7	4.6	5.0	**4.8**	6.1	5.0	5.5	**5.5**	5.0	5.0	4.4	**4.8**	6.2	5.9	6.0	**6.0**	5.7	5.1	5.2	**5.3**
AT3G03060	4.2	5.1	4.9	**4.7**	4.3	4.5	4.4	**4.4**	4.3	4.7	5.1	**4.7**	4.8	4.9	4.6	**4.7**	5.0	4.8	5.4	**5.1**	4.3	4.5	4.7	**4.5**	6.0	5.3	6.0	**5.8**	6.0	4.7	4.8	**5.2**
AT3G09440	5.0	4.5	5.1	**4.9**	5.3	5.4	5.1	**5.3**	4.8	5.0	5.3	**5.0**	5.7	6.4	6.1	**6.1**	4.9	4.9	5.1	**5.0**	6.0	5.7	5.6	**5.8**	6.4	6.5	6.4	**6.4**	7.0	6.3	6.3	**6.5**
AT3G11340	4.1	3.7	3.6	**3.8**	5.8	5.8	5.7	**5.8**	4.2	4.6	5.1	**4.6**	6.4	7.1	6.2	**6.6**	4.3	4.4	4.1	**4.3**	6.4	6.8	6.4	**6.5**	7.1	7.6	7.0	**7.2**	6.8	6.8	6.5	**6.7**
AT3G12860	4.8	5.3	4.9	**5.0**	4.1	4.4	3.8	**4.1**	5.0	4.6	5.0	**4.9**	4.6	5.2	4.5	**4.7**	5.2	4.8	5.0	**5.0**	4.5	4.7	4.2	**4.5**	6.3	5.6	6.0	**6.0**	6.0	5.1	5.2	**5.4**
AT3G13432	3.2	3.5	3.2	**3.3**	3.6	3.1	3.6	**3.4**	3.2	2.9	3.4	**3.2**	2.9	3.4	3.6	**3.3**	3.1	2.9	3.1	**3.0**	4.0	3.5	3.5	**3.7**	4.7	5.3	4.1	**4.7**	4.9	3.1	4.3	**4.1**
AT3G14540	3.2	4.1	2.8	**3.4**	2.7	3.7	2.4	**2.9**	3.0	2.8	2.7	**2.9**	3.1	3.5	2.8	**3.2**	3.2	3.9	2.5	**3.2**	3.1	2.8	2.7	**2.9**	4.8	4.0	4.4	**4.4**	2.9	3.1	3.3	**3.1**
AT3G15440	5.6	5.9	6.1	**5.9**	5.8	6.3	6.5	**6.2**	5.9	5.9	5.6	**5.8**	5.7	5.7	6.2	**5.8**	5.7	5.9	5.9	**5.8**	6.5	6.4	6.3	**6.4**	4.9	4.6	4.8	**4.7**	5.1	5.4	5.1	**5.2**
AT3G19430	3.0	2.9	2.3	**2.7**	3.4	2.7	2.7	**2.9**	2.7	2.2	2.7	**2.5**	2.5	2.9	2.8	**2.7**	3.0	2.6	2.8	**2.8**	2.9	2.8	2.5	**2.7**	4.7	3.5	4.0	**4.1**	2.5	2.6	2.8	**2.6**
AT3G21930	2.0	3.8	2.6	**2.8**	2.5	2.9	3.3	**2.9**	2.1	2.6	2.8	**2.5**	3.9	3.3	2.1	**3.1**	3.2	3.3	3.2	**3.3**	2.8	2.5	3.0	**2.8**	3.2	3.8	4.0	**3.7**	3.2	2.7	2.7	**2.9**
AT3G22600	4.9	5.4	5.5	**5.2**	6.5	6.1	6.3	**6.3**	5.6	4.9	5.8	**5.4**	6.6	7.1	6.8	**6.8**	6.2	5.2	5.3	**5.6**	6.6	6.5	6.5	**6.5**	6.6	6.9	6.0	**6.5**	6.7	6.4	6.8	**6.6**
AT3G23175	4.0	5.0	3.8	**4.3**	5.0	3.7	4.5	**4.4**	4.6	3.4	4.9	**4.3**	4.9	4.5	4.7	**4.7**	4.9	4.6	5.0	**4.8**	4.5	4.4	3.4	**4.1**	6.7	5.4	5.9	**6.0**	4.7	3.8	4.7	**4.4**
AT3G23470	4.7	4.8	4.2	**4.6**	4.4	4.2	4.7	**4.4**	4.5	3.6	5.1	**4.4**	5.3	4.1	4.4	**4.6**	5.1	4.9	4.7	**4.9**	4.7	4.0	3.3	**4.0**	6.9	5.5	6.2	**6.2**	5.0	4.3	3.9	**4.4**
AT3G25610	3.9	3.9	3.8	**3.9**	5.9	5.6	4.9	**5.5**	4.5	4.6	3.9	**4.3**	6.0	6.1	5.8	**6.0**	4.6	4.6	4.8	**4.7**	6.1	6.0	6.0	**6.0**	5.3	6.1	5.4	**5.6**	6.7	6.0	6.4	**6.4**
AT3G27950	3.8	4.5	3.3	**3.9**	3.9	3.6	3.6	**3.7**	4.0	3.2	4.0	**3.7**	4.0	3.5	4.0	**3.8**	4.4	3.9	3.8	**4.0**	3.3	4.3	3.3	**3.6**	5.5	4.4	5.0	**5.0**	4.7	3.8	3.5	**4.0**
AT3G28510	2.9	2.8	2.4	**2.7**	3.9	2.9	3.1	**3.3**	1.7	2.9	2.8	**2.5**	3.8	3.5	3.0	**3.4**	2.6	2.3	2.6	**2.5**	4.2	3.9	4.1	**4.0**	5.0	5.3	5.1	**5.1**	5.1	3.4	3.7	**4.0**
AT3G28918	2.5	3.1	2.4	**2.6**	2.4	3.2	2.0	**2.5**	2.0	2.0	2.4	**2.2**	2.2	3.3	4.0	**3.2**	1.3	2.5	3.5	**2.4**	2.7	3.1	3.1	**3.0**	3.6	3.5	2.8	**3.3**	2.6	2.9	3.2	**2.9**
AT3G43850	5.0	5.0	5.2	**5.1**	5.1	5.0	4.8	**5.0**	5.0	5.4	4.7	**5.1**	4.6	4.3	4.8	**4.6**	5.1	4.7	4.9	**4.9**	5.3	4.7	4.7	**4.9**	3.9	3.8	4.1	**4.0**	3.9	4.2	4.0	**4.0**
AT3G48020	3.9	3.6	4.0	**3.8**	4.5	4.2	4.3	**4.3**	3.5	3.5	3.9	**3.6**	4.4	4.8	4.5	**4.6**	4.3	3.2	3.6	**3.7**	5.1	4.6	4.9	**4.9**	4.9	5.0	4.4	**4.7**	5.5	4.8	5.1	**5.1**
AT3G48390	6.5	6.6	6.7	**6.6**	6.6	6.3	6.4	**6.5**	6.0	6.6	6.6	**6.4**	6.5	6.1	6.5	**6.4**	6.1	6.0	6.6	**6.2**	6.4	6.4	6.3	**6.4**	5.2	5.5	5.3	**5.3**	5.2	5.5	5.6	**5.4**
AT3G48450	3.3	2.8	3.3	**3.1**	3.7	3.8	3.0	**3.5**	3.2	2.5	3.4	**3.0**	2.8	3.0	3.2	**3.0**	3.7	3.3	3.5	**3.5**	3.3	3.8	3.5	**3.5**	4.4	5.0	3.8	**4.4**	4.8	3.5	3.7	**4.0**
AT3G50400	2.9	3.5	3.0	**3.2**	3.0	2.9	3.1	**3.0**	3.1	2.8	2.4	**2.8**	3.0	2.9	2.7	**2.9**	3.4	3.3	3.7	**3.5**	2.9	3.1	2.8	**3.0**	4.2	4.3	4.2	**4.2**	4.1	3.2	2.8	**3.3**
AT3G51350	5.3	5.2	5.1	**5.2**	4.8	4.6	4.3	**4.6**	4.7	4.6	4.7	**4.7**	4.7	4.9	4.6	**4.7**	5.5	4.8	5.4	**5.2**	4.6	4.9	4.6	**4.7**	5.5	5.8	6.1	**5.8**	4.9	4.3	4.9	**4.7**
AT3G53230	4.1	4.4	4.9	**4.5**	4.1	4.4	4.7	**4.4**	4.4	4.3	4.5	**4.4**	5.1	5.3	4.9	**5.1**	4.9	4.2	4.3	**4.5**	5.2	5.2	5.1	**5.2**	5.9	5.4	5.7	**5.7**	6.4	5.8	5.1	**5.8**
AT3G54520	1.9	1.5	1.9	**1.8**	2.0	1.8	1.8	**1.9**	1.7	2.2	1.7	**1.8**	2.0	1.5	1.9	**1.8**	1.4	2.0	1.4	**1.6**	2.2	1.9	1.9	**2.0**	3.5	3.8	3.4	**3.6**	3.0	2.1	2.6	**2.6**
AT3G54770	4.8	5.1	4.5	**4.8**	4.4	4.8	4.5	**4.6**	4.8	4.1	5.3	**4.7**	5.0	4.9	4.9	**5.0**	5.0	5.2	5.4	**5.2**	4.6	4.8	4.5	**4.6**	7.0	5.9	6.3	**6.4**	5.2	4.9	4.6	**4.9**
AT3G55090	3.1	2.7	2.9	**2.9**	2.9	2.8	3.1	**2.9**	2.4	2.5	2.5	**2.5**	3.3	2.6	2.9	**2.9**	2.9	3.0	2.6	**2.8**	2.7	3.1	2.6	**2.8**	3.7	4.4	3.9	**4.0**	4.2	3.3	3.0	**3.5**
AT3G60270	3.7	3.3	3.7	**3.6**	3.5	3.6	3.9	**3.7**	3.1	3.4	3.6	**3.4**	3.6	3.1	3.4	**3.4**	3.6	3.5	3.8	**3.6**	3.0	2.8	3.6	**3.1**	4.6	4.3	4.2	**4.4**	3.0	3.0	3.5	**3.2**
AT3G62460	2.7	2.8	2.9	**2.8**	2.5	3.2	2.6	**2.8**	2.2	2.5	2.6	**2.4**	3.4	3.0	3.1	**3.2**	2.4	2.7	2.6	**2.6**	3.0	3.1	3.2	**3.1**	3.5	3.6	3.4	**3.5**	3.5	2.5	2.9	**3.0**
AT3G62950	6.6	6.7	7.0	**6.8**	8.0	7.5	8.2	**7.9**	6.9	7.0	7.0	**7.0**	7.8	7.6	7.9	**7.7**	7.2	7.1	7.0	**7.1**	7.1	7.4	7.5	**7.4**	6.2	5.5	5.5	**5.7**	5.0	6.3	6.7	**6.0**
AT3G63380	2.9	2.5	3.5	**3.0**	5.1	4.8	4.5	**4.8**	2.6	3.1	3.0	**2.9**	4.8	5.2	5.0	**5.0**	3.5	3.0	2.4	**3.0**	5.3	5.2	5.0	**5.2**	3.9	4.8	3.9	**4.2**	5.4	4.9	4.5	**4.9**
AT4G00700	4.9	5.1	5.0	**5.0**	6.0	5.7	5.4	**5.7**	4.8	4.5	5.1	**4.8**	5.8	5.8	5.8	**5.8**	5.4	4.7	5.0	**5.0**	6.8	6.4	6.8	**6.7**	6.1	6.2	5.7	**6.0**	6.1	6.0	6.2	**6.1**
AT4G00940	4.5	4.5	3.7	**4.2**	3.7	4.2	4.4	**4.1**	3.8	3.1	4.2	**3.7**	4.8	3.1	3.6	**3.8**	4.0	4.3	4.2	**4.2**	4.3	4.7	4.2	**4.4**	5.1	5.0	4.7	**4.9**	4.8	4.3	4.4	**4.5**
AT4G01380	4.5	3.9	3.9	**4.1**	4.1	3.3	3.1	**3.5**	3.4	3.1	4.1	**3.5**	3.7	4.0	3.2	**3.6**	3.3	3.3	3.5	**3.4**	3.2	2.9	3.6	**3.2**	4.4	4.9	4.7	**4.7**	4.2	5.2	4.8	**4.7**
AT4G01390	5.8	5.6	4.5	**5.3**	5.6	4.9	4.7	**5.0**	4.7	4.7	5.0	**4.8**	5.2	5.4	5.6	**5.4**	4.8	4.5	5.1	**4.8**	4.5	5.0	5.2	**4.9**	5.9	6.5	6.4	**6.3**	5.7	6.7	6.6	**6.4**
AT4G01700	5.0	5.0	5.3	**5.1**	7.0	6.8	6.2	**6.7**	5.5	5.7	5.9	**5.7**	7.6	7.5	6.8	**7.3**	5.5	5.7	5.3	**5.5**	7.3	7.1	7.1	**7.2**	6.8	6.8	6.7	**6.8**	7.5	7.5	7.3	**7.4**
AT4G02230	8.1	8.6	8.2	**8.3**	8.3	8.4	8.0	**8.3**	8.4	8.0	8.4	**8.2**	8.1	8.3	7.9	**8.1**	6.3	6.3	6.3	**6.3**	6.2	6.2	6.1	**6.1**	6.1	5.6	6.0	**5.9**	5.9	6.0	5.8	**5.9**
AT4G02830	4.2	4.6	4.1	**4.3**	4.0	4.0	4.3	**4.1**	4.2	4.3	4.2	**4.2**	4.2	3.6	4.4	**4.1**	4.3	4.7	4.3	**4.4**	3.8	4.2	3.8	**4.0**	5.4	5.4	5.3	**5.3**	5.3	3.9	4.0	**4.4**
AT4G03292	3.2	3.2	2.8	**3.1**	3.1	3.1	3.1	**3.1**	3.2	3.1	2.9	**3.0**	2.6	4.1	3.0	**3.2**	2.6	2.9	3.0	**2.8**	3.2	3.1	3.0	**3.1**	4.4	4.2	4.1	**4.2**	3.7	3.2	3.4	**3.4**
AT4G05370	3.5	3.3	3.1	**3.3**	3.2	3.2	3.3	**3.2**	3.7	3.0	3.1	**3.3**	3.2	3.0	3.1	**3.1**	3.1	3.5	3.6	**3.4**	3.2	3.1	3.0	**3.1**	6.0	5.6	5.5	**5.7**	6.6	4.5	4.5	**5.2**
AT4G05380	3.4	2.2	2.7	**2.8**	2.7	2.6	2.5	**2.6**	2.3	2.4	2.1	**2.2**	2.4	2.5	2.4	**2.4**	2.5	2.5	2.0	**2.3**	2.7	2.4	3.0	**2.7**	3.3	4.1	3.6	**3.7**	4.1	3.1	2.7	**3.3**
AT4G08770	4.8	4.3	4.3	**4.5**	6.4	6.4	5.8	**6.2**	5.4	5.1	5.6	**5.3**	6.7	7.3	6.9	**7.0**	5.4	4.8	4.8	**5.0**	6.7	6.7	6.5	**6.6**	7.1	7.0	7.4	**7.2**	7.4	6.7	6.8	**7.0**
AT4G08780	3.8	3.8	3.4	**3.7**	3.4	3.9	3.3	**3.5**	4.0	2.8	3.9	**3.6**	4.2	3.2	4.7	**4.1**	4.6	3.6	3.8	**4.0**	4.3	4.5	3.8	**4.2**	6.0	6.1	6.4	**6.2**	6.0	5.3	4.8	**5.4**
AT4G10500	3.6	3.3	3.4	**3.4**	3.6	3.7	3.5	**3.6**	3.8	3.8	3.4	**3.7**	4.5	4.1	3.9	**4.2**	3.2	3.5	3.5	**3.4**	5.0	4.5	4.6	**4.7**	4.8	5.0	5.5	**5.1**	5.2	5.1	4.8	**5.0**
AT4G12490	4.2	4.9	6.2	**5.1**	7.8	7.6	8.4	**8.0**	8.1	7.8	8.9	**8.3**	7.2	8.0	7.5	**7.6**	6.6	6.9	5.3	**6.3**	9.4	8.7	8.8	**9.0**	3.5	4.8	4.3	**4.2**	8.4	7.1	7.3	**7.6**
AT4G12500	4.9	3.9	5.1	**4.6**	6.2	6.0	6.6	**6.3**	6.7	6.7	8.3	**7.2**	5.8	6.7	6.4	**6.3**	5.4	6.6	5.5	**5.8**	8.1	7.1	6.7	**7.3**	4.5	5.1	4.4	**4.6**	6.6	5.6	5.7	**6.0**
AT4G15660	5.2	4.5	5.1	**4.9**	4.6	4.0	4.1	**4.2**	5.0	4.7	5.0	**4.9**	4.6	4.3	3.8	**4.2**	4.5	4.5	4.5	**4.5**	4.2	4.4	4.8	**4.5**	3.1	1.9	2.5	**2.5**	3.6	2.7	3.1	**3.1**
AT4G15670	5.4	5.2	5.3	**5.3**	5.4	5.0	4.8	**5.1**	4.5	5.1	4.9	**4.8**	4.9	4.4	4.2	**4.5**	5.2	4.4	4.9	**4.8**	4.6	4.6	4.6	**4.6**	3.4	3.6	2.9	**3.3**	4.0	3.7	4.0	**3.9**

AT4G15680	6.0	6.4	6.1	**6.2**	5.5	4.9	4.9	**5.1**	5.6	5.3	5.7	**5.5**	5.7	5.3	4.8	**5.3**	5.2	5.0	6.0	**5.4**	4.9	5.4	4.9	**5.1**	3.1	3.7	3.3	**3.4**	4.1	3.6	4.2	**4.0**
AT4G15700	7.0	7.5	7.7	**7.4**	6.5	6.3	7.1	**6.6**	7.1	6.9	7.2	**7.1**	6.6	6.9	6.7	**6.7**	7.1	6.9	7.6	**7.2**	7.0	7.0	7.2	**7.1**	5.5	5.7	5.9	**5.7**	6.1	6.1	6.2	**6.2**
AT4G16260	4.5	4.6	4.8	**4.6**	6.5	6.3	6.1	**6.3**	4.7	4.8	4.9	**4.8**	6.2	6.5	6.5	**6.4**	4.6	4.8	4.1	**4.5**	6.4	7.1	7.1	**6.9**	5.9	6.1	6.3	**6.1**	5.6	5.9	5.9	**5.8**
AT4G18360	3.0	3.4	2.9	**3.1**	2.7	2.8	3.4	**3.0**	2.6	2.9	3.1	**2.9**	3.2	2.8	2.7	**2.9**	3.2	3.1	3.0	**3.1**	2.9	3.1	2.8	**2.9**	3.9	4.0	4.0	**4.0**	3.7	3.5	3.4	**3.5**
AT4G19720	3.7	3.5	3.5	**3.6**	3.8	3.6	3.5	**3.6**	3.8	3.6	3.3	**3.6**	3.9	4.2	3.6	**3.9**	4.0	3.8	3.8	**3.8**	3.9	4.2	3.4	**3.8**	4.8	4.7	4.4	**4.6**	4.7	3.5	4.5	**4.2**
AT4G21250	2.0	2.5	2.6	**2.3**	2.9	2.1	2.6	**2.5**	2.4	2.5	1.9	**2.3**	2.8	2.2	2.3	**2.4**	2.7	2.6	2.0	**2.4**	2.7	2.2	2.2	**2.4**	4.1	3.8	4.4	**4.1**	3.2	3.0	2.8	**3.0**
AT4G22470	4.9	4.9	5.8	**5.2**	6.3	6.0	7.3	**6.5**	6.0	6.7	7.1	**6.6**	5.6	6.3	7.4	**6.4**	5.6	6.3	6.1	**6.0**	7.7	6.7	7.1	**7.2**	4.2	4.9	4.6	**4.6**	7.7	5.6	5.7	**6.3**
AT4G26290	7.2	7.2	7.2	**7.2**	7.8	7.7	7.4	**7.6**	6.7	7.1	6.9	**6.9**	7.2	7.1	7.4	**7.2**	7.3	7.3	6.7	**7.1**	6.8	6.8	6.9	**6.9**	6.0	5.4	5.5	**5.6**	5.1	5.2	5.3	**5.2**
AT4G30800	3.7	4.0	2.9	**3.5**	3.0	3.5	3.7	**3.4**	3.2	3.0	2.8	**3.0**	3.0	3.2	3.0	**3.1**	3.2	3.9	3.5	**3.5**	3.4	2.8	2.9	**3.0**	4.4	4.3	3.8	**4.2**	3.2	3.4	2.9	**3.2**
AT4G31180	8.7	9.1	9.1	**8.9**	9.2	9.1	9.0	**9.1**	9.1	9.1	9.4	**9.2**	9.3	9.3	9.3	**9.3**	7.6	7.9	7.1	**7.5**	7.3	7.8	7.6	**7.6**	7.7	7.6	7.3	**7.5**	7.6	7.7	7.6	**7.6**
AT4G31910	4.9	4.8	4.7	**4.8**	4.1	4.2	4.6	**4.3**	4.4	3.6	4.7	**4.3**	4.1	4.3	4.4	**4.2**	5.0	5.0	5.3	**5.1**	4.3	4.0	4.1	**4.1**	5.9	5.6	5.6	**5.7**	5.0	4.7	4.8	**4.8**
AT4G32860	4.9	5.1	4.6	**4.9**	5.1	4.5	4.7	**4.8**	4.3	4.0	5.0	**4.5**	4.7	4.1	4.9	**4.6**	4.6	4.8	4.8	**4.8**	4.9	4.8	4.8	**4.8**	5.9	5.6	6.0	**5.8**	5.6	4.7	5.0	**5.1**
AT4G33610	3.3	3.4	3.4	**3.4**	2.7	2.8	3.4	**3.0**	3.4	2.5	3.2	**3.0**	2.9	2.8	3.1	**2.9**	4.2	3.6	3.4	**3.7**	3.0	3.7	3.0	**3.3**	4.0	4.5	4.7	**4.4**	4.8	2.8	3.0	**3.6**
AT4G35200	3.3	3.5	3.0	**3.3**	2.9	3.4	3.3	**3.2**	1.9	2.3	2.3	**2.2**	2.7	3.0	2.3	**2.7**	2.9	3.5	2.9	**3.1**	2.3	2.4	2.6	**2.4**	4.1	3.1	3.5	**3.6**	2.1	2.9	3.1	**2.7**
AT4G36430	3.6	4.3	4.4	**4.1**	4.6	4.3	4.5	**4.5**	3.9	3.6	5.1	**4.2**	4.9	4.1	4.2	**4.4**	4.3	4.1	4.2	**4.2**	4.5	4.5	3.5	**4.2**	5.6	5.7	6.4	**5.9**	6.7	4.5	4.9	**5.4**
AT4G36670	6.7	6.6	6.8	**6.7**	7.3	7.0	7.3	**7.2**	6.8	7.1	7.1	**7.0**	7.2	7.2	7.5	**7.3**	6.4	6.9	6.8	**6.7**	7.5	7.3	7.4	**7.4**	5.7	5.8	5.8	**5.8**	6.6	6.7	6.7	**6.7**
AT4G37220	7.1	7.3	7.3	**7.2**	7.6	7.6	7.9	**7.7**	7.0	6.5	7.0	**6.9**	7.4	7.2	7.6	**7.4**	7.3	7.5	7.3	**7.4**	7.4	7.7	7.5	**7.6**	5.7	5.8	6.0	**5.8**	5.4	5.6	6.5	**5.8**
AT5G04120	2.3	2.4	2.4	**2.3**	2.4	2.3	2.1	**2.3**	2.1	2.4	2.5	**2.3**	2.2	2.1	2.4	**2.3**	2.7	2.4	2.0	**2.4**	2.2	2.3	2.6	**2.4**	4.0	3.9	3.0	**3.6**	2.3	2.7	2.6	**2.5**
AT5G05340	3.7	3.9	4.0	**3.9**	5.6	4.4	5.0	**5.0**	4.4	3.6	4.1	**4.0**	4.6	5.1	4.7	**4.8**	3.6	3.5	3.7	**3.6**	4.2	4.5	4.3	**4.3**	4.7	5.2	5.5	**5.1**	6.9	4.3	4.8	**5.3**
AT5G05500	2.1	2.5	1.8	**2.1**	2.8	1.8	2.0	**2.2**	1.7	1.8	2.6	**2.0**	2.0	2.3	2.3	**2.2**	2.2	2.3	2.2	**2.2**	2.1	1.8	2.1	**2.0**	3.8	3.0	2.9	**3.2**	2.7	2.2	2.3	**2.4**
AT5G05965	5.2	5.3	5.4	**5.3**	4.9	4.9	5.1	**5.0**	5.0	5.2	5.4	**5.2**	4.6	4.9	4.9	**4.8**	5.2	5.3	5.5	**5.3**	5.2	5.5	5.3	**5.4**	3.6	4.3	4.0	**4.0**	5.3	5.1	4.4	**4.9**
AT5G06640	3.9	4.4	4.1	**4.1**	4.4	3.4	3.6	**3.8**	3.7	3.4	4.6	**3.9**	4.0	4.9	3.8	**4.2**	4.6	4.4	4.1	**4.4**	3.4	3.2	2.3	**3.0**	7.1	6.6	5.9	**6.5**	4.4	4.6	4.2	**4.4**
AT5G07475	3.6	4.4	3.9	**3.9**	3.5	3.4	3.5	**3.5**	4.2	3.5	3.9	**3.9**	3.3	3.6	3.6	**3.5**	4.5	4.1	4.2	**4.3**	3.6	3.8	3.0	**3.5**	5.0	5.5	5.2	**5.2**	4.6	4.1	4.0	**4.2**
AT5G09520	6.4	6.9	6.9	**6.7**	5.2	6.1	5.5	**5.6**	6.6	5.1	6.3	**6.0**	5.6	5.2	5.9	**5.6**	7.5	6.7	7.1	**7.1**	5.7	6.3	5.1	**5.7**	8.0	8.3	8.2	**8.2**	7.6	6.5	6.7	**6.9**
AT5G10580	2.9	3.0	2.9	**3.0**	2.7	2.7	2.4	**2.6**	2.6	2.3	3.3	**2.8**	2.5	2.8	2.8	**2.7**	3.2	2.6	3.0	**2.9**	2.8	2.4	2.3	**2.5**	4.5	3.5	3.8	**3.9**	3.6	3.2	3.1	**3.3**
AT5G13210	2.0	2.6	2.3	**2.3**	2.5	2.6	2.3	**2.5**	2.6	2.9	2.7	**2.7**	2.8	2.8	2.6	**2.7**	2.6	3.0	2.2	**2.6**	2.8	3.2	2.7	**2.9**	3.8	4.8	4.0	**4.2**	5.7	4.2	3.4	**4.5**
AT5G13580	3.7	3.1	2.7	**3.2**	2.7	3.1	3.2	**3.0**	2.4	3.0	2.7	**2.7**	2.8	3.2	2.7	**2.9**	3.3	3.2	3.2	**3.2**	3.0	2.9	2.7	**2.9**	3.9	3.8	3.8	**3.8**	4.0	3.1	2.8	**3.3**
AT5G14580	4.5	4.8	4.7	**4.6**	3.7	3.7	3.8	**3.8**	4.4	3.9	4.7	**4.3**	4.2	3.4	4.1	**3.9**	4.3	4.3	4.3	**4.3**	3.5	3.7	3.6	**3.6**	5.5	5.4	5.5	**5.5**	4.9	4.5	4.3	**4.6**
AT5G16930	5.8	6.0	5.9	**5.9**	5.2	5.6	5.0	**5.3**	5.3	5.2	6.0	**5.5**	5.2	5.1	5.3	**5.2**	5.7	5.4	5.7	**5.6**	5.3	5.3	5.4	**5.3**	6.7	6.7	6.6	**6.6**	6.5	5.7	5.7	**6.0**
AT5G19410	3.8	4.0	3.8	**3.9**	3.8	3.7	3.8	**3.8**	4.2	3.1	4.1	**3.8**	3.7	3.8	3.6	**3.7**	4.5	4.1	4.2	**4.3**	4.0	4.0	2.9	**3.6**	5.5	5.6	5.6	**5.6**	5.7	4.3	4.4	**4.8**
AT5G19890	2.5	3.1	2.4	**2.7**	2.2	2.6	2.7	**2.5**	2.4	2.5	2.8	**2.6**	3.0	2.5	2.6	**2.7**	3.2	2.5	2.5	**2.7**	2.5	3.2	2.2	**2.6**	4.0	3.3	3.5	**3.6**	2.5	2.3	2.7	**2.5**
AT5G20160	5.5	5.3	5.2	**5.4**	5.1	5.0	4.8	**5.0**	5.3	5.2	5.4	**5.3**	5.3	5.2	5.4	**5.3**	5.3	5.6	5.3	**5.4**	5.1	5.2	5.1	**5.2**	6.5	6.2	6.3	**6.3**	6.3	5.4	5.5	**5.7**
AT5G26300	2.4	2.4	1.4	**2.1**	1.5	2.3	2.2	**2.0**	1.5	2.3	2.1	**2.0**	1.7	1.9	2.0	**1.9**	2.0	2.1	2.6	**2.2**	1.8	2.6	2.0	**2.1**	5.1	3.3	4.3	**4.2**	3.1	2.7	2.1	**2.7**
AT5G36320	3.4	4.8	4.0	**4.1**	3.5	5.0	3.5	**4.0**	3.7	3.9	3.4	**3.6**	2.8	4.0	3.6	**3.5**	4.3	4.2	3.8	**4.1**	4.3	3.4	3.9	**3.9**	4.3	4.9	4.8	**4.7**	3.5	4.0	3.7	**3.7**
AT5G37690	4.3	4.2	4.3	**4.3**	3.6	4.1	3.8	**3.9**	3.8	3.3	4.4	**3.8**	4.2	3.3	4.0	**3.9**	4.8	4.5	4.1	**4.5**	3.7	4.7	3.9	**4.1**	5.3	5.5	6.0	**5.6**	5.0	4.5	4.7	**4.8**
AT5G39120	2.0	2.4	2.5	**2.3**	3.0	3.2	3.3	**3.1**	1.7	2.6	2.1	**2.1**	3.1	1.9	2.0	**2.3**	1.2	3.3	1.7	**2.1**	2.8	2.6	1.6	**2.3**	3.9	4.2	5.5	**4.6**	5.3	3.3	1.6	**3.4**
AT5G39480	2.3	2.0	2.0	**2.1**	1.7	1.8	1.7	**1.7**	2.8	2.8	2.2	**2.6**	2.1	1.5	1.9	**1.8**	1.9	1.8	2.5	**2.1**	2.0	1.6	1.4	**1.7**	1.9	1.3	1.4	**1.6**	1.6	1.3	2.0	**1.6**
AT5G40000	2.4	2.6	2.5	**2.5**	2.8	2.5	2.6	**2.6**	2.0	1.8	3.1	**2.3**	2.5	2.7	3.0	**2.7**	2.2	2.6	2.9	**2.6**	2.6	2.4	2.4	**2.4**	4.0	5.2	4.4	**4.5**	5.0	3.1	4.3	**4.1**
AT5G41040	5.3	5.5	5.4	**5.4**	4.9	4.7	5.2	**5.0**	5.5	4.6	5.6	**5.2**	5.3	4.8	5.0	**5.0**	5.8	5.4	5.8	**5.7**	5.0	4.7	4.9	**4.9**	7.4	7.4	6.8	**7.2**	6.8	5.7	5.9	**6.1**
AT5G42830	4.9	5.1	4.9	**5.0**	7.4	7.1	6.7	**7.1**	5.9	6.4	6.4	**6.2**	8.0	8.3	7.6	**7.9**	5.7	5.6	5.2	**5.5**	8.0	7.7	8.1	**7.9**	7.8	8.5	7.9	**8.1**	8.7	7.7	7.7	**8.0**
AT5G44585	5.6	5.9	5.1	**5.6**	7.9	7.6	7.0	**7.5**	6.4	7.1	7.1	**6.9**	8.6	8.7	8.2	**8.5**	6.3	6.4	6.8	**6.5**	8.8	8.3	8.1	**8.4**	5.9	5.4	5.6	**5.6**	6.6	6.9	7.2	**6.9**
AT5G46090	4.0	4.1	3.7	**3.9**	3.7	3.6	4.0	**3.7**	4.2	3.6	4.3	**4.0**	4.1	3.2	4.1	**3.8**	4.9	3.2	4.4	**4.2**	3.4	4.1	3.6	**3.7**	5.3	4.7	5.2	**5.1**	4.1	3.3	3.9	**3.8**
AT5G47635	4.4	4.5	4.6	**4.5**	3.6	4.6	4.2	**4.1**	4.7	3.9	4.5	**4.4**	4.6	4.1	4.6	**4.5**	4.8	4.6	4.3	**4.6**	4.1	4.9	4.7	**4.6**	5.2	5.7	5.5	**5.5**	6.3	4.6	4.5	**5.1**
AT5G49780	3.8	3.2	3.8	**3.6**	3.1	3.0	3.5	**3.2**	2.9	2.5	3.5	**3.0**	3.5	2.0	3.0	**2.8**	3.5	3.6	3.7	**3.6**	3.3	3.9	2.9	**3.4**	4.6	3.7	4.9	**4.4**	3.8	2.9	3.5	**3.4**
AT5G50560	3.1	3.6	3.2	**3.3**	3.5	2.9	3.3	**3.2**	2.7	2.5	3.2	**2.8**	3.6	3.0	3.3	**3.3**	3.1	3.2	3.4	**3.2**	3.2	3.2	3.6	**3.3**	2.9	3.1	3.1	**3.0**	3.3	2.8	3.7	**3.3**
AT5G51440	2.8	2.7	2.9	**2.8**	2.8	2.7	2.7	**2.7**	3.3	2.7	2.7	**2.9**	3.6	4.0	4.3	**4.0**	2.7	3.0	3.0	**2.9**	3.2	3.7	2.7	**3.2**	4.1	5.2	4.6	**4.7**	5.6	4.9	4.4	**5.0**
AT5G54100	6.4	6.2	6.0	**6.2**	6.3	5.9	5.8	**6.0**	6.0	5.6	6.3	**6.0**	6.3	6.1	6.2	**6.2**	6.1	6.3	6.0	**6.1**	5.7	6.1	6.1	**6.0**	6.7	7.3	6.9	**7.0**	7.6	6.7	6.3	**6.9**
AT5G54370	3.2	3.4	3.1	**3.2**	4.8	3.3	2.8	**3.6**	2.8	3.0	3.6	**3.2**	3.9	4.2	3.8	**3.9**	3.8	3.2	3.8	**3.6**	3.5	3.7	3.1	**3.4**	6.5	5.3	6.0	**5.9**	3.9	3.5	3.6	**3.7**
AT5G57510	3.5	3.8	3.0	**3.5**	6.2	5.4	5.8	**5.8**	3.8	3.3	4.2	**3.8**	5.8	6.0	5.3	**5.7**	4.4	3.9	3.7	**4.0**	6.2	6.0	6.3	**6.2**	5.5	6.6	4.9	**5.7**	6.9	5.1	5.8	**6.0**
AT5G57625	4.9	5.6	4.6	**5.0**	5.0	4.6	4.8	**4.8**	4.5	4.5	5.2	**4.7**	4.9	5.3	5.1	**5.1**	5.4	5.1	5.0	**5.2**	4.6	4.7	3.8	**4.4**	7.1	5.8	6.1	**6.3**	4.6	4.5	4.9	**4.7**
AT5G59490	4.0	3.9	3.3	**3.8**	4.0	3.9	3.5	**3.8**	3.8	3.7	3.6	**3.7**	3.9	3.7	3.3	**3.6**	3.6	3.7	3.3	**3.5**	3.8	3.5	3.5	**3.6**	4.8	5.7	4.7	**5.1**	4.3	3.9	3.8	**4.0**
AT5G59680	4.0	4.4	4.1	**4.2**	5.0	4.8	4.6	**4.8**	3.7	2.8	4.7	**3.7**	4.8	4.4	5.1	**4.8**	4.8	5.1	4.3	**4.7**	4.5	4.1	4.3	**4.3**	6.2	5.6	5.6	**5.8**	5.2	4.5	4.8	**4.8**
AT5G63560	5.0	5.6	4.6	**5.1**	4.6	4.8	5.3	**4.9**	4.8	4.2	4.9	**4.6**	5.2	3.4	4.9	**4.5**	5.6	5.0	5.4	**5.3**	5.1	4.9	4.6	**4.9**	5.8	5.6	6.0	**5.8**	5.9	5.0	5.2	**5.4**
AT5G64120	6.8	7.1	7.4	**7.1**	8.6	8.5	8.5	**8.6**	7.6	8.3	8.4	**8.1**	8.5	8.9	8.8	**8.8**	7.1	8.0	7.8	**7.6**	9.4	9.0	9.0	**9.1**	6.7	7.1	6.5	**6.8**	8.9	8.2	8.3	**8.5**
ATC	2.7	3.0	2.2	**2.7**	3.0	2.6	2.3	**2.6**	2.7	2.8	2.6	**2.7**	2.3	3.2	2.6	**2.7**	2.7	2.3	2.6	**2.5**	3.2	2.4	2.6	**2.7**	3.9	3.7	4.4	**4.0**	3.6	2.5	3.0	**3.0**
ATH6	2.8	4.3	3.6	**3.6**	3.7	4.0	4.5	**4.1**	3.5	3.1	3.8	**3.4**	4.5	4.6	4.1	**4.4**	3.9	4.0	4.3	**4.1**	4.2	4.4	4.7	**4.5**	5.0	5.1	5.1	**5.1**	5.5	4.6	4.4	**4.8**
ATLP-1	3.0	3.5	3.2	**3.2**	3.2	3.0	3.4	**3.2**	3.0	3.0	3.1	**3.0**	3.1	2.9	3.4	**3.2**	2.7	2.8	3.3	**3.0**	3.4	3.2	2.8	**3.1**	3.9	4.1	4.1	**4.0**	3.6	3.6	3.5	**3.6**

BCAT-2	10.2	10.3	10.2	**10.3**	11.0	11.2	10.8	**11.0**	10.3	10.6	10.4	**10.4**	10.9	11.0	11.0	**11.0**	10.4	10.5	10.2	**10.4**	11.0	11.0	11.3	**11.1**	9.0	8.8	9.5	**9.1**	9.3	10.0	10.2	**9.8**
BCAT4	6.0	6.2	6.3	**6.2**	4.1	4.3	5.1	**4.5**	5.5	4.7	5.4	**5.2**	5.2	4.5	4.9	**4.9**	5.1	5.5	5.5	**5.4**	3.7	4.2	3.7	**3.9**	6.8	6.6	6.6	**6.7**	6.7	5.4	5.4	**5.8**
CEL5	4.5	4.4	4.1	**4.3**	4.3	4.3	4.4	**4.3**	4.4	4.2	4.5	**4.4**	4.8	4.0	4.4	**4.4**	4.9	4.3	4.5	**4.5**	4.2	4.1	4.0	**4.1**	6.2	5.6	5.3	**5.7**	5.0	4.8	4.1	**4.6**
CP1	2.9	2.0	2.1	**2.3**	2.2	2.4	2.6	**2.4**	2.2	2.4	1.9	**2.2**	2.1	2.4	2.4	**2.3**	2.0	2.2	2.2	**2.1**	2.5	2.1	2.8	**2.5**	2.2	2.0	2.0	**2.1**	2.1	2.2	2.4	**2.2**
cwINV6	3.2	3.5	3.4	**3.4**	4.6	4.7	4.4	**4.6**	3.7	3.1	4.4	**3.7**	5.7	5.2	4.8	**5.3**	4.0	4.0	3.5	**3.8**	5.1	5.5	4.9	**5.2**	5.6	5.0	5.3	**5.3**	4.9	4.4	4.8	**4.7**
CYP710A1	4.8	5.2	4.4	**4.8**	5.8	4.9	5.4	**5.4**	4.7	4.3	4.7	**4.6**	5.4	5.5	5.6	**5.5**	5.0	4.2	4.7	**4.6**	5.2	5.8	5.8	**5.6**	6.3	7.2	6.5	**6.7**	6.7	6.0	6.1	**6.3**
CYP710A3	4.7	4.8	4.1	**4.5**	4.2	4.4	3.6	**4.1**	3.9	3.7	4.8	**4.1**	4.3	4.4	3.9	**4.2**	4.2	4.0	4.3	**4.2**	3.6	4.4	3.3	**3.8**	5.8	4.9	5.8	**5.5**	3.6	3.9	4.3	**3.9**
CYP71A14	3.8	3.6	3.6	**3.7**	3.1	4.4	3.4	**3.6**	4.2	3.7	3.6	**3.8**	3.4	3.5	3.5	**3.5**	4.0	3.5	3.9	**3.8**	3.0	3.4	3.2	**3.2**	5.3	4.8	4.6	**4.9**	3.8	3.3	3.3	**3.5**
CYP71A27	4.0	4.5	3.8	**4.1**	3.6	3.5	3.6	**3.5**	3.7	3.0	3.9	**3.5**	4.2	3.5	3.2	**3.6**	4.7	4.1	4.3	**4.4**	3.7	3.4	3.5	**3.5**	5.4	4.7	4.6	**4.9**	3.7	4.2	4.1	**4.0**
CYP79B2	4.0	3.6	3.1	**3.6**	6.9	6.3	6.1	**6.4**	4.1	4.6	4.4	**4.4**	6.8	6.7	6.4	**6.6**	4.0	4.3	4.0	**4.1**	6.9	6.7	6.7	**6.8**	5.8	5.9	5.1	**5.6**	6.6	6.8	7.0	**6.8**
CYP81F2	5.0	4.6	4.5	**4.7**	7.0	6.9	6.4	**6.7**	5.1	5.4	5.1	**5.2**	7.6	7.6	7.0	**7.4**	4.6	5.7	4.9	**5.1**	7.6	7.3	7.1	**7.3**	5.8	6.7	6.1	**6.2**	7.4	7.6	7.4	**7.5**
CYP86A1	3.8	3.9	3.9	**3.9**	3.6	3.9	4.3	**3.9**	3.4	1.9	3.6	**3.0**	3.9	3.2	4.1	**3.7**	4.3	3.7	4.0	**4.0**	4.0	4.5	3.7	**4.1**	4.7	5.3	5.4	**5.1**	5.8	4.2	4.7	**4.9**
CYP86A4	3.9	3.9	3.3	**3.7**	4.3	3.6	4.3	**4.1**	3.4	3.1	4.1	**3.6**	3.9	3.7	3.7	**3.8**	4.3	3.7	3.9	**4.0**	3.8	3.9	3.4	**3.7**	4.9	4.8	4.2	**4.7**	4.4	3.4	3.8	**3.9**
DI21	4.5	5.1	4.8	**4.8**	4.9	4.7	5.3	**5.0**	4.3	4.4	4.6	**4.4**	5.0	4.9	5.3	**5.1**	4.5	5.0	4.3	**4.6**	4.5	4.7	4.6	**4.6**	5.7	5.0	5.7	**5.5**	5.6	5.2	5.0	**5.2**
DOX1	5.6	5.2	5.6	**5.5**	6.0	4.9	6.1	**5.7**	5.2	4.8	5.1	**5.0**	5.7	5.0	5.5	**5.4**	4.8	5.1	5.3	**5.0**	4.7	5.1	5.0	**4.9**	7.6	7.0	7.2	**7.3**	6.6	5.1	5.6	**5.8**
ENODL13	3.6	4.5	3.6	**3.9**	3.8	4.1	4.4	**4.1**	3.6	3.7	4.4	**3.9**	4.9	3.9	3.8	**4.2**	3.8	3.4	4.1	**3.8**	3.2	4.2	3.5	**3.6**	4.8	4.8	5.1	**4.9**	4.7	4.4	4.1	**4.4**
EXT4	6.2	6.1	7.3	**6.5**	8.7	8.0	9.0	**8.6**	7.9	8.1	8.7	**8.2**	8.4	9.1	8.7	**8.7**	6.5	7.4	6.8	**6.9**	9.4	9.1	8.9	**9.1**	7.2	6.9	6.9	**7.0**	9.0	8.0	8.4	**8.5**
FAR4	2.9	3.5	2.8	**3.1**	2.6	2.4	2.4	**2.5**	2.8	2.5	2.7	**2.6**	3.1	2.0	3.2	**2.7**	3.1	3.1	2.7	**2.9**	2.3	2.4	2.2	**2.3**	3.9	3.7	3.7	**3.8**	3.5	3.1	2.8	**3.1**
FAR5	4.5	4.8	4.5	**4.6**	3.6	4.4	4.4	**4.1**	4.9	4.4	4.7	**4.6**	4.3	4.0	4.3	**4.2**	5.2	4.8	4.9	**5.0**	4.2	4.2	4.1	**4.2**	6.2	6.0	6.0	**6.0**	6.1	4.8	4.4	**5.1**
FIB2	5.1	5.0	5.3	**5.1**	4.7	4.6	4.9	**4.7**	5.1	5.2	5.7	**5.3**	5.4	4.7	5.1	**5.1**	4.8	5.0	5.2	**5.0**	4.5	4.7	4.2	**4.5**	6.5	6.1	6.4	**6.3**	6.2	4.9	5.1	**5.4**
FRO5	2.9	2.8	3.7	**3.1**	2.4	4.5	4.1	**3.7**	3.1	3.3	3.6	**3.3**	3.4	3.2	3.4	**3.3**	3.4	3.3	3.8	**3.5**	2.1	2.3	3.4	**2.6**	4.7	4.8	5.2	**4.9**	4.7	5.0	5.0	**4.9**
FRU	4.5	5.1	4.6	**4.8**	5.2	4.5	4.2	**4.6**	4.0	3.6	4.8	**4.1**	5.0	4.5	5.1	**4.9**	5.1	4.5	4.5	**4.7**	4.9	4.7	4.5	**4.7**	6.4	5.6	5.6	**5.9**	4.6	4.7	4.9	**4.7**
GA20OX2	6.8	6.3	6.6	**6.6**	6.9	6.5	6.8	**6.7**	6.1	5.9	6.0	**6.0**	6.6	6.9	6.7	**6.7**	6.7	6.6	6.2	**6.5**	6.9	7.0	7.2	**7.0**	6.9	7.0	7.1	**7.0**	6.5	6.8	7.2	**6.8**
GSTF7	4.7	4.5	5.1	**4.8**	8.2	8.3	7.8	**8.1**	6.1	6.1	5.7	**6.0**	8.5	9.0	8.8	**8.8**	5.3	5.5	5.1	**5.3**	8.9	8.6	8.8	**8.8**	6.6	7.5	7.1	**7.0**	8.1	8.6	8.8	**8.5**
GSTU22	2.7	2.2	1.9	**2.3**	2.1	2.3	2.0	**2.2**	2.2	2.0	2.1	**2.1**	2.0	2.5	2.3	**2.3**	2.2	2.4	2.2	**2.3**	2.3	2.2	2.3	**2.3**	3.9	3.1	3.0	**3.3**	2.8	1.8	2.4	**2.4**
GSTU9	3.3	3.6	3.6	**3.5**	4.4	3.6	3.9	**3.9**	3.0	2.7	3.7	**3.1**	4.1	4.2	4.7	**4.3**	3.3	3.1	3.2	**3.2**	4.5	4.4	3.9	**4.2**	6.6	7.7	6.6	**7.0**	7.8	6.3	5.4	**6.5**
HDA3	6.4	7.1	6.8	**6.8**	6.0	5.8	6.1	**6.0**	6.8	6.2	6.4	**6.5**	6.3	6.4	6.3	**6.3**	6.7	6.6	6.7	**6.7**	5.8	5.9	6.2	**6.0**	7.7	7.4	7.8	**7.6**	7.3	6.5	6.5	**6.8**
HDT4	5.6	6.0	5.6	**5.8**	5.3	5.4	5.2	**5.3**	5.5	5.2	5.6	**5.4**	5.4	5.2	5.2	**5.3**	5.6	5.5	5.8	**5.6**	5.0	5.1	5.3	**5.1**	6.6	6.4	6.5	**6.5**	6.3	5.3	5.8	**5.8**
HTA4	4.7	4.2	5.3	**4.7**	4.0	4.4	4.7	**4.3**	4.7	4.8	4.2	**4.6**	4.0	4.9	4.4	**4.4**	4.7	4.4	4.4	**4.5**	4.4	3.8	3.8	**4.0**	4.5	4.7	4.3	**4.5**	4.7	4.3	4.4	**4.5**
IMS2	3.7	3.9	4.5	**4.0**	3.4	3.6	3.1	**3.4**	3.6	3.7	4.4	**3.9**	3.5	3.1	3.5	**3.3**	3.9	3.9	3.7	**3.8**	3.7	3.1	3.3	**3.4**	6.0	5.0	5.8	**5.6**	5.0	4.6	4.6	**4.7**
IVD	9.5	9.8	9.8	**9.7**	10.9	10.8	10.8	**10.8**	9.8	10.3	10.1	**10.1**	10.6	10.6	10.7	**10.6**	10.0	9.7	9.7	**9.8**	10.6	10.7	10.8	**10.7**	9.2	9.0	9.1	**9.1**	10.1	10.0	10.2	**10.1**
LAC5	4.3	5.0	4.6	**4.6**	4.6	4.2	4.4	**4.4**	4.5	3.6	4.9	**4.3**	3.7	4.0	4.4	**4.0**	5.3	4.7	4.8	**4.9**	4.6	5.1	4.3	**4.7**	5.9	6.5	6.5	**6.3**	6.8	5.0	4.8	**5.5**
LAC7	5.8	5.9	5.7	**5.8**	6.5	5.4	6.6	**6.2**	5.4	4.7	5.8	**5.3**	6.6	5.7	6.3	**6.2**	6.4	5.5	5.9	**5.9**	5.3	5.9	4.9	**5.4**	7.1	6.4	6.5	**6.7**	6.2	4.7	5.6	**5.5**
LDOX	3.0	3.1	2.9	**3.0**	2.0	3.0	2.6	**2.5**	2.8	2.9	2.7	**2.8**	3.1	2.9	2.5	**2.8**	2.4	2.8	2.5	**2.5**	1.8	2.3	2.2	**2.1**	3.5	4.0	3.9	**3.8**	2.0	2.3	2.4	**2.3**
LRX1	2.2	3.7	3.0	**3.0**	3.0	3.0	3.0	**3.0**	3.2	2.7	3.7	**3.2**	3.1	3.6	3.9	**3.6**	4.2	3.2	3.1	**3.5**	2.9	2.5	2.0	**2.4**	5.5	4.4	4.2	**4.7**	2.9	3.2	3.4	**3.2**
LTP4	2.2	2.2	2.5	**2.3**	2.0	2.5	4.8	**3.1**	2.2	2.5	2.3	**2.3**	2.5	2.6	6.0	**3.7**	2.0	2.3	2.0	**2.1**	2.2	2.0	2.9	**2.4**	3.4	3.6	3.5	**3.5**	5.3	4.5	4.8	**4.9**
MPL1	5.0	5.1	5.3	**5.1**	6.2	6.0	7.3	**6.5**	5.1	6.1	5.7	**5.6**	5.2	5.8	7.2	**6.1**	5.5	5.3	5.3	**5.4**	6.4	6.3	6.1	**6.3**	4.3	4.8	4.3	**4.5**	7.0	5.0	5.5	**5.8**
MRD1	3.5	3.2	2.9	**3.2**	2.2	3.2	3.0	**2.8**	3.2	3.3	2.7	**3.1**	3.2	3.2	2.7	**3.0**	6.1	5.8	5.7	**5.9**	5.7	5.9	5.7	**5.8**	5.4	5.8	6.1	**5.8**	6.0	5.9	5.8	**5.9**
MRP7	3.6	3.8	3.1	**3.5**	5.0	5.0	4.2	**4.7**	3.9	4.1	3.6	**3.9**	4.9	5.2	4.8	**5.0**	4.1	2.9	4.0	**3.6**	5.3	5.3	5.3	**5.3**	4.7	5.1	4.8	**4.9**	5.1	5.2	5.1	**5.2**
mtHsc70-1	8.5	8.6	8.6	**8.6**	8.0	7.9	7.8	**7.9**	8.5	8.1	8.5	**8.4**	8.3	8.3	8.2	**8.3**	8.3	8.4	8.5	**8.4**	8.2	8.1	7.9	**8.1**	9.6	9.6	9.3	**9.5**	9.8	8.6	8.4	**8.9**
MYB14	2.9	3.4	3.5	**3.3**	3.2	3.9	3.5	**3.5**	3.1	3.2	3.3	**3.2**	3.5	3.0	2.8	**3.1**	3.4	3.7	3.0	**3.4**	3.1	2.9	3.7	**3.2**	4.0	4.5	4.2	**4.2**	5.0	3.3	2.9	**3.7**
MYB28	5.5	6.0	6.1	**5.9**	4.9	5.1	5.2	**5.1**	5.7	5.5	5.8	**5.7**	5.4	5.3	5.3	**5.3**	5.8	5.7	5.7	**5.7**	4.9	4.9	4.9	**4.9**	6.7	6.9	7.0	**6.9**	6.4	6.4	6.0	**6.3**
MYB45	1.8	2.0	2.0	**1.9**	1.9	2.1	1.7	**1.9**	1.6	2.3	2.1	**2.0**	2.0	1.8	2.2	**2.0**	2.1	1.8	2.0	**1.9**	1.6	1.9	1.7	**1.7**	3.1	3.4	3.0	**3.1**	2.9	2.3	2.0	**2.4**
MYB56	2.8	3.7	2.8	**3.1**	3.1	3.4	3.2	**3.2**	3.2	2.9	2.7	**2.9**	3.3	3.4	3.5	**3.4**	3.4	3.9	3.1	**3.5**	3.0	3.4	3.0	**3.1**	4.2	3.9	4.0	**4.0**	3.2	3.1	3.6	**3.3**
NAC038	2.9	2.6	2.6	**2.7**	2.4	2.1	2.7	**2.4**	2.7	2.4	2.3	**2.5**	2.6	2.5	2.4	**2.5**	2.8	2.8	2.8	**2.8**	2.3	3.0	2.5	**2.6**	3.8	3.4	3.6	**3.6**	3.5	2.8	2.5	**2.9**
NAC044	4.2	3.7	4.1	**4.0**	3.6	3.7	3.5	**3.6**	4.1	3.9	3.6	**3.9**	3.5	3.0	3.9	**3.5**	4.1	3.9	4.0	**4.0**	3.5	3.7	3.2	**3.4**	5.5	5.6	5.9	**5.7**	6.5	4.8	4.7	**5.3**
NAS1	4.5	4.6	4.4	**4.5**	3.3	3.9	3.8	**3.7**	4.6	4.4	5.4	**4.8**	4.2	3.8	3.9	**4.0**	4.5	5.2	5.0	**4.9**	3.4	2.9	3.2	**3.2**	6.9	6.7	6.5	**6.7**	5.2	5.5	5.1	**5.3**
NDB2	3.0	4.2	3.9	**3.7**	4.7	4.3	5.0	**4.6**	4.1	4.6	4.4	**4.4**	4.9	5.1	4.9	**5.0**	4.5	4.2	4.3	**4.3**	4.8	4.9	4.9	**4.9**	5.3	5.8	5.5	**5.5**	6.4	5.5	5.0	**5.6**
NIC3	4.2	4.3	4.0	**4.2**	4.9	3.9	4.5	**4.4**	4.5	3.3	4.3	**4.0**	5.2	3.9	4.7	**4.6**	4.4	4.3	4.5	**4.4**	4.1	4.1	3.3	**3.8**	6.7	5.3	6.2	**6.1**	5.3	4.8	4.7	**4.9**
NRT2:1	5.1	5.4	5.5	**5.3**	4.8	4.5	4.7	**4.6**	5.1	4.8	5.8	**5.2**	4.3	4.5	4.8	**4.5**	5.4	5.4	5.0	**5.2**	4.4	4.3	4.4	**4.4**	8.5	8.8	8.4	**8.6**	7.4	6.9	6.6	**7.0**
NSP4	3.9	3.6	3.9	**3.8**	3.2	3.9	3.6	**3.6**	3.7	3.4	4.3	**3.8**	3.5	3.6	4.4	**3.8**	4.2	3.6	4.4	**4.1**	3.7	3.9	3.3	**3.6**	5.3	4.7	4.6	**4.9**	4.0	4.1	3.7	**3.9**
NUC-L2	2.7	2.5	3.4	**2.9**	2.7	2.7	2.8	**2.7**	2.6	2.5	2.6	**2.6**	2.9	2.3	2.5	**2.6**	3.4	2.8	2.8	**3.0**	3.4	3.2	2.7	**3.1**	3.7	3.7	3.7	**3.7**	4.5	3.7	3.5	**3.9**
PDF1.4	6.4	6.0	6.7	**6.4**	7.9	7.5	8.3	**7.9**	6.3	7.7	7.7	**7.2**	7.4	7.6	7.8	**7.6**	6.6	6.7	6.9	**6.7**	8.2	8.1	7.9	**8.1**	5.3	5.5	5.2	**5.3**	7.5	6.6	7.1	**7.0**
PDF2.5	3.6	4.7	3.5	**3.9**	4.2	4.1	3.8	**4.0**	3.7	2.9	4.4	**3.7**	3.5	3.7	3.7	**3.6**	3.8	3.8	3.5	**3.7**	3.2	3.5	2.8	**3.2**	5.9	4.6	5.3	**5.3**	4.2	3.7	3.5	**3.8**
pEARLI1	7.6	7.4	8.3	**7.8**	9.0	8.8	9.9	**9.2**	9.1	9.6	10.0	**9.6**	8.3	9.5	9.6	**9.1**	8.5	9.0	8.3	**8.6**	10.3	9.6	9.9	**9.9**	7.3	7.7	7.6	**7.5**	10.3	8.7	8.9	**9.3**
PGP11	1.9	1.7	1.8	**1.8**	2.1	1.6	1.3	**1.7**	1.7	1.4	1.6	**1.6**	1.7	1.2	1.2	**1.4**	1.2	1.5	1.2	**1.3**	1.9	1.8	1.3	**1.6**	3.3	2.7	2.3	**2.8**	2.7	1.5	1.5	**1.9**
PIN5	4.1	3.1	3.8	**3.7**	3.4	3.0	2.9	**3.1**	3.3	3.2	3.1	**3.2**	3.0	2.8	3.3	**3.0**	3.4	3.2	3.3	**3.3**	3.3	3.2	3.5	**3.3**	3.9	4.4	4.4	**4.2**	3.3	4.0	3.3	**3.5**

PMZ	3.0	3.0	2.8	**2.9**	3.0	3.0	2.6	**2.9**	2.7	2.7	2.3	**2.6**	3.2	3.3	3.3	**3.3**	2.3	2.5	2.3	**2.4**	3.2	3.6	3.9	**3.6**	3.6	4.2	3.2	**3.7**	4.6	3.6	3.9	**4.0**
PP2-A6	4.5	4.5	3.8	**4.3**	3.8	3.7	4.6	**4.0**	3.8	3.6	4.4	**3.9**	4.1	3.9	3.9	**3.9**	4.6	3.9	4.4	**4.3**	3.9	4.1	3.1	**3.7**	5.3	4.6	5.2	**5.1**	4.1	3.3	3.9	**3.8**
PPa5	6.8	7.2	6.9	**7.0**	6.8	6.7	6.7	**6.7**	7.4	6.5	7.2	**7.0**	7.0	6.7	6.9	**6.9**	6.6	7.0	6.6	**6.8**	6.6	6.5	6.5	**6.5**	8.3	8.0	7.9	**8.1**	7.3	6.8	6.8	**6.9**
PPC3	6.1	5.9	5.8	**5.9**	5.3	4.7	4.7	**4.9**	5.6	5.3	5.3	**5.4**	5.8	5.2	5.3	**5.4**	5.5	5.6	5.9	**5.7**	3.1	4.8	4.9	**4.3**	7.3	6.6	6.7	**6.9**	5.8	5.5	5.7	**5.7**
PRMT1A	6.9	7.3	7.0	**7.1**	6.4	6.4	6.3	**6.4**	7.0	6.5	7.0	**6.8**	6.6	6.4	6.6	**6.5**	7.2	7.1	7.2	**7.2**	6.2	6.5	6.2	**6.3**	8.0	7.6	7.9	**7.9**	7.2	6.7	6.9	**6.9**
RBOHA	2.8	2.6	2.9	**2.8**	2.8	2.4	2.7	**2.7**	2.4	2.6	2.6	**2.5**	2.8	2.8	2.3	**2.6**	3.1	2.7	2.6	**2.8**	2.6	2.8	2.3	**2.6**	3.8	3.8	3.8	**3.8**	3.1	2.5	2.7	**2.8**
RFNR2	7.1	7.0	7.0	**7.0**	6.3	6.7	6.8	**6.6**	6.5	6.2	7.1	**6.6**	6.6	6.6	6.5	**6.6**	7.0	7.1	7.0	**7.0**	6.4	6.6	6.5	**6.5**	8.0	7.6	7.3	**7.6**	6.6	7.0	6.7	**6.8**
RGP1	7.3	7.0	7.4	**7.2**	6.9	6.7	7.2	**6.9**	7.4	6.6	7.4	**7.1**	7.4	7.6	7.1	**7.3**	7.1	7.4	7.8	**7.4**	6.8	7.2	6.9	**7.0**	8.4	8.3	8.1	**8.3**	8.0	7.6	7.6	**7.7**
RLP21	4.8	5.0	4.9	**4.9**	5.9	6.1	6.1	**6.0**	5.5	6.1	6.0	**5.9**	6.2	6.6	6.1	**6.3**	5.6	5.4	5.7	**5.6**	7.2	6.6	6.6	**6.8**	4.5	4.7	4.6	**4.6**	6.2	6.0	6.0	**6.0**
scpl28	4.4	4.2	4.5	**4.4**	3.6	4.1	3.9	**3.9**	4.2	3.9	3.8	**3.9**	4.2	3.8	4.3	**4.1**	4.6	4.3	4.4	**4.4**	3.4	3.9	3.7	**3.7**	5.6	6.2	5.3	**5.7**	5.0	4.3	4.9	**4.7**
SRO3	1.9	2.0	2.1	**2.0**	2.1	2.4	2.1	**2.2**	1.9	2.2	2.3	**2.1**	2.3	2.1	2.6	**2.3**	2.7	2.6	2.3	**2.5**	2.6	2.2	2.3	**2.4**	3.4	3.8	3.8	**3.7**	4.6	3.0	2.2	**3.3**
SUFE2	3.9	3.8	4.2	**3.9**	5.1	4.9	4.5	**4.8**	4.1	3.9	4.2	**4.1**	5.0	4.7	4.9	**4.9**	3.8	4.1	3.8	**3.9**	5.3	5.3	5.3	**5.3**	5.0	5.7	4.8	**5.2**	5.4	5.5	5.5	**5.5**
SULTR1;2	5.5	5.8	5.0	**5.5**	5.7	5.1	5.7	**5.5**	5.2	4.6	5.9	**5.3**	6.7	5.4	6.2	**6.1**	5.9	5.5	5.9	**5.7**	4.9	5.8	4.7	**5.2**	7.6	6.6	6.5	**6.9**	6.2	5.7	5.2	**5.7**
TH8	3.6	3.7	3.8	**3.7**	3.8	3.7	4.8	**4.1**	3.3	3.4	3.0	**3.2**	3.9	4.0	4.8	**4.2**	3.3	3.5	3.5	**3.4**	3.2	3.9	4.1	**3.7**	4.0	4.4	4.5	**4.3**	5.3	3.7	3.6	**4.2**
THA1	8.6	8.7	8.6	**8.6**	9.7	9.7	9.5	**9.6**	8.6	8.8	8.7	**8.7**	9.3	9.6	9.4	**9.4**	8.7	8.8	8.6	**8.7**	9.7	9.6	9.9	**9.7**	7.2	6.9	7.8	**7.3**	7.5	8.3	8.6	**8.1**
tic20-IV	4.5	4.7	4.4	**4.5**	4.9	4.8	4.7	**4.8**	4.4	4.1	4.4	**4.3**	4.9	4.6	4.7	**4.8**	4.4	4.5	4.5	**4.5**	5.0	5.3	5.0	**5.1**	5.7	5.4	5.5	**5.6**	6.2	5.3	4.9	**5.5**
TLL1	8.0	7.9	8.2	**8.0**	7.2	7.4	7.7	**7.5**	7.7	7.4	7.6	**7.6**	8.0	7.3	7.6	**7.7**	7.9	8.0	7.9	**7.9**	7.6	7.3	7.1	**7.3**	8.7	8.8	8.4	**8.6**	7.9	7.7	8.0	**7.9**
TOM7-2	6.1	6.1	7.0	**6.4**	5.6	4.6	5.1	**5.1**	6.0	5.3	6.0	**5.8**	5.4	5.1	5.7	**5.4**	6.1	6.7	6.4	**6.4**	5.5	5.4	5.5	**5.5**	7.5	7.5	7.5	**7.5**	7.0	5.5	6.0	**6.2**
TUA4	6.3	7.1	7.5	**7.0**	6.5	6.4	6.9	**6.6**	7.1	6.8	7.4	**7.1**	6.2	5.9	6.5	**6.2**	7.2	6.9	6.6	**6.9**	6.2	6.0	6.0	**6.1**	5.9	6.3	6.1	**6.1**	6.0	6.3	6.2	**6.2**
WRKY59	2.7	2.4	1.9	**2.4**	2.6	3.1	2.8	**2.8**	2.8	2.1	2.8	**2.6**	2.9	3.4	2.8	**3.0**	3.4	2.2	2.5	**2.7**	2.9	3.7	3.0	**3.2**	4.4	5.2	3.9	**4.5**	4.1	3.4	3.7	**3.7**
XTR8	5.7	6.1	6.4	**6.1**	4.6	3.9	4.9	**4.5**	6.1	5.8	6.9	**6.3**	5.0	4.5	4.6	**4.7**	6.7	6.2	6.2	**6.3**	5.0	4.9	4.3	**4.7**	7.9	8.1	7.5	**7.8**	6.5	6.5	6.1	**6.4**

water-treated and *Hpa*-inoculated

	CHM 1	CHM 2	CHM 3	**CHM avg**	CHP 1	CHP 2	CHP 3	**CHP avg**	CBM 1	CBM 2	CBM 3	**CBM avg**	CBP 1	CBP 2	CBP 3	**CBP avg**	IHM 1	IHM 2	IHM 3	**IHM avg**	IHP1	IHP2	IHP3	**IHP avg**	IBM 1	IBM 2	IBM 3	**IBM avg**	IBP1	IBP2	IBP3	**IBP avg**
ArthMp003	9.8	10.5	10.7	10.3	10.6	10.6	10.6	10.6	10.9	10.8	10.7	10.8	10.5	9.5	10.7	10.3	10.7	10.8	7.6	9.7	9.0	9.0	9.5	9.2	8.1	8.4	8.7	8.4	9.1	9.7	9.1	9.3
AT1G27260	2.8	2.6	2.7	2.7	3.0	3.4	3.5	3.3	3.1	4.0	2.1	3.1	2.7	2.8	2.3	2.6	2.0	3.0	2.5	2.5	2.0	2.0	2.6	2.2	2.6	2.0	2.6	2.4	2.5	2.2	2.6	2.4
AT1G51620	2.7	2.7	2.6	2.7	3.9	3.3	3.2	3.5	3.2	3.4	3.8	3.4	4.3	4.7	4.4	4.5	3.1	2.9	3.1	3.0	5.0	4.1	4.6	4.5	2.9	3.2	2.6	2.9	4.2	3.6	3.7	3.8
AT1G55980	3.2	3.3	3.2	3.2	3.3	2.8	2.4	2.8	2.8	2.4	3.2	2.8	3.0	4.1	4.2	3.7	4.4	3.0	3.0	3.5	4.6	5.0	4.6	4.7	2.9	2.9	2.9	2.9	2.9	3.9	3.4	3.4
AT1G57860	1.6	3.6	3.5	2.9	3.9	3.2	3.4	3.5	2.9	1.6	1.8	2.1	2.2	3.2	3.2	2.9	3.1	2.6	1.7	2.5	2.4	2.4	2.1	2.3	3.1	2.7	3.7	3.2	3.5	2.3	3.1	3.0
AT2G13422	3.7	2.9	2.8	3.2	2.4	1.9	1.3	1.8	3.2	3.6	3.0	3.3	2.8	2.1	3.4	2.8	2.3	1.3	2.5	2.0	3.0	2.9	3.6	3.2	2.0	3.7	2.6	2.8	1.6	1.1	2.0	1.6
AT2G23321	2.3	2.8	2.8	2.6	4.5	4.2	4.0	4.2	2.8	2.5	3.3	2.9	5.0	5.2	4.3	4.8	3.2	2.6	3.6	3.1	5.4	5.1	5.4	5.3	2.9	3.8	3.3	3.3	5.1	4.8	4.7	4.9
AT3G41762	8.4	8.7	9.0	8.7	8.7	9.3	8.6	8.9	8.2	8.9	8.4	8.5	8.4	8.4	8.6	8.5	8.8	8.7	7.6	8.3	7.9	7.5	8.1	7.9	7.9	8.0	8.0	8.0	8.0	8.5	8.1	8.2
AT3G56790	3.5	3.7	2.5	3.2	2.8	3.2	2.4	2.8	3.5	3.1	3.2	3.3	4.1	3.8	3.7	3.9	3.5	3.2	2.9	3.2	4.3	3.6	4.5	4.1	3.5	3.4	3.2	3.3	3.5	4.4	3.9	3.9
AT4G02230	8.1	8.6	8.2	8.3	8.3	8.4	8.0	8.3	8.4	8.0	8.4	8.2	8.1	8.3	7.9	8.1	6.3	6.3	6.3	6.3	6.2	6.2	6.1	6.1	6.1	5.6	6.0	5.9	5.9	6.0	5.8	5.9
AT4G03450	3.1	2.5	3.2	2.9	4.2	3.9	3.2	3.7	3.4	3.3	3.2	3.3	5.0	4.6	5.1	4.9	3.2	3.1	3.5	3.2	4.6	5.1	5.2	5.0	3.4	3.2	3.2	3.3	3.7	4.3	4.3	4.1
AT4G08875	3.2	3.7	2.9	3.2	4.1	4.0	3.8	4.0	3.3	2.8	2.9	3.0	3.2	3.4	2.5	3.0	2.9	3.4	3.5	3.2	3.0	2.5	2.7	2.8	3.4	3.6	3.7	3.6	2.8	3.2	3.5	3.2
AT4G10500	3.6	3.3	3.4	3.4	3.6	3.7	3.5	3.6	3.8	3.8	3.4	3.7	4.5	4.1	3.9	4.2	3.2	3.5	3.5	3.4	5.0	4.5	4.6	4.7	4.8	5.0	5.5	5.1	5.2	5.1	4.8	5.0
AT4G16960	2.5	2.5	3.9	3.0	3.5	3.4	3.5	3.5	4.1	4.0	3.6	3.9	4.5	4.8	4.3	4.5	3.5	3.6	3.9	3.7	4.5	4.8	4.6	4.6	3.6	4.2	4.1	4.0	4.4	4.7	4.5	4.5
AT4G22510	6.5	6.9	7.1	6.8	7.5	7.5	7.2	7.4	6.9	6.2	6.5	6.5	7.4	6.8	7.4	7.2	6.8	7.3	6.7	6.9	6.0	6.3	6.7	6.4	6.5	6.0	5.5	6.0	4.6	6.3	6.7	5.8
AT4G30640	3.0	2.6	2.8	2.8	2.5	2.5	2.2	2.4	2.9	2.8	2.5	2.7	3.4	3.3	3.4	3.4	2.9	3.1	3.0	3.0	3.5	3.6	3.6	3.6	2.8	2.8	2.6	2.7	2.8	2.8	3.3	2.9
AT4G31180	8.7	9.1	9.1	8.9	9.2	9.1	9.0	9.1	9.1	9.1	9.4	9.2	9.3	9.3	9.3	9.3	7.6	7.9	7.1	7.5	7.3	7.8	7.6	7.6	7.7	7.6	7.3	7.5	7.6	7.7	7.6	7.6
AT5G09290	3.4	3.6	2.6	3.2	4.8	4.6	4.6	4.7	4.6	4.2	3.9	4.2	5.5	5.8	5.2	5.5	4.2	4.2	4.1	4.2	5.7	5.8	6.3	5.9	5.5	4.5	4.9	5.0	5.1	5.4	5.6	5.3
AT5G19240	2.9	2.7	3.5	3.0	6.2	5.8	5.5	5.8	4.0	4.8	4.6	4.5	6.9	7.2	6.5	6.9	4.1	3.8	3.3	3.7	7.1	6.6	6.9	6.9	4.9	4.9	4.8	4.9	6.4	7.0	7.0	6.8
AT5G28160	2.4	1.8	1.8	2.0	3.4	2.8	3.4	3.2	3.5	2.0	2.9	2.8	3.3	1.8	2.8	2.6	2.5	2.2	2.8	2.5	2.0	1.9	2.4	2.1	2.0	2.8	2.7	2.5	2.7	2.2	2.4	2.4
AT5G52760	1.2	1.1	1.4	1.2	3.3	2.3	2.5	2.7	1.9	1.5	1.5	1.6	3.8	3.8	3.6	3.7	1.3	0.9	1.1	1.1	3.7	4.0	4.2	4.0	1.6	1.8	1.4	1.6	2.2	3.6	3.6	3.2
AT5G67460	3.2	3.7	2.9	3.2	3.3	3.2	3.4	3.3	3.2	3.2	3.3	3.2	3.3	3.3	3.6	3.4	3.0	2.7	3.1	2.9	3.3	3.8	3.1	3.4	2.8	3.5	3.6	3.3	3.1	2.9	3.8	3.3
BIP3	2.6	2.6	2.5	2.6	4.0	4.4	3.6	4.0	3.4	4.0	3.2	3.5	5.0	4.7	4.3	4.7	3.2	3.0	2.2	2.8	4.9	5.5	5.4	5.3	3.5	3.1	3.5	3.4	3.7	4.5	4.4	4.2
CCR4	2.5	1.9	1.8	2.1	2.6	2.9	2.5	2.7	2.4	2.4	2.3	2.4	4.2	3.7	3.5	3.8	2.2	2.0	2.1	2.1	4.2	3.6	4.3	4.0	2.2	2.0	1.8	2.0	2.6	2.7	3.4	2.9
CTF2B	5.2	4.6	4.2	4.7	5.8	5.6	5.2	5.5	4.1	5.2	4.3	4.5	6.4	6.8	6.1	6.4	4.7	4.5	5.4	4.8	6.9	6.3	6.4	6.5	5.2	5.4	4.5	5.0	5.8	6.2	6.4	6.1
CYP71B19	2.3	1.6	2.3	2.1	2.8	2.0	2.5	2.4	1.8	2.9	2.7	2.5	3.9	3.8	2.7	3.5	2.7	1.7	2.2	2.2	3.4	4.1	3.5	3.6	2.4	2.6	2.2	2.4	3.5	3.8	3.2	3.5
CYP96A5	2.9	4.0	3.8	3.6	3.5	3.6	2.5	3.2	3.0	4.2	3.6	3.6	3.5	4.2	3.5	3.7	3.9	2.8	3.7	3.5	5.0	4.5	4.7	4.7	2.9	3.2	3.2	3.1	3.6	4.2	3.4	3.7
MRD1	3.5	3.2	2.9	3.2	2.2	3.2	3.0	2.8	3.2	3.3	2.7	3.1	3.2	3.2	2.7	3.0	6.1	5.8	5.7	5.9	5.7	5.9	5.7	5.8	5.4	5.8	6.1	5.8	6.0	5.9	5.8	5.9
NAC036	2.8	2.7	2.9	2.8	4.8	4.6	4.3	4.6	3.7	3.4	3.6	3.6	5.5	5.8	4.9	5.4	3.8	3.3	3.0	3.4	5.8	5.5	6.0	5.7	4.0	3.9	3.9	3.9	5.2	6.6	6.3	6.0
NAC061	3.1	3.0	2.8	3.0	3.7	3.4	3.5	3.5	3.2	3.0	3.3	3.1	4.9	4.5	4.2	4.5	3.4	2.9	2.9	3.1	4.9	4.1	4.8	4.6	4.4	4.2	3.3	3.9	4.1	4.9	4.8	4.6
NAC090	3.4	2.8	3.1	3.1	3.9	3.7	3.4	3.7	3.6	3.6	2.9	3.4	5.9	6.0	4.5	5.5	3.0	1.9	3.1	2.7	5.5	5.1	6.3	5.6	3.7	3.3	3.2	3.4	3.9	4.8	5.5	4.7
NRT1.8	5.1	4.9	4.6	4.9	6.3	5.8	6.0	6.0	5.2	5.6	5.8	5.5	5.7	5.9	6.0	5.9	5.1	5.6	5.1	5.3	7.4	7.2	6.9	7.2	5.5	5.7	5.2	5.5	5.7	4.8	5.3	5.3
PAD3	1.2	1.3	1.2	1.2	3.4	2.7	2.7	2.9	2.0	1.4	1.9	1.7	4.0	4.4	3.3	3.9	1.7	1.8	1.5	1.7	4.3	3.9	3.8	4.0	2.0	3.4	2.8	2.7	3.6	3.9	4.0	3.8
PR5	3.8	3.4	4.1	3.8	4.1	4.0	3.7	3.9	5.3	4.1	3.2	4.2	5.9	4.8	5.0	5.2	3.3	3.5	3.5	3.4	5.4	4.6	5.1	5.0	3.7	3.2	3.2	3.4	3.6	4.2	5.1	4.3
RLK1	2.8	3.3	2.6	2.9	5.2	4.6	4.4	4.7	3.6	3.7	3.3	3.5	5.7	6.0	5.3	5.7	3.4	3.3	3.2	3.3	5.8	5.7	5.8	5.8	4.1	4.1	3.2	3.8	5.0	5.9	5.9	5.6
RLP23	4.3	3.6	3.7	3.9	5.5	5.4	4.8	5.2	4.7	4.9	4.1	4.6	7.5	6.7	6.6	7.0	3.6	4.0	4.4	4.0	7.3	6.5	6.8	6.9	4.6	4.9	4.4	4.6	5.1	6.0	6.5	5.9
RLP39	3.3	3.8	4.2	3.8	5.5	4.9	4.5	5.0	5.4	4.8	3.4	4.5	5.4	5.6	6.2	5.7	4.0	4.0	4.3	4.1	6.2	6.8	6.8	6.6	4.9	5.0	4.5	4.8	5.0	5.8	5.8	5.5
RLP7	3.9	3.7	4.1	3.9	5.2	5.0	5.5	5.2	4.2	4.1	3.9	4.0	5.7	5.9	5.4	5.7	3.4	3.8	3.5	3.6	6.4	6.2	6.2	6.2	4.2	4.8	4.2	4.4	5.7	6.1	6.0	5.9
TPX2	6.9	7.1	6.8	6.9	7.2	6.8	7.1	7.0	6.5	6.0	6.7	6.4	7.1	6.9	7.1	7.0	6.3	6.7	6.9	6.6	6.4	6.8	6.9	6.7	7.2	7.1	7.3	7.2	7.1	7.1	6.9	7.1

BABA-treated and *Hpa*-inoculated

	CHM 1	CHM 2	CHM 3	**CHM avg**	CHP 1	CHP 2	CHP 3	**CHP avg**	CBM 1	CBM 2	CBM 3	**CBM avg**	CBP 1	CBP 2	CBP 3	**CBP avg**	IHM 1	IHM 2	IHM 3	**IHM avg**	IHP1	IHP2	IHP3	**IHP avg**	IBM 1	IBM 2	IBM 3	**IBM avg**	IBP1	IBP2	IBP3	**IBP avg**
ABF2	4.7	4.5	4.3	**4.5**	5.0	4.5	4.9	**4.8**	4.5	4.4	4.6	**4.5**	4.2	4.6	4.4	**4.4**	4.5	4.8	4.8	**4.7**	4.4	5.1	5.1	**4.9**	4.7	4.8	4.6	**4.7**	5.7	5.7	5.6	**5.7**
ABF3	4.2	4.5	4.6	**4.4**	4.6	4.5	4.6	**4.6**	4.4	5.4	5.2	**5.0**	4.4	4.1	4.5	**4.4**	5.0	4.6	4.5	**4.7**	4.0	4.3	4.6	**4.3**	4.7	4.9	4.3	**4.6**	5.7	5.7	5.3	**5.6**
ABI1	6.6	6.1	6.3	**6.4**	7.1	7.0	7.0	**7.0**	6.4	6.4	6.6	**6.5**	7.1	7.2	7.1	**7.1**	6.3	6.4	6.5	**6.4**	7.3	7.3	7.2	**7.3**	6.9	6.9	6.6	**6.8**	8.7	8.3	8.4	**8.5**
ABI2	4.9	5.3	4.9	**5.0**	5.1	4.9	5.4	**5.1**	5.0	5.5	4.9	**5.1**	4.9	5.0	5.5	**5.1**	4.7	4.4	5.1	**4.7**	4.7	5.0	5.1	**4.9**	5.1	5.4	4.9	**5.2**	6.8	6.2	5.8	**6.3**
ACHT5	8.6	8.5	8.5	**8.5**	8.5	8.3	8.3	**8.3**	8.2	8.2	8.2	**8.2**	8.7	8.0	8.3	**8.3**	8.3	8.5	8.4	**8.4**	8.0	8.2	8.3	**8.2**	7.8	8.0	8.0	**8.0**	6.9	7.1	7.4	**7.2**
AFP1	4.5	4.3	4.3	**4.3**	4.8	4.2	5.1	**4.7**	4.7	4.7	4.5	**4.6**	4.7	4.7	4.4	**4.6**	4.1	4.6	4.7	**4.5**	4.3	4.6	5.0	**4.6**	4.7	4.3	4.7	**4.6**	6.8	6.7	5.9	**6.5**
AGP1	7.4	7.5	7.0	**7.3**	7.9	7.3	7.7	**7.6**	7.4	7.0	7.7	**7.4**	7.9	7.6	7.9	**7.8**	7.0	7.4	6.8	**7.1**	8.0	7.6	8.0	**7.9**	7.7	7.9	7.8	**7.8**	9.2	8.5	8.9	**8.8**
AHL20	5.4	5.2	5.6	**5.4**	4.9	5.2	5.5	**5.2**	5.5	5.1	5.8	**5.4**	5.4	5.1	5.3	**5.3**	5.4	5.3	6.0	**5.6**	5.5	5.5	5.5	**5.5**	6.3	6.2	6.5	**6.3**	6.8	6.4	6.1	**6.4**
AK3	5.0	4.9	4.9	**5.0**	3.6	3.9	4.3	**3.9**	4.7	3.9	4.5	**4.4**	4.3	3.5	4.1	**4.0**	4.7	4.6	4.0	**4.5**	3.2	3.5	3.6	**3.4**	5.5	5.1	5.2	**5.3**	6.1	5.1	4.8	**5.4**
AT1G02650	6.6	6.6	6.6	**6.6**	6.2	6.5	6.1	**6.3**	6.4	6.1	6.0	**6.2**	6.2	6.0	6.3	**6.2**	6.4	6.2	6.7	**6.5**	6.5	6.3	6.1	**6.3**	5.8	5.7	5.7	**5.7**	4.8	5.5	5.1	**5.1**
AT1G08890	6.4	6.8	6.8	**6.6**	6.3	6.1	6.7	**6.4**	6.6	7.5	7.0	**7.0**	6.1	6.3	6.2	**6.2**	6.6	6.5	6.8	**6.6**	6.7	6.7	6.7	**6.7**	6.3	6.8	6.7	**6.6**	7.7	7.3	7.0	**7.3**
AT1G11210	3.2	3.2	3.3	**3.2**	3.7	3.3	4.0	**3.6**	3.1	3.4	3.2	**3.2**	3.1	3.7	3.9	**3.5**	2.7	3.0	3.2	**3.0**	4.3	3.7	3.9	**4.0**	3.4	3.4	3.1	**3.3**	5.6	5.6	5.6	**5.6**
AT1G12200	4.0	3.5	3.8	**3.8**	4.3	3.9	4.2	**4.1**	4.6	4.4	4.2	**4.4**	3.8	4.6	4.6	**4.4**	3.4	4.4	4.4	**4.0**	5.4	4.5	4.3	**4.7**	4.6	5.6	5.5	**5.2**	5.7	5.3	5.2	**5.4**
AT1G19530	4.3	4.7	4.7	**4.6**	5.7	5.2	5.7	**5.5**	4.5	4.9	4.9	**4.7**	5.4	5.0	5.8	**5.4**	4.8	5.3	5.1	**5.1**	6.0	6.2	6.1	**6.1**	4.0	3.8	4.2	**4.0**	4.4	4.2	4.1	**4.2**
AT1G24580	4.0	3.5	4.0	**3.8**	3.7	3.8	3.5	**3.7**	3.9	3.5	3.7	**3.7**	3.7	3.6	3.8	**3.7**	3.9	4.2	3.7	**3.9**	3.4	3.5	3.7	**3.6**	3.9	3.9	3.8	**3.9**	6.4	5.5	5.5	**5.8**
AT1G25400	5.6	5.6	5.4	**5.5**	6.7	6.5	6.3	**6.5**	5.5	5.6	5.5	**5.5**	6.7	6.6	6.5	**6.6**	5.5	5.7	5.4	**5.5**	6.6	6.5	6.5	**6.5**	4.8	4.6	4.8	**4.7**	5.0	5.3	5.6	**5.3**
AT1G29195	3.6	3.2	3.4	**3.4**	3.1	3.6	3.3	**3.3**	2.8	3.5	3.2	**3.2**	2.6	3.1	3.3	**3.0**	2.5	3.2	2.9	**2.9**	3.4	3.1	3.3	**3.2**	4.3	4.2	4.1	**4.2**	5.0	4.3	3.9	**4.4**
AT1G30720	6.6	6.6	6.9	**6.7**	8.0	7.4	7.8	**7.7**	6.8	6.8	7.2	**6.9**	8.0	7.9	7.5	**7.8**	6.3	6.7	6.7	**6.6**	8.5	8.0	7.9	**8.1**	5.6	5.2	5.9	**5.6**	6.8	6.3	6.8	**6.6**
AT1G53490	4.7	4.8	4.3	**4.6**	4.9	4.7	4.5	**4.7**	4.2	4.4	4.7	**4.4**	4.6	4.5	4.5	**4.5**	5.2	5.1	5.4	**5.2**	5.3	5.4	5.9	**5.5**	5.3	5.1	5.3	**5.2**	5.5	5.9	5.4	**5.6**
AT1G60190	1.6	1.2	1.1	**1.3**	1.2	1.4	1.9	**1.5**	1.5	1.4	1.1	**1.3**	1.5	1.4	1.9	**1.6**	1.4	1.3	1.2	**1.3**	1.8	1.4	1.6	**1.6**	1.5	1.5	1.5	**1.5**	3.9	3.5	4.1	**3.8**
AT1G64380	4.2	4.2	4.5	**4.3**	4.8	4.7	4.9	**4.8**	4.4	4.3	4.6	**4.4**	4.1	4.6	4.8	**4.5**	4.8	4.6	4.6	**4.7**	4.9	5.0	4.9	**4.9**	3.9	4.5	3.8	**4.1**	6.0	5.7	5.7	**5.8**
AT1G66460	3.6	3.4	3.0	**3.3**	3.1	4.4	3.3	**3.6**	3.5	3.0	3.4	**3.3**	4.1	3.7	3.4	**3.7**	3.4	3.5	3.2	**3.4**	3.0	3.8	2.6	**3.1**	3.7	2.9	3.0	**3.2**	2.4	2.5	3.1	**2.7**
AT1G68600	3.6	3.4	3.3	**3.4**	2.7	3.0	3.3	**3.0**	3.8	3.6	3.3	**3.6**	2.3	2.2	2.8	**2.4**	3.5	3.7	3.5	**3.5**	2.8	2.4	2.6	**2.6**	3.5	4.0	3.6	**3.7**	3.4	3.5	3.3	**3.4**
AT1G77200	7.4	7.4	7.4	**7.4**	6.6	6.7	7.2	**6.9**	7.0	7.5	7.4	**7.3**	6.5	6.5	6.6	**6.5**	7.4	7.4	7.7	**7.5**	6.7	6.7	6.9	**6.8**	7.6	7.8	7.2	**7.5**	7.7	7.6	7.4	**7.6**
AT1G78070	6.3	6.5	7.0	**6.6**	6.1	6.1	7.0	**6.4**	6.9	7.8	7.2	**7.3**	6.1	6.7	6.3	**6.4**	6.4	6.0	6.9	**6.5**	6.7	7.0	6.7	**6.8**	6.5	6.9	6.6	**6.7**	9.6	8.8	8.8	**9.1**
AT1G78230	7.6	7.8	8.1	**7.8**	7.2	7.4	7.7	**7.4**	7.8	8.1	8.1	**8.0**	6.9	7.3	7.6	**7.3**	7.7	7.6	7.9	**7.8**	7.2	7.3	7.0	**7.2**	8.2	8.3	8.1	**8.2**	8.7	7.9	8.1	**8.3**
AT1G78450	6.6	6.5	6.7	**6.6**	6.4	6.5	6.3	**6.4**	6.4	6.0	6.3	**6.2**	5.9	6.0	6.3	**6.1**	6.5	6.6	6.4	**6.5**	6.1	6.7	6.6	**6.5**	5.2	5.3	5.6	**5.4**	4.2	4.8	5.2	**4.8**
AT1G79520	4.1	5.1	4.6	**4.6**	4.4	5.6	5.6	**5.2**	4.4	5.0	5.3	**4.9**	4.8	5.3	6.0	**5.4**	5.1	5.0	5.0	**5.0**	4.7	5.0	5.0	**4.9**	5.3	6.0	5.0	**5.4**	7.1	6.4	6.4	**6.7**
AT1G80130	4.5	5.2	5.1	**4.9**	5.6	5.5	5.3	**5.5**	4.9	6.0	5.1	**5.4**	5.2	5.6	5.1	**5.3**	4.9	4.7	4.8	**4.8**	5.7	5.4	5.5	**5.5**	5.5	5.7	5.5	**5.6**	7.2	7.1	7.0	**7.1**
AT2G04050	2.4	2.7	2.3	**2.4**	1.6	2.7	1.8	**2.0**	2.5	2.2	2.6	**2.4**	2.5	2.1	2.1	**2.3**	1.6	1.4	2.4	**1.8**	1.7	1.8	1.8	**1.7**	4.4	5.5	4.3	**4.7**	5.7	4.6	3.7	**4.7**
AT2G11851	2.1	2.1	2.0	**2.1**	2.3	2.1	2.0	**2.1**	1.9	2.7	1.8	**2.1**	1.9	2.3	2.1	**2.1**	2.0	2.3	2.5	**2.3**	2.2	2.2	1.8	**2.1**	2.3	2.5	2.2	**2.4**	2.2	2.2	1.6	**2.0**
AT2G15830	6.8	6.7	6.9	**6.8**	6.9	6.8	7.2	**7.0**	6.5	7.5	7.0	**7.0**	6.0	6.7	6.8	**6.5**	6.6	6.5	6.8	**6.6**	7.1	6.6	7.0	**6.9**	6.4	7.0	7.0	**6.8**	7.7	7.5	7.5	**7.6**
AT2G18969	4.4	4.5	3.9	**4.3**	4.2	4.0	4.3	**4.2**	4.7	4.8	4.7	**4.7**	3.8	3.6	4.0	**3.8**	4.3	4.5	4.4	**4.4**	4.2	3.7	4.2	**4.0**	4.6	4.4	4.4	**4.5**	4.8	5.1	5.3	**5.1**
AT2G20835	8.9	9.2	9.1	**9.1**	8.6	8.5	8.5	**8.5**	8.9	9.6	9.1	**9.2**	7.9	8.2	8.1	**8.0**	9.1	8.9	9.3	**9.1**	8.7	8.3	8.2	**8.4**	8.3	8.7	8.8	**8.6**	9.3	9.0	9.0	**9.1**
AT2G23830	2.9	2.9	2.8	**2.9**	3.3	3.9	3.2	**3.5**	2.3	2.4	3.1	**2.6**	3.0	3.4	3.5	**3.3**	3.9	3.0	2.7	**3.2**	3.8	4.3	4.5	**4.2**	4.0	4.8	4.3	**4.4**	5.1	4.7	4.7	**4.8**
AT2G28400	4.4	4.1	4.3	**4.3**	6.3	5.8	6.2	**6.1**	5.2	6.0	4.9	**5.4**	5.5	6.3	6.1	**6.0**	3.9	4.9	5.0	**4.6**	6.4	6.4	6.7	**6.5**	4.7	5.3	4.8	**4.9**	7.7	7.4	7.0	**7.3**
AT2G35070	3.4	4.3	5.6	**4.4**	5.9	5.6	7.5	**6.3**	5.1	6.8	5.5	**5.8**	6.0	5.9	6.7	**6.2**	4.6	4.9	4.9	**4.8**	6.5	6.3	6.7	**6.5**	5.5	5.8	5.3	**5.5**	8.6	7.7	7.7	**8.0**
AT2G40475	5.9	5.8	6.0	**5.9**	5.6	5.6	5.8	**5.6**	5.8	5.7	6.0	**5.8**	5.7	5.6	5.5	**5.6**	5.9	6.1	5.7	**5.9**	6.0	5.7	5.6	**5.7**	5.9	6.5	6.0	**6.1**	6.9	6.8	6.5	**6.7**
AT2G40955	1.8	2.0	2.1	**2.0**	1.7	1.8	1.9	**1.8**	2.1	2.3	1.6	**2.0**	2.4	2.9	2.1	**2.5**	1.8	1.7	2.0	**1.8**	1.7	2.0	2.2	**2.0**	2.0	1.7	1.2	**1.6**	1.2	1.7	1.4	**1.4**
AT2G41870	5.3	5.2	5.0	**5.2**	5.1	5.2	5.3	**5.2**	5.1	5.3	5.1	**5.1**	5.3	5.6	5.3	**5.4**	5.0	5.2	5.3	**5.2**	5.3	5.7	5.4	**5.5**	5.5	5.5	5.1	**5.4**	6.6	6.4	6.2	**6.4**
AT2G44940	2.2	2.5	2.3	**2.3**	3.1	2.4	2.7	**2.8**	3.1	2.5	2.3	**2.6**	2.7	3.1	2.1	**2.6**	2.9	2.5	2.6	**2.7**	2.6	3.3	2.5	**2.8**	3.8	3.0	3.0	**3.3**	3.5	4.1	3.9	**3.8**
AT2G46740	5.0	4.7	5.0	**4.9**	5.2	5.3	5.4	**5.3**	5.2	4.8	5.3	**5.1**	6.2	5.8	5.8	**5.9**	5.9	4.8	5.9	**5.5**	6.5	6.3	6.1	**6.3**	5.5	4.0	5.2	**4.9**	3.4	4.2	4.6	**4.1**
AT3G03341	2.6	2.8	3.2	**2.9**	2.5	2.6	3.2	**2.7**	2.9	3.2	2.5	**2.9**	2.7	2.5	2.6	**2.6**	2.8	2.2	2.9	**2.6**	2.0	2.0	2.4	**2.1**	4.2	4.2	2.7	**3.7**	4.2	3.7	3.4	**3.7**
AT3G19390	6.6	7.1	7.0	**6.9**	7.3	7.1	6.8	**7.1**	6.9	6.8	7.0	**6.9**	7.2	6.8	7.6	**7.2**	7.1	7.0	6.7	**7.0**	7.7	7.5	7.4	**7.5**	6.7	5.9	6.2	**6.3**	5.6	5.5	6.5	**5.9**
AT3G19620	4.6	4.4	4.2	**4.4**	4.7	4.5	5.2	**4.8**	4.8	4.6	4.7	**4.7**	4.9	4.8	5.1	**4.9**	4.2	4.8	4.2	**4.4**	4.7	4.3	4.7	**4.6**	5.7	5.4	5.2	**5.4**	6.7	6.1	5.8	**6.2**
AT3G25190	7.9	8.0	7.9	**7.9**	8.2	8.5	8.4	**8.4**	8.2	6.8	7.9	**7.6**	8.7	8.6	8.9	**8.7**	7.8	8.1	7.9	**7.9**	8.0	8.6	8.5	**8.4**	8.3	7.3	8.0	**7.9**	6.9	7.6	7.8	**7.4**
AT3G27250	1.6	2.0	1.8	**1.8**	2.4	2.2	1.9	**2.2**	1.9	2.3	1.6	**1.9**	2.0	1.8	1.3	**1.7**	2.1	2.0	1.6	**1.9**	1.6	1.6	1.6	**1.6**	1.9	1.9	1.8	**1.9**	3.1	2.5	2.5	**2.7**
AT3G45690	2.8	1.8	1.7	**2.1**	2.3	2.6	2.2	**2.4**	2.3	1.7	2.1	**2.0**	2.8	2.7	3.4	**3.0**	2.3	1.5	2.1	**1.9**	2.0	1.9	2.1	**2.0**	2.2	2.1	1.8	**2.0**	1.6	1.8	2.0	**1.8**
AT3G51540	4.6	4.5	4.9	**4.6**	5.6	5.5	5.4	**5.5**	4.8	4.4	4.8	**4.6**	5.7	5.1	5.6	**5.5**	4.8	4.9	4.7	**4.8**	5.7	5.6	5.8	**5.7**	5.2	4.4	4.7	**4.7**	4.5	4.1	4.8	**4.5**
AT3G53980	5.3	5.7	5.0	**5.3**	6.1	4.7	6.0	**5.6**	5.3	4.7	6.7	**5.6**	5.7	5.1	5.3	**5.4**	6.2	5.0	5.3	**5.5**	3.7	4.5	4.5	**4.2**	7.7	7.3	6.5	**7.2**	7.8	7.5	7.3	**7.5**
AT3G54880	9.2	9.3	9.1	**9.2**	9.4	9.4	9.4	**9.4**	9.1	9.0	9.0	**9.0**	9.5	9.3	9.4	**9.4**	9.3	9.3	9.1	**9.2**	8.9	9.2	9.3	**9.1**	8.7	8.5	8.8	**8.7**	7.7	8.4	8.5	**8.2**

AT3G55646	4.6	3.7	4.8	**4.4**	4.7	5.0	4.9	**4.9**	4.1	3.9	3.9	**3.9**	4.3	4.3	4.6	**4.4**	3.9	4.1	4.1	**4.0**	4.6	4.5	4.6	**4.6**	3.3	3.1	3.0	**3.1**	3.2	3.6	3.2	**3.3**
AT3G57460	3.5	3.2	3.0	**3.3**	5.6	5.7	4.4	**5.2**	4.0	5.2	4.3	**4.5**	6.2	7.0	5.9	**6.3**	3.6	4.1	2.8	**3.5**	5.9	5.8	6.8	**6.2**	3.8	3.3	3.5	**3.5**	4.3	4.9	5.3	**4.9**
AT4G01380	4.5	3.9	3.9	**4.1**	4.1	3.3	3.1	**3.5**	3.4	3.1	4.1	**3.5**	3.7	4.0	3.2	**3.6**	3.3	3.3	3.5	**3.4**	3.2	2.9	3.6	**3.2**	4.4	4.9	4.7	**4.7**	4.2	5.2	4.8	**4.7**
AT4G02230	8.1	8.6	8.2	**8.3**	8.3	8.4	8.0	**8.3**	8.4	8.0	8.4	**8.2**	8.1	8.3	7.9	**8.1**	6.3	6.3	6.3	**6.3**	6.2	6.2	6.1	**6.1**	6.1	5.6	6.0	**5.9**	5.9	6.0	5.8	**5.9**
AT4G15660	5.2	4.5	5.1	**4.9**	4.6	4.0	4.1	**4.2**	5.0	4.7	5.0	**4.9**	4.6	4.3	3.8	**4.2**	4.5	4.5	4.5	**4.5**	4.2	4.4	4.8	**4.5**	3.1	1.9	2.5	**2.5**	3.6	2.7	3.1	**3.1**
AT4G15680	6.0	6.4	6.1	**6.2**	5.5	4.9	4.9	**5.1**	5.6	5.3	5.7	**5.5**	5.7	5.3	4.8	**5.3**	5.2	5.0	6.0	**5.4**	4.9	5.4	4.9	**5.1**	3.1	3.7	3.3	**3.4**	4.1	3.6	4.2	**4.0**
AT4G16750	3.6	4.0	4.0	**3.9**	3.9	3.8	4.4	**4.0**	4.3	3.7	3.6	**3.9**	4.7	4.4	3.9	**4.3**	4.4	4.0	4.3	**4.2**	3.9	4.0	4.3	**4.1**	4.7	4.7	4.3	**4.5**	5.7	5.9	5.4	**5.7**
AT4G26288	7.4	7.3	7.6	**7.5**	8.1	7.9	7.9	**8.0**	7.2	7.6	7.5	**7.4**	7.6	7.5	7.8	**7.6**	7.5	7.6	7.7	**7.6**	7.7	7.8	7.5	**7.7**	6.7	6.6	6.5	**6.6**	5.8	6.0	6.4	**6.1**
AT4G26290	7.2	7.2	7.2	**7.2**	7.8	7.7	7.4	**7.6**	6.7	7.1	6.9	**6.9**	7.2	7.1	7.4	**7.2**	7.3	7.3	6.7	**7.1**	6.8	6.8	6.9	**6.9**	6.0	5.4	5.5	**5.6**	5.1	5.2	5.3	**5.2**
AT4G27657	4.9	5.0	5.3	**5.0**	5.5	5.1	5.2	**5.3**	5.0	4.9	4.9	**4.9**	6.2	5.3	4.8	**5.4**	5.2	5.5	4.6	**5.1**	6.6	5.7	6.2	**6.2**	4.7	4.9	5.0	**4.8**	7.6	7.6	7.4	**7.5**
AT4G28140	2.8	3.1	3.0	**3.0**	2.9	2.6	3.2	**2.9**	3.3	2.9	2.9	**3.0**	3.0	2.5	3.0	**2.8**	2.9	2.7	3.3	**3.0**	3.0	2.9	3.1	**3.0**	2.9	2.9	2.9	**2.9**	3.6	3.6	4.3	**3.8**
AT4G30650	5.6	5.9	6.1	**5.9**	5.3	5.6	6.2	**5.7**	6.2	6.6	6.2	**6.4**	5.5	6.0	6.0	**5.8**	6.2	5.9	6.0	**6.1**	5.9	5.7	6.0	**5.8**	6.5	6.9	6.1	**6.5**	7.7	6.7	6.8	**7.1**
AT4G31180	8.7	9.1	9.1	**8.9**	9.2	9.1	9.0	**9.1**	9.1	9.1	9.4	**9.2**	9.3	9.3	9.3	**9.3**	7.6	7.9	7.1	**7.5**	7.3	7.8	7.6	**7.6**	7.7	7.6	7.3	**7.5**	7.6	7.7	7.6	**7.6**
AT4G36850	9.0	9.0	8.7	**8.9**	9.0	9.1	9.3	**9.1**	8.9	8.9	9.1	**9.0**	9.2	9.2	9.2	**9.2**	8.6	8.9	8.5	**8.7**	8.9	8.7	8.9	**8.8**	8.7	8.4	8.5	**8.5**	8.0	8.1	8.4	**8.2**
AT4G37220	7.1	7.3	7.3	**7.2**	7.6	7.6	7.9	**7.7**	7.0	6.5	7.0	**6.9**	7.4	7.2	7.6	**7.4**	7.3	7.5	7.3	**7.4**	7.4	7.7	7.5	**7.6**	5.7	5.8	6.0	**5.8**	5.4	5.6	6.5	**5.8**
AT5G02890	3.9	3.6	3.3	**3.6**	3.3	3.5	3.8	**3.5**	3.9	3.5	3.7	**3.7**	3.4	3.6	2.8	**3.3**	3.9	3.4	3.5	**3.6**	3.3	3.0	3.0	**3.1**	4.0	4.0	4.4	**4.1**	4.2	4.1	4.7	**4.3**
AT5G03210	2.4	2.4	2.5	**2.5**	2.4	2.7	3.2	**2.8**	2.6	2.4	2.3	**2.4**	2.8	3.0	2.9	**2.9**	2.6	2.7	2.5	**2.6**	3.3	2.7	3.4	**3.1**	2.6	2.9	2.2	**2.5**	5.2	4.7	4.8	**4.9**
AT5G08350	8.1	7.8	8.0	**8.0**	8.2	7.9	8.1	**8.1**	7.9	7.9	7.9	**7.9**	8.0	7.7	7.7	**7.8**	8.0	8.1	8.1	**8.1**	7.9	7.9	8.0	**7.9**	7.3	7.5	7.4	**7.4**	6.6	6.6	7.0	**6.7**
AT5G09520	6.4	6.9	6.9	**6.7**	5.2	6.1	5.5	**5.6**	6.6	5.1	6.3	**6.0**	5.6	5.2	5.9	**5.6**	7.5	6.7	7.1	**7.1**	5.7	6.3	5.1	**5.7**	8.0	8.3	8.2	**8.2**	7.6	6.5	6.7	**6.9**
AT5G14470	5.8	6.0	5.6	**5.8**	6.5	6.2	6.0	**6.2**	4.9	5.4	5.8	**5.4**	5.5	6.0	6.3	**5.9**	5.7	5.7	6.1	**5.8**	6.3	6.6	6.2	**6.3**	4.6	4.5	4.6	**4.6**	4.7	4.9	5.1	**4.9**
AT5G16200	5.0	4.8	5.0	**4.9**	5.1	5.3	5.3	**5.2**	4.9	4.8	5.2	**5.0**	5.6	5.4	5.2	**5.4**	5.1	5.1	5.0	**5.0**	5.5	5.4	5.5	**5.5**	5.2	5.2	5.3	**5.2**	6.9	6.2	6.2	**6.4**
AT5G23360	6.6	7.0	6.9	**6.8**	6.8	6.6	7.1	**6.8**	6.6	6.6	6.8	**6.7**	6.6	6.6	6.5	**6.6**	7.1	6.7	7.2	**7.0**	6.2	6.5	6.3	**6.4**	6.6	6.4	6.2	**6.4**	5.3	5.0	5.6	**5.3**
AT5G27920	6.4	7.0	6.9	**6.8**	7.0	7.0	7.2	**7.1**	7.0	6.8	6.8	**6.9**	7.3	7.1	7.3	**7.2**	6.8	7.0	6.7	**6.8**	7.0	7.1	6.9	**7.0**	6.3	6.0	6.3	**6.2**	6.0	6.2	6.4	**6.2**
AT5G38710	4.3	4.5	4.7	**4.5**	5.3	5.3	5.2	**5.2**	4.7	4.2	4.6	**4.5**	5.8	5.6	5.5	**5.6**	4.5	4.4	4.4	**4.4**	6.0	6.0	5.9	**6.0**	4.2	4.3	3.9	**4.1**	4.5	4.5	4.7	**4.6**
AT5G41080	10.9	11.2	11.1	**11.1**	11.4	11.3	11.2	**11.3**	11.1	10.9	11.1	**11.0**	11.5	11.2	11.2	**11.3**	11.3	11.1	11.0	**11.1**	11.0	11.2	11.2	**11.1**	10.7	10.2	10.7	**10.5**	8.1	9.2	9.9	**9.1**
AT5G44585	5.6	5.9	5.1	**5.6**	7.9	7.6	7.0	**7.5**	6.4	7.1	7.1	**6.9**	8.6	8.7	8.2	**8.5**	6.3	6.4	6.8	**6.5**	8.8	8.3	8.1	**8.4**	5.9	5.4	5.6	**5.6**	6.6	6.9	7.2	**6.9**
AT5G44973	5.7	6.0	6.1	**6.0**	6.5	6.4	6.4	**6.4**	5.8	6.0	5.9	**5.9**	6.5	6.5	6.7	**6.6**	6.9	5.7	5.9	**6.2**	6.5	6.5	6.5	**6.5**	5.8	5.7	5.4	**5.6**	4.8	5.5	5.4	**5.2**
AT5G46871	3.7	2.9	3.5	**3.3**	4.3	4.5	4.2	**4.4**	4.4	4.0	3.3	**3.9**	4.9	5.2	4.6	**4.9**	3.2	3.9	3.6	**3.6**	4.1	3.9	3.3	**3.8**	3.9	3.2	3.4	**3.5**	3.5	2.9	3.7	**3.3**
AT5G63130	3.3	2.5	2.1	**2.6**	3.5	3.7	2.4	**3.2**	2.8	3.1	2.6	**2.8**	4.3	4.6	4.0	**4.3**	2.0	2.9	2.8	**2.5**	4.5	4.7	4.9	**4.7**	3.9	2.4	2.4	**2.9**	6.0	5.9	5.6	**5.8**
BCE2	9.0	9.6	9.4	**9.4**	9.9	9.9	10.0	**9.9**	9.4	9.3	9.6	**9.4**	9.8	9.7	9.7	**9.7**	9.5	9.4	9.3	**9.4**	9.8	10.0	10.1	**9.9**	8.5	8.4	8.8	**8.6**	8.2	8.6	9.0	**8.6**
BGLU34	2.3	3.8	3.0	**3.0**	3.4	2.9	2.6	**2.9**	3.0	2.4	4.2	**3.2**	4.5	3.4	3.9	**3.9**	2.7	4.1	3.7	**3.5**	2.4	2.6	1.9	**2.3**	6.0	3.9	5.0	**5.0**	2.7	3.0	2.4	**2.7**
BT2	10.4	10.4	10.4	**10.4**	10.3	10.0	10.3	**10.2**	10.1	9.9	10.1	**10.0**	10.2	10.0	10.3	**10.2**	10.0	10.3	10.5	**10.2**	10.3	10.2	10.2	**10.2**	9.4	9.1	9.4	**9.3**	8.9	9.2	9.2	**9.1**
BT5	7.6	7.8	7.8	**7.7**	8.1	8.0	8.0	**8.1**	7.4	7.6	7.7	**7.5**	8.0	7.8	8.0	**7.9**	7.5	7.8	7.8	**7.7**	7.9	7.9	7.9	**7.9**	7.1	6.9	7.2	**7.1**	6.1	6.7	7.0	**6.6**
CBF1	1.8	1.8	2.3	**2.0**	2.0	2.1	2.5	**2.2**	2.1	1.8	2.1	**2.0**	2.2	2.0	2.3	**2.2**	1.8	2.1	1.9	**1.9**	2.6	2.2	2.5	**2.4**	1.6	2.0	1.6	**1.7**	3.7	4.0	4.1	**3.9**
CBF2	2.1	2.4	2.5	**2.3**	2.0	2.5	2.3	**2.3**	2.6	2.5	2.1	**2.4**	2.3	2.4	2.6	**2.4**	2.1	2.2	2.4	**2.2**	2.8	2.9	2.7	**2.8**	1.9	1.7	2.1	**1.9**	3.6	4.2	4.5	**4.1**
CCOAMT	2.7	2.5	2.4	**2.5**	5.4	4.9	5.0	**5.1**	3.2	3.8	3.5	**3.5**	5.4	5.7	5.5	**5.5**	3.0	3.0	2.7	**2.9**	5.8	5.7	5.8	**5.7**	3.0	3.4	3.4	**3.3**	4.2	4.8	4.5	**4.5**
CDF1	8.9	8.8	9.0	**8.9**	9.0	9.0	9.0	**9.0**	9.2	9.1	9.1	**9.1**	9.0	9.0	9.1	**9.0**	9.2	9.2	8.9	**9.1**	8.9	8.9	9.0	**8.9**	8.8	9.0	9.0	**8.9**	9.0	9.1	8.9	**9.0**
CHX11	2.6	2.6	2.2	**2.4**	2.0	3.4	3.6	**3.0**	2.2	1.5	2.6	**2.1**	2.8	2.6	3.6	**3.0**	2.4	3.0	2.1	**2.5**	3.6	3.3	2.5	**3.1**	2.7	2.1	2.2	**2.3**	2.0	2.1	1.4	**1.8**
CID9	5.6	6.0	5.5	**5.7**	5.3	5.1	6.0	**5.4**	6.3	6.6	5.9	**6.3**	4.7	4.9	4.9	**4.9**	6.1	6.0	5.7	**6.0**	5.2	5.2	5.2	**5.2**	5.9	6.0	5.8	**5.9**	6.4	5.9	5.6	**6.0**
COR15A	9.3	9.0	9.8	**9.4**	8.8	9.0	11.2	**9.6**	9.9	10.6	10.0	**10.2**	9.5	9.3	10.3	**9.7**	9.4	8.7	9.5	**9.2**	9.1	8.4	9.3	**8.9**	10.7	11.2	10.5	**10.8**	11.7	11.1	10.8	**11.2**
COR15B	3.7	4.2	4.0	**3.9**	3.7	4.0	3.9	**3.9**	4.3	5.2	4.5	**4.7**	3.9	4.2	4.0	**4.0**	4.3	3.9	3.6	**3.9**	4.0	3.9	4.0	**3.9**	4.8	5.0	4.6	**4.8**	6.1	5.3	5.0	**5.5**
CPuORF10	3.0	2.9	3.3	**3.1**	3.2	3.1	3.4	**3.3**	3.3	3.3	3.0	**3.2**	3.3	3.4	2.9	**3.2**	3.4	3.2	2.9	**3.1**	3.1	2.9	3.1	**3.0**	3.2	3.4	3.5	**3.4**	5.1	4.7	4.1	**4.6**
CRK5	2.0	2.0	2.2	**2.1**	3.3	3.1	3.3	**3.3**	3.0	2.8	3.0	**2.9**	4.1	4.5	4.2	**4.3**	2.5	3.4	2.3	**2.8**	4.2	3.5	4.4	**4.0**	2.6	2.5	2.3	**2.5**	2.8	3.4	3.4	**3.2**
CYP78A8	4.0	4.1	4.2	**4.1**	3.6	3.5	3.8	**3.6**	4.4	3.6	4.1	**4.0**	4.0	3.8	4.1	**4.0**	4.0	4.4	3.9	**4.1**	4.2	3.9	3.7	**3.9**	4.0	3.7	3.8	**3.9**	2.7	3.0	3.1	**2.9**
DIN11	3.1	2.8	3.4	**3.1**	4.6	4.7	4.3	**4.5**	3.0	3.0	3.0	**3.0**	4.7	4.9	4.5	**4.7**	3.0	3.5	2.9	**3.1**	5.1	5.0	5.6	**5.2**	2.6	3.1	2.6	**2.8**	3.1	3.5	3.3	**3.3**
DOGT1	4.8	3.5	4.3	**4.2**	4.7	4.9	5.1	**4.9**	4.1	3.8	4.1	**4.0**	4.3	4.9	4.0	**4.4**	4.5	4.9	4.3	**4.5**	4.8	4.5	4.5	**4.6**	4.1	5.9	4.7	**4.9**	6.1	6.0	5.2	**5.8**
DREB2A	4.2	4.3	4.5	**4.3**	4.5	4.2	4.8	**4.5**	4.2	4.8	4.6	**4.5**	4.5	4.7	4.5	**4.6**	4.4	4.0	4.3	**4.2**	5.0	5.2	4.7	**5.0**	3.8	4.2	4.0	**4.0**	6.0	5.8	5.2	**5.7**
DREB2B	3.7	4.2	4.4	**4.1**	4.7	4.5	4.4	**4.5**	4.2	4.5	4.3	**4.3**	4.6	4.3	4.4	**4.4**	3.4	4.1	4.8	**4.1**	5.1	4.7	4.6	**4.8**	4.0	4.7	4.2	**4.3**	5.3	5.4	5.7	**5.5**
EDL3	2.8	2.1	2.2	**2.4**	2.4	2.4	2.9	**2.6**	2.2	2.5	2.4	**2.4**	2.6	2.0	2.4	**2.3**	2.7	2.1	2.3	**2.4**	2.4	2.5	3.0	**2.6**	2.0	2.0	2.4	**2.1**	5.5	4.5	4.4	**4.8**
ERD10	6.7	6.8	7.0	**6.8**	6.5	6.5	7.0	**6.7**	7.0	7.6	7.3	**7.3**	6.3	6.7	6.9	**6.6**	6.7	6.6	6.7	**6.7**	6.5	6.5	6.7	**6.6**	6.9	7.0	6.8	**6.9**	8.4	7.6	7.5	**7.8**
ERF2	5.7	5.6	5.6	**5.6**	7.7	7.6	6.8	**7.3**	6.1	5.9	5.9	**6.0**	8.0	7.6	7.3	**7.6**	5.7	6.1	5.3	**5.7**	7.3	7.2	7.4	**7.3**	6.1	5.9	5.8	**5.9**	6.2	6.6	6.5	**6.4**
ERF38	5.7	5.1	5.3	**5.3**	4.9	5.3	5.3	**5.1**	5.3	5.0	5.2	**5.2**	4.9	4.9	5.2	**5.0**	5.2	5.6	5.4	**5.4**	5.0	5.6	5.2	**5.3**	5.5	6.0	5.4	**5.7**	6.8	6.1	6.1	**6.3**
ERF4	7.3	7.2	7.6	**7.4**	8.3	8.4	8.5	**8.4**	7.6	7.8	7.7	**7.7**	8.7	8.7	8.4	**8.6**	7.5	7.7	7.7	**7.6**	9.1	8.8	8.8	**8.9**	7.5	8.1	7.6	**7.7**	9.7	9.6	9.7	**9.7**
ERF8	3.0	2.7	3.1	**2.9**	3.5	3.8	3.2	**3.5**	3.0	2.9	3.4	**3.1**	4.0	4.1	3.8	**4.0**	3.0	3.3	3.4	**3.3**	3.8	4.0	4.3	**4.0**	3.3	3.6	3.0	**3.3**	5.1	5.2	5.3	**5.2**
EXPA8	8.8	8.2	8.6	**8.6**	8.0	7.7	8.1	**7.9**	8.4	8.5	8.6	**8.5**	7.5	8.0	7.5	**7.7**	8.6	8.2	8.6	**8.5**	8.3	8.3	8.5	**8.3**	8.8	9.0	8.9	**8.9**	8.8	9.0	8.7	**8.8**
EXPB1	5.2	5.7	5.4	**5.4**	5.4	5.5	5.1	**5.3**	5.4	5.5	5.4	**5.4**	4.7	5.5	5.1	**5.1**	5.6	5.6	5.6	**5.6**	5.4	5.6	5.2	**5.4**	6.3	6.1	5.8	**6.1**	5.8	6.4	6.1	**6.1**
FRO5	2.9	2.8	3.7	**3.1**	2.4	4.5	4.1	**3.7**	3.1	3.3	3.6	**3.3**	3.4	3.2	3.4	**3.3**	3.4	3.3	3.8	**3.5**	2.1	2.3	3.4	**2.6**	4.7	4.8	5.2	**4.9**	4.7	5.0	5.0	**4.9**

FRUCT4	10.7	10.8	10.8	**10.8**	10.9	10.9	10.8	**10.9**	10.8	10.8	10.7	**10.8**	11.0	11.0	10.9	**11.0**	10.7	10.8	10.7	**10.8**	10.9	11.1	11.0	**11.0**	10.2	10.2	10.3	**10.2**	9.7	10.0	10.1	**9.9**
GAPCP-1	7.4	7.1	7.1	**7.2**	6.8	6.8	7.4	**7.0**	7.0	6.9	7.1	**7.0**	6.7	6.6	6.7	**6.7**	7.0	6.8	7.5	**7.1**	6.5	7.1	6.9	**6.8**	6.9	7.0	7.0	**7.0**	8.0	7.7	7.7	**7.8**
GDH2	9.2	9.4	9.4	**9.3**	10.0	9.8	9.8	**9.8**	9.6	9.3	9.7	**9.6**	10.1	9.8	10.0	**10.0**	9.4	9.6	9.4	**9.5**	9.9	9.9	10.0	**9.9**	9.3	8.9	9.1	**9.1**	8.5	8.8	9.4	**8.9**
GH9C2	7.9	8.3	8.5	**8.3**	6.6	6.8	7.7	**7.0**	8.3	8.0	8.4	**8.2**	7.1	7.0	7.0	**7.0**	8.3	8.2	8.5	**8.3**	7.0	6.9	6.9	**6.9**	8.5	8.8	8.5	**8.6**	8.6	8.2	8.0	**8.3**
GolS2	3.0	3.2	3.0	**3.1**	3.6	3.3	3.3	**3.4**	3.0	3.0	3.1	**3.0**	3.4	3.1	3.4	**3.3**	3.0	2.7	2.9	**2.9**	3.8	3.4	3.8	**3.7**	3.4	3.9	3.4	**3.6**	4.4	5.3	5.4	**5.1**
GPAT2	4.6	4.6	4.6	**4.6**	4.7	5.0	5.0	**4.9**	5.1	5.2	4.7	**5.0**	5.3	5.0	4.8	**5.0**	4.3	4.6	4.9	**4.6**	4.7	5.2	5.1	**5.0**	5.2	4.9	5.0	**5.1**	6.7	5.8	6.1	**6.2**
HAI1	3.1	3.3	3.4	**3.2**	3.9	3.8	4.2	**4.0**	3.4	3.9	3.3	**3.5**	3.4	3.3	3.5	**3.4**	3.6	3.5	3.5	**3.6**	3.8	3.7	3.7	**3.8**	3.4	3.9	3.6	**3.6**	6.4	5.5	5.6	**5.8**
HAI2	3.3	2.7	2.8	**2.9**	3.0	2.9	3.4	**3.1**	2.5	3.0	2.6	**2.7**	2.6	2.8	2.7	**2.7**	2.7	2.8	2.8	**2.8**	2.8	2.7	2.4	**2.6**	2.7	2.7	2.3	**2.6**	5.0	4.6	4.5	**4.7**
HAT1	9.4	9.5	9.5	**9.5**	8.9	9.1	8.9	**9.0**	9.5	9.1	9.5	**9.4**	8.5	8.7	8.8	**8.7**	9.8	9.6	9.6	**9.7**	8.6	8.7	8.6	**8.6**	9.1	9.3	9.2	**9.2**	7.3	7.8	7.9	**7.7**
HB-7	3.8	3.4	3.9	**3.7**	4.3	3.7	4.8	**4.3**	4.1	3.7	3.9	**3.9**	4.1	3.8	4.9	**4.3**	4.0	4.1	4.0	**4.0**	3.7	4.5	4.2	**4.1**	4.5	4.3	3.6	**4.1**	6.1	5.7	5.5	**5.8**
HFR1	8.2	8.5	8.7	**8.5**	8.2	8.2	8.8	**8.4**	8.4	9.7	8.8	**9.0**	7.3	8.2	8.0	**7.8**	8.5	8.3	8.8	**8.5**	8.8	8.8	8.6	**8.7**	8.0	8.8	8.6	**8.4**	9.2	9.1	8.5	**8.9**
HSFC1	3.3	3.4	3.5	**3.4**	3.1	3.3	3.4	**3.3**	3.3	2.9	3.1	**3.1**	3.2	3.1	3.1	**3.1**	3.2	3.4	3.2	**3.3**	2.9	3.2	3.4	**3.2**	3.0	3.2	2.9	**3.0**	4.7	4.7	4.8	**4.7**
IMS2	3.7	3.9	4.5	**4.0**	3.4	3.6	3.1	**3.4**	3.6	3.7	4.4	**3.9**	3.5	3.1	3.5	**3.3**	3.9	3.9	3.7	**3.8**	3.7	3.1	3.3	**3.4**	6.0	5.0	5.8	**5.6**	5.0	4.6	4.6	**4.7**
IRT3	4.7	5.3	5.6	**5.2**	5.1	4.9	5.7	**5.2**	5.1	6.1	6.0	**5.8**	5.0	5.4	5.1	**5.2**	5.4	4.7	5.3	**5.1**	5.3	5.1	4.9	**5.1**	5.6	5.9	5.9	**5.8**	6.9	6.2	6.1	**6.4**
KTI1	6.0	5.9	6.4	**6.1**	7.6	6.2	7.6	**7.1**	5.5	5.3	6.1	**5.6**	6.6	6.0	6.8	**6.5**	5.9	5.6	5.9	**5.8**	6.5	7.1	6.6	**6.7**	5.2	5.3	5.3	**5.3**	5.8	4.9	5.6	**5.4**
LBD11	4.2	4.5	4.2	**4.3**	4.2	4.1	4.8	**4.4**	4.1	4.4	4.1	**4.2**	4.2	4.1	4.8	**4.4**	3.8	4.2	4.0	**4.0**	4.1	4.5	4.3	**4.3**	3.9	4.1	3.9	**4.0**	6.3	5.5	5.4	**5.7**
LTI65	4.8	4.4	4.6	**4.6**	4.4	4.8	5.2	**4.8**	4.7	5.2	5.0	**4.9**	4.7	5.0	5.7	**5.1**	4.7	4.4	4.8	**4.6**	4.7	4.8	4.4	**4.7**	5.2	5.1	3.8	**4.7**	8.0	7.2	6.2	**7.1**
LTI78	4.5	4.2	5.0	**4.6**	4.1	3.8	5.3	**4.4**	4.6	5.8	5.2	**5.2**	3.6	4.4	4.7	**4.3**	4.0	3.9	4.7	**4.2**	4.9	4.2	4.3	**4.5**	4.7	4.6	4.1	**4.5**	7.4	6.0	5.8	**6.4**
MIOX4	4.9	4.7	4.6	**4.7**	4.5	5.0	4.6	**4.7**	5.0	4.0	4.3	**4.4**	5.8	5.4	5.8	**5.7**	4.8	5.0	4.4	**4.7**	4.5	4.5	4.5	**4.5**	3.9	2.6	2.8	**3.1**	2.5	2.7	3.0	**2.7**
MRD1	3.5	3.2	2.9	**3.2**	2.2	3.2	3.0	**2.8**	3.2	3.3	2.7	**3.1**	3.2	3.2	2.7	**3.0**	6.1	5.8	5.7	**5.9**	5.7	5.9	5.7	**5.8**	5.4	5.8	6.1	**5.8**	6.0	5.9	5.8	**5.9**
MYB34	5.3	5.0	5.4	**5.3**	4.5	4.9	4.6	**4.7**	5.4	4.7	5.2	**5.1**	4.7	4.5	4.8	**4.7**	5.9	5.0	4.6	**5.2**	4.5	5.3	4.6	**4.8**	5.3	5.5	5.3	**5.4**	6.0	5.9	5.7	**5.8**
NAC019	3.3	2.7	3.1	**3.0**	3.8	3.9	4.3	**4.0**	3.2	4.2	2.8	**3.4**	4.2	4.3	3.9	**4.1**	3.1	2.7	3.5	**3.1**	4.7	4.8	5.0	**4.9**	3.2	3.1	3.2	**3.2**	6.7	6.0	5.4	**6.0**
NAC032	4.5	4.1	4.3	**4.3**	4.5	4.7	4.6	**4.6**	4.6	4.5	4.6	**4.6**	4.5	4.6	4.5	**4.5**	4.7	4.6	4.6	**4.6**	4.6	5.1	4.5	**4.7**	4.4	4.9	4.4	**4.6**	6.4	6.0	6.2	**6.2**
NAC102	5.4	5.5	6.0	**5.6**	6.6	6.4	6.9	**6.6**	5.7	6.0	6.0	**5.9**	6.9	7.2	7.0	**7.0**	5.4	5.4	5.5	**5.4**	6.9	6.7	7.1	**6.9**	5.8	6.2	5.5	**5.8**	8.2	8.1	8.0	**8.1**
NAS1	4.5	4.6	4.4	**4.5**	3.3	3.9	3.8	**3.7**	4.6	4.4	5.4	**4.8**	4.2	3.8	3.9	**4.0**	4.5	5.2	5.0	**4.9**	3.4	2.9	3.2	**3.2**	6.9	6.7	6.5	**6.7**	5.2	5.5	5.1	**5.3**
NCED3	6.1	5.7	6.2	**6.0**	6.1	6.2	6.7	**6.3**	5.6	6.3	6.1	**6.0**	6.0	6.4	6.2	**6.2**	5.9	5.5	6.4	**5.9**	6.1	6.6	6.5	**6.4**	5.8	6.5	5.8	**6.0**	8.0	7.7	7.8	**7.8**
NF-YB2	5.2	5.5	5.7	**5.5**	5.7	5.6	5.5	**5.6**	5.5	5.5	5.7	**5.6**	5.7	5.5	5.6	**5.6**	5.7	5.5	5.8	**5.7**	5.4	5.8	5.6	**5.6**	5.5	5.6	5.4	**5.5**	6.7	6.9	6.9	**6.8**
NF-YC2	4.0	4.1	4.2	**4.1**	4.1	4.1	4.1	**4.1**	4.1	3.8	4.5	**4.1**	4.0	3.8	3.6	**3.8**	4.2	4.1	4.4	**4.2**	3.9	3.8	3.9	**3.9**	4.2	4.2	4.5	**4.3**	5.5	4.7	4.8	**5.0**
NRT1.5	6.0	6.3	5.2	**5.8**	4.8	5.3	5.4	**5.2**	5.8	4.0	6.3	**5.4**	5.4	4.5	5.2	**5.0**	6.3	5.6	6.0	**6.0**	5.2	5.5	4.8	**5.2**	7.2	6.8	6.9	**7.0**	6.0	6.0	6.3	**6.1**
NRT2.6	2.6	2.7	3.0	**2.8**	4.8	4.2	4.4	**4.5**	2.7	2.3	3.8	**3.0**	4.9	4.3	4.4	**4.5**	3.2	3.7	3.2	**3.4**	4.9	4.9	4.5	**4.8**	2.4	1.7	2.1	**2.1**	3.4	2.2	2.8	**2.8**
NRT2:1	5.1	5.4	5.5	**5.3**	4.8	4.5	4.7	**4.6**	5.1	4.8	5.8	**5.2**	4.3	4.5	4.8	**4.5**	5.4	5.4	5.0	**5.2**	4.4	4.3	4.4	**4.4**	8.5	8.8	8.4	**8.6**	7.4	6.9	6.6	**7.0**
NUC-L2	2.7	2.5	3.4	**2.9**	2.7	2.7	2.8	**2.7**	2.6	2.5	2.6	**2.6**	2.9	2.3	2.5	**2.6**	3.4	2.8	2.8	**3.0**	3.4	3.2	2.7	**3.1**	3.7	3.7	3.7	**3.7**	4.5	3.7	3.5	**3.9**
OXS3	7.6	7.5	7.5	**7.5**	7.5	7.6	7.7	**7.6**	7.0	7.3	7.2	**7.2**	7.3	7.4	7.4	**7.4**	7.1	7.2	7.7	**7.3**	7.3	7.6	7.4	**7.4**	6.7	6.5	6.5	**6.6**	6.0	6.3	6.6	**6.3**
P5CS1	7.5	8.2	8.5	**8.1**	8.1	7.4	8.6	**8.0**	8.4	9.3	8.6	**8.8**	8.0	7.7	8.5	**8.0**	7.9	7.4	8.2	**7.9**	7.7	7.9	7.6	**7.7**	8.8	8.7	8.4	**8.6**	9.8	9.2	9.1	**9.4**
PAP17	3.8	3.5	3.4	**3.6**	4.8	5.0	4.4	**4.7**	3.6	4.9	4.1	**4.2**	4.8	5.4	5.7	**5.3**	3.9	3.3	3.1	**3.4**	5.8	5.5	5.9	**5.7**	4.6	4.6	4.8	**4.7**	6.9	6.7	6.4	**6.6**
PCK2	4.0	4.1	4.8	**4.3**	5.8	5.3	5.6	**5.6**	4.4	4.0	5.0	**4.5**	6.0	5.1	5.8	**5.6**	4.3	4.1	4.2	**4.2**	5.4	5.4	5.2	**5.3**	4.3	4.1	4.3	**4.2**	4.2	3.7	4.9	**4.2**
PHT3;2	3.4	3.0	2.2	**2.9**	3.7	3.6	2.5	**3.3**	3.0	2.3	3.2	**2.8**	3.2	3.5	4.0	**3.6**	2.5	2.9	2.3	**2.5**	4.1	3.7	4.3	**4.0**	3.4	4.8	3.7	**4.0**	4.8	4.7	4.4	**4.6**
PIL1	6.2	6.2	6.9	**6.4**	5.9	6.4	7.0	**6.4**	6.8	8.1	7.4	**7.4**	5.6	6.6	6.3	**6.1**	6.4	6.4	7.0	**6.6**	7.3	7.1	6.9	**7.1**	6.6	7.1	7.2	**7.0**	7.7	7.4	7.4	**7.5**
PIL2	4.5	4.4	4.5	**4.5**	4.6	4.9	5.2	**4.9**	4.7	5.1	4.7	**4.8**	4.1	4.8	4.6	**4.5**	4.5	4.8	4.8	**4.7**	4.8	5.0	5.6	**5.1**	4.8	5.1	5.0	**4.9**	6.4	6.2	5.6	**6.1**
PNP-A	3.0	2.6	3.0	**2.9**	5.5	4.8	4.1	**4.8**	3.8	4.0	3.5	**3.8**	6.5	6.2	5.8	**6.2**	3.1	2.9	3.0	**3.0**	6.1	5.6	5.9	**5.8**	4.0	3.7	3.6	**3.8**	4.5	5.3	5.4	**5.1**
PP2-A13	8.5	8.9	9.1	**8.8**	8.9	8.9	8.9	**8.9**	8.8	8.6	8.7	**8.7**	8.7	8.6	8.7	**8.7**	8.9	8.7	8.7	**8.7**	8.8	8.9	8.8	**8.8**	7.8	7.7	7.9	**7.8**	6.8	7.3	7.6	**7.2**
PP2CA	6.6	7.0	6.9	**6.8**	6.5	6.6	7.2	**6.8**	7.2	7.4	7.1	**7.2**	6.8	6.8	7.3	**7.0**	6.9	6.6	7.2	**6.9**	6.7	7.0	7.0	**6.9**	6.7	6.9	6.6	**6.7**	9.2	9.2	9.0	**9.1**
PUM1	3.2	2.6	4.0	**3.3**	2.5	4.3	2.8	**3.2**	3.9	4.2	4.2	**4.1**	3.7	3.1	3.2	**3.3**	3.4	4.5	3.7	**3.9**	3.0	3.8	3.2	**3.3**	4.5	4.2	4.8	**4.5**	4.7	4.5	4.0	**4.4**
PYL5	9.2	9.2	9.2	**9.2**	9.1	9.1	8.9	**9.0**	9.2	9.1	9.3	**9.2**	9.0	9.0	8.9	**9.0**	9.5	9.5	9.8	**9.6**	9.1	8.9	8.7	**8.9**	9.1	9.1	8.9	**9.1**	8.0	7.6	8.0	**7.9**
RD22	8.8	8.9	9.1	**8.9**	8.6	8.5	9.4	**8.8**	9.3	9.5	9.4	**9.4**	8.6	8.6	8.9	**8.7**	9.0	8.8	9.0	**9.0**	8.9	8.8	8.4	**8.7**	9.3	9.8	9.2	**9.4**	10.2	9.6	9.4	**9.7**
RHA2B	6.2	6.0	6.7	**6.3**	6.4	6.7	6.6	**6.6**	6.6	6.5	6.7	**6.6**	6.8	6.6	6.8	**6.7**	6.2	6.4	6.5	**6.4**	6.7	6.9	6.9	**6.8**	6.4	7.1	6.8	**6.8**	7.7	7.7	7.8	**7.8**
SIP2	12.1	12.2	11.9	**12.0**	12.2	12.1	12.1	**12.1**	12.0	11.9	11.9	**12.0**	12.3	12.1	12.2	**12.2**	12.1	12.0	11.8	**12.0**	12.0	12.1	12.2	**12.1**	11.8	11.5	11.9	**11.7**	10.9	11.2	11.5	**11.2**
UGT85A1	6.5	6.5	6.2	**6.4**	6.5	6.8	6.7	**6.7**	6.4	6.4	6.2	**6.3**	6.7	6.5	6.4	**6.6**	6.5	6.5	6.2	**6.4**	6.5	6.3	6.5	**6.4**	5.6	5.6	5.5	**5.6**	5.4	5.4	5.8	**5.5**
XTR8	5.7	6.1	6.4	**6.1**	4.6	3.9	4.9	**4.5**	6.1	5.8	6.9	**6.3**	5.0	4.5	4.6	**4.7**	6.7	6.2	6.2	**6.3**	5.0	4.9	4.3	**4.7**	7.9	8.1	7.5	**7.8**	6.5	6.5	6.1	**6.4**
ZF2	3.3	3.3	3.5	**3.4**	4.3	4.3	4.4	**4.3**	3.6	3.6	3.7	**3.6**	3.9	4.4	4.4	**4.2**	3.3	3.4	3.7	**3.5**	4.8	5.1	4.6	**4.8**	3.9	4.2	3.9	**4.0**	5.8	5.4	5.2	**5.5**
ZIP1	4.5	4.4	4.7	**4.5**	4.5	4.8	4.7	**4.7**	5.1	4.1	4.6	**4.6**	4.1	4.7	4.8	**4.5**	4.9	4.4	4.2	**4.5**	4.6	4.6	4.8	**4.6**	5.6	6.0	5.5	**5.7**	6.1	5.7	6.2	**6.0**

Appendix 4

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Ion intensities from MALDI-MS analysis

(Chapter 7)

Mean intensities and standard deviation of ions that significantly differed in abundance under at least one condition (ANOVA; p<0.01; no FDR)

Ions that accumulated only in BABA-pre-treated, *Hpa*-infected Col-0 wild-type plants are shown in red.

Ions obtained in positive ionisation mode:

m/z	CHM mean	CHM std.dev.	CBM mean	CBM std.dev.	CHP mean	CHP std.dev.	CBP mean	CBP std.dev.	IHM mean	IHM std.dev.	IBM mean	IBM std.dev.	IHP mean	IHP std.dev.	IBP mean	IBP std.dev.	Raw p value
50.4712	0.5017	0.7767	2.1194	0.3429	-0.6928	0.1573	-0.4151	0.6743	-0.2250	0.5500	-0.7698	0.0654	0.1341	1.4638	-0.5664	0.1563	0.0015
50.7156	-0.2730	0.2149	-0.2881	0.0494	-0.2375	0.1285	-0.3786	0.0972	-0.4384	0.1360	-0.3735	0.1432	-0.2295	0.0247	2.9300	2.7279	0.0083
50.9181	-0.6489	0.1568	-0.4088	0.4844	-0.6540	0.1548	1.7181	0.7931	-0.3164	0.8260	-0.7360	0.0720	1.4020	1.8497	0.1324	0.5253	0.0079
51.4758	-0.4061	0.1943	-0.0547	0.6793	2.8131	1.5355	-0.5115	0.0785	-0.5634	0.0974	-0.5018	0.1179	-0.1388	0.4555	-0.1249	0.2350	0.0001
52.1433	-0.4512	0.3400	1.6428	0.6205	1.2530	1.8075	0.0736	0.2154	-0.8973	0.0074	-0.5222	0.5091	-0.5065	0.5361	-0.4798	0.1687	0.0049
52.5	-0.5072	0.1787	-0.0587	0.8466	-0.4970	0.1449	-0.6126	0.0649	-0.0276	1.0282	-0.4688	0.2824	2.3576	1.3000	0.0099	0.4644	0.0015
52.9204	-0.5644	0.1699	-0.5424	0.0702	-0.4869	0.2760	-0.4260	0.4457	1.4919	0.5479	0.3469	0.6039	-0.1219	0.4269	-0.1454	0.1757	0.0001
54.5363	1.8674	1.0715	0.4604	1.0508	0.2970	1.0273	-0.5254	0.4031	-0.6272	0.2390	-0.7254	0.0741	-0.5051	0.2997	-0.3665	0.3530	0.0040
55.386	2.0224	1.3242	-0.4365	0.0362	-0.3950	0.1384	-0.5205	0.0772	-0.5719	0.0948	-0.1447	0.5177	-0.3045	0.1621	0.2297	0.8890	0.0012
55.6353	0.9077	0.9610	-0.5664	0.0247	-0.5327	0.1471	-0.6447	0.0608	-0.6886	0.0588	1.3280	0.9853	0.1187	0.5583	-0.2061	0.1378	0.0012
57.9135	-0.3056	0.2099	-0.3221	0.0464	-0.2736	0.1308	-0.4111	0.0925	1.5487	1.4529	-0.4049	0.1370	-0.2228	0.0767	0.0105	0.2634	0.0057
62.5808	-0.0228	0.7416	2.2366	1.4301	0.3735	1.5755	-0.6832	0.0561	-0.7248	0.0477	-0.6675	0.0854	-0.5948	0.0224	0.5828	0.2350	0.0044
62.8842	-0.2081	0.4816	2.1755	1.7295	0.0296	0.5911	-0.4763	0.0833	-0.4397	0.2093	-0.4678	0.1246	-0.3467	0.0238	-0.1250	0.2073	0.0031
63.8065	-0.5751	0.1682	1.7484	0.9857	1.1969	1.8621	-0.6803	0.0564	-0.7221	0.0485	-0.1875	0.7413	-0.5914	0.0224	-0.0049	0.1274	0.0082
63.9823	0.9161	1.0938	1.5626	0.6865	-0.4567	0.1667	-0.8664	0.1470	0.5115	1.4838	-0.3191	0.6802	-0.7687	0.2201	-0.4981	0.1989	0.0085
64.1714	-0.4166	0.2790	-0.4953	0.0310	-0.1323	0.5045	-0.2341	0.6134	2.2680	1.3391	-0.5648	0.1055	-0.4671	0.0231	0.0082	0.3232	0.0003
64.6642	-0.4878	0.3435	-0.3173	0.2627	0.6255	1.2640	-0.6956	0.0546	1.6122	0.8507	-0.6795	0.0830	-0.0393	0.9695	-0.1574	0.4059	0.0096
65.3118	0.1367	1.3343	2.2434	0.9295	-0.5253	0.1467	-0.6381	0.0617	-0.6824	0.0607	-0.3875	0.4567	0.0671	1.0346	0.1495	0.8423	0.0051
69.6702	-0.8048	0.1327	-0.8437	0.0007	-0.0308	0.5553	-0.3575	0.5383	0.4376	1.3310	0.7276	1.5031	1.9129	0.5763	-0.5999	0.3204	0.0084
69.7791	0.5136	1.5115	-0.4350	0.0364	-0.3934	0.1383	-0.5191	0.0774	-0.5705	0.0952	-0.5091	0.1165	-0.3980	0.0235	2.8438	0.9854	0.0001
72.8305	-0.5205	0.1766	2.2390	0.2120	0.4236	1.5504	-0.6258	0.0632	-0.1006	1.0023	-0.6122	0.0962	-0.5260	0.0227	0.0100	0.7377	0.0023
77.2107	-0.4415	0.4345	1.7053	1.0462	0.8192	0.9901	-0.0951	0.8777	-0.7663	0.1645	-0.2450	0.9211	-0.7713	0.0216	-0.2544	0.6402	0.0099
81.2513	-0.1701	0.4399	2.1227	1.9262	-0.2705	0.1306	-0.4083	0.0929	-0.4664	0.1274	-0.4022	0.1375	-0.2651	0.0244	-0.0250	0.2084	0.0063
83.976	1.9008	1.0069	-0.4078	0.2989	0.1807	1.3844	-0.6455	0.0607	-0.6893	0.0586	0.4361	1.1251	-0.4270	0.2332	-0.3246	0.0476	0.0094
85.2202	1.2340	1.5251	1.2598	0.5756	-0.8253	0.2106	-0.3859	0.3797	0.0122	0.5298	-0.5128	0.0619	-0.5010	0.4642	-0.5732	0.1753	0.0043
86.7886	-0.4272	0.4597	-0.1625	0.8224	-0.5970	0.1512	-0.7027	0.0538	-0.7431	0.0420	1.9831	1.2309	0.3526	0.9338	0.5576	0.8029	0.0031
89.0389	-0.6220	0.3188	-0.8122	0.2097	-0.1154	0.5153	0.4076	0.2176	1.0597	0.5845	0.2264	0.9696	-0.6081	0.3705	-0.1553	0.0945	0.0037
93.369	-0.4281	0.1909	2.5432	0.6918	-0.4093	0.1393	-0.5335	0.0754	-0.5841	0.0910	-0.1805	0.4799	-0.4153	0.0234	0.0571	0.1857	0.0000
95.4786	-0.4774	0.1833	2.4776	0.7946	-0.4639	0.1428	-0.5827	0.0688	0.0684	1.2285	-0.4266	0.3203	-0.4743	0.0230	0.1285	0.3752	0.0001
95.5219	0.3311	1.0868	-0.3837	0.4782	2.2483	1.5542	-0.3398	0.7196	-0.2996	0.5930	-0.7233	0.0745	-0.2920	0.6441	-0.2940	0.3711	0.0097
96.0491	-0.4628	0.4055	-0.3786	0.2728	-0.6023	0.1516	-0.2425	0.3882	-0.3122	0.5222	1.4627	1.2288	-0.1308	0.4611	0.3448	0.5112	0.0099
96.4442	-0.6636	0.1545	-0.2311	0.5403	-0.6702	0.1559	1.7508	1.1828	-0.1750	1.0708	0.3127	0.3355	-0.6974	0.0219	-0.1147	0.5320	0.0044
96.5686	-0.4171	0.3693	-0.1213	0.7495	-0.2120	0.4817	1.8697	1.1833	-0.3152	0.6778	-0.2657	0.5204	-0.3070	0.3793	-0.3714	0.1517	0.0068
98.9054	-0.5103	0.1782	1.7276	0.8411	-0.5005	0.1451	-0.6157	0.0645	-0.5551	0.1220	0.2291	1.3424	0.4078	0.9840	-0.3477	0.1512	0.0072
99.5151	-0.7440	0.4067	0.2756	1.2449	1.7731	0.4501	0.4142	0.0822	-0.7958	0.5466	-0.5834	0.5970	0.9558	1.4153	-0.9669	0.1673	0.0036
100.4839	0.6161	0.7822	1.8125	1.6538	-0.4870	0.3298	0.0165	0.2314	-0.7375	0.0438	-0.6806	0.0828	-0.4407	0.3154	-0.1620	0.4946	0.0057
101.4623	1.0626	1.2061	-0.5534	0.4903	-0.2015	0.3670	-0.8910	0.3143	-0.8963	0.3077	1.2830	1.0969	0.3454	0.6886	-0.0750	0.4410	0.0078
103.0244	-0.4300	0.1906	-0.4521	0.0348	0.3465	1.2516	-0.5354	0.0752	-0.5859	0.0905	2.1814	0.5774	-0.4176	0.0234	-0.1107	0.0887	0.0001
106.587	-0.5046	0.5807	1.7084	0.6895	-0.5104	0.4047	-0.6769	0.2883	-0.2706	0.6050	-0.3544	0.7950	1.1430	1.3303	-0.6613	0.1588	0.0031
109.7073	0.4703	1.6842	1.8804	0.4671	-0.4081	0.2099	-0.6562	0.0594	-0.1111	0.5609	-0.6415	0.0905	-0.3128	0.4142	-0.3592	0.1929	0.0049
109.8158	-0.6776	0.1524	0.4517	1.0234	2.3010	0.6059	-0.5167	0.4822	-0.0957	1.2523	-0.6278	0.2818	0.2047	1.0466	-0.5584	0.1561	0.0026
111.5338	-0.3957	0.1960	2.3685	1.7006	-0.3734	0.1371	0.1712	1.0901	-0.5536	0.1004	-0.4917	0.1199	-0.3764	0.0236	-0.2031	0.1481	0.0024
112.3962	-0.8189	0.1578	-0.6266	0.4353	0.4861	1.3097	1.2776	0.5620	0.5006	0.6475	0.5197	0.2687	-0.9582	0.2278	-0.7907	0.3617	0.0017
113.7444	-0.0745	0.7707	-0.5915	0.0224	-0.1562	0.4281	-0.6687	0.0578	1.4292	0.2176	0.5333	1.2163	-0.5775	0.0225	-0.4146	0.1527	0.0027
115.4817	1.6862	1.4750	0.6577	1.0971	0.3245	0.3886	-0.4686	0.5597	-0.4964	0.2731	-0.7577	0.0678	-0.5975	0.1708	-0.3775	0.3992	0.0092
116.3527	-0.6839	0.3218	0.6067	0.8114	-0.8125	0.1649	1.8561	0.2729	-0.7480	0.2998	-0.5709	0.5387	0.4936	0.4911	0.1068	1.2106	0.0006
118.0651	-0.6593	0.3969	-0.7282	0.3407	-0.1739	0.4774	0.3698	0.3378	0.9614	0.5498	0.3643	0.9663	-0.6523	0.4820	-0.1138	0.1899	0.0086
118.4263	0.5607	1.0614	2.0032	1.2199	-0.2115	0.8091	-0.4317	0.6165	-0.8457	0.0111	0.0437	0.4086	-0.5353	0.3515	-0.5952	0.1571	0.0033
120.743	-0.7894	0.2168	0.1453	0.9111	-0.4746	0.3340	2.0458	1.3619	-0.5359	0.6057	-0.2986	0.7211	0.2394	0.2591	-0.5118	0.0711	0.0031
121.8617	-0.5339	0.1746	-0.5606	0.0252	0.3978	1.5310	1.8756	0.6515	-0.6834	0.0604	0.1502	1.0066	-0.5420	0.0226	-0.0498	0.4625	0.0074
122.0617	-0.6726	0.3653	-0.7082	0.1084	-0.2899	0.2651	0.1990	0.1271	1.0765	0.6518	0.2595	1.1035	-0.3929	0.3607	-0.0713	0.2051	0.0097
127.7668	-0.5105	0.2030	-0.4176	0.2579	0.1799	0.5007	0.2586	0.8368	-0.6762	0.0626	1.5921	1.2419	-0.1839	0.3160	-0.3677	0.1516	0.0053

130.4161	**-0.8516**	0.2758	**-0.6123**	0.6451	**0.2216**	0.9329	**1.3828**	0.3710	**0.5195**	0.9853	**0.4463**	0.6750	**-0.9280**	0.1685	**-0.5133**	0.1639	0.0027
130.832	**-0.4665**	0.2661	**-0.5336**	0.3222	**0.0541**	0.7633	**2.1927**	1.3126	**-0.4580**	0.6289	**-0.5495**	0.4201	**0.5061**	0.4427	**-0.4795**	0.1234	0.0009
131.7121	**0.1740**	0.7319	**1.8470**	1.1448	**-0.3319**	0.1816	**0.1870**	1.0142	**-0.7019**	0.2808	**-0.2567**	0.8945	**-0.7447**	0.0584	**-0.4706**	0.0834	0.0065
132.6884	**-0.4174**	0.8325	**2.2002**	0.6545	**-0.6762**	0.4630	**-0.0801**	1.0453	**-0.2000**	0.6662	**-0.4870**	0.7519	**-0.0505**	0.7409	**-0.1159**	0.3712	0.0045
133.0807	**-0.4411**	0.1889	**-0.3049**	0.3064	**-0.4237**	0.1402	**-0.5465**	0.0737	**1.4760**	0.4892	**0.5827**	1.0835	**-0.4308**	0.0233	**-0.2604**	0.1493	0.0004
134.0568	**-0.6191**	0.1124	**-0.5651**	0.0646	**-0.3531**	0.3169	**-0.1536**	0.3008	**1.0601**	0.7037	**0.5239**	0.8621	**-0.2677**	0.4530	**-0.2685**	0.1387	0.0045
134.1572	**-0.6740**	0.1529	**-0.0426**	1.1619	**1.2566**	0.4082	**-0.5174**	0.4080	**-0.8150**	0.0201	**-0.1862**	1.0377	**2.0745**	1.7943	**-0.4186**	0.2734	0.0084
135.3426	**-0.2292**	0.3722	**2.1773**	1.6829	**-0.2028**	0.4166	**-0.2833**	0.5945	**-0.5318**	0.3109	**-0.3079**	0.4428	**-0.1663**	0.5013	**-0.3443**	0.2183	0.0057
135.9833	**-0.3966**	0.3740	**-0.3771**	0.2717	**-0.3593**	0.2095	**-0.4504**	0.3091	**-0.3648**	0.4488	**1.6037**	1.0729	**0.2344**	0.6501	**-0.2525**	0.2386	0.0025
136.2471	**-0.3543**	0.2024	**2.7697**	0.4947	**-0.3275**	0.1342	**-0.4598**	0.0856	**-0.5148**	0.1124	**-0.4518**	0.1277	**-0.3268**	0.0240	**-0.1510**	0.1471	0.0000
136.8233	**-0.2890**	0.8189	**1.8714**	1.7414	**-0.1623**	0.2605	**1.0156**	0.1028	**-0.9301**	0.0166	**-0.2585**	0.7094	**-0.6549**	0.3676	**-0.4491**	0.1588	0.0046
137.0995	**-0.4210**	0.5271	**-0.3530**	0.5716	**2.2088**	1.1161	**-0.6769**	0.5897	**0.0108**	1.1755	**-0.7242**	0.4594	**-0.5148**	0.8590	**0.8573**	0.4221	0.0030
137.4807	**-0.6229**	0.1608	**0.1889**	0.3162	**-0.3652**	0.6034	**-0.7281**	0.0509	**1.2883**	0.6734	**0.8008**	0.1928	**-0.6487**	0.0221	**-0.4895**	0.1544	0.0000
139.6583	**-0.4423**	0.1887	**-0.4649**	0.0337	**-0.3981**	0.1870	**0.0163**	1.0327	**-0.3735**	0.4151	**2.0321**	1.0040	**-0.2906**	0.2424	**-0.1540**	0.3000	0.0007
140.2934	**0.0212**	0.9975	**-0.4690**	0.0333	**-0.2078**	0.5243	**-0.5516**	0.0730	**-0.6011**	0.0858	**1.8894**	0.7063	**-0.4370**	0.0232	**0.2669**	0.4524	0.0003
140.4101	**-0.4545**	0.1868	**-0.4777**	0.0326	**-0.0725**	0.5741	**2.4051**	0.7274	**-0.6089**	0.0834	**-0.1581**	0.7300	**-0.2932**	0.2881	**-0.2403**	0.1855	0.0000
143.5633	**-0.4521**	0.1872	**2.2740**	1.6260	**0.3439**	1.0856	**-0.5575**	0.0722	**-0.6066**	0.0841	**-0.3198**	0.2833	**-0.4440**	0.0232	**-0.1023**	0.4041	0.0025
144.6265	**-0.5301**	0.1752	**-0.3809**	0.3281	**-0.5224**	0.1465	**0.0089**	1.0555	**1.9094**	1.3680	**-0.2094**	0.7578	**-0.5375**	0.0227	**0.1665**	0.4427	0.0091
147.1174	**-0.6309**	0.4529	**-0.6990**	0.1709	**0.0288**	0.2429	**0.1377**	0.3876	**1.0441**	0.6559	**0.1775**	0.9572	**-0.9819**	0.2955	**0.3864**	0.6225	0.0050
147.4171	**-0.5849**	0.3861	**-0.7592**	0.0075	**-0.0719**	0.5421	**0.0716**	0.6850	**0.3247**	0.2129	**1.4542**	1.0813	**-0.4898**	0.4861	**-0.2734**	0.2774	0.0043
147.9618	**-0.4928**	0.1809	**2.1436**	1.4456	**-0.4240**	0.2427	**-0.3852**	0.3914	**-0.3527**	0.5621	**-0.1595**	0.8000	**0.0903**	1.0072	**-0.2289**	0.0216	0.0076
150.9216	**-0.6669**	0.1317	**-0.6391**	0.1716	**-0.2785**	0.2730	**-0.5062**	0.1314	**1.0077**	0.3863	**1.4733**	1.2852	**-0.1673**	0.1189	**-0.5853**	0.1568	0.0003
151.8207	**-0.2196**	0.5825	**1.7877**	1.3617	**-0.1140**	1.1482	**1.0440**	0.2026	**-0.9317**	0.0171	**-0.1364**	0.7073	**-0.5916**	0.4805	**-0.7105**	0.1601	0.0043
152.1416	**-0.0491**	0.1000	**0.2494**	0.8133	**-0.3148**	0.1398	**-0.5243**	0.0767	**-0.5755**	0.0937	**1.5619**	1.3734	**-0.4043**	0.0235	**-0.2046**	0.1737	0.0064
152.9965	**0.2266**	0.7097	**-0.5379**	0.2253	**0.0068**	0.7198	**-0.4099**	0.5377	**1.3068**	0.7728	**-0.4791**	0.3631	**-0.4976**	0.3041	**-0.2986**	0.1142	0.0078
153.1717	**-0.4469**	0.3526	**-0.2266**	0.8567	**0.1388**	1.3638	**-0.2680**	0.8544	**-0.8224**	0.0179	**0.0876**	0.8102	**2.5566**	0.9686	**-0.4219**	0.2955	0.0032
154.0208	**-0.5808**	0.1673	**0.8010**	1.3297	**-0.3269**	0.3817	**1.7936**	1.0372	**-0.4406**	0.5068	**-0.6703**	0.0848	**-0.0608**	0.9294	**-0.4046**	0.1849	0.0084
154.5141	**-0.5950**	0.2159	**-0.5135**	0.2493	**-0.6299**	0.1533	**-0.4863**	0.4516	**0.2614**	0.9080	**1.6689**	1.1968	**0.2873**	0.3451	**-0.3673**	0.2680	0.0028
155.4578	**-0.4414**	0.1889	**-0.2909**	0.2739	**-0.4241**	0.1403	**2.3751**	1.2615	**-0.5966**	0.0872	**-0.3521**	0.3804	**-0.1280**	0.5472	**-0.0143**	0.3718	0.0001
157.3575	**-0.5022**	0.1795	**-0.2722**	0.4682	**-0.2924**	0.3022	**2.4045**	0.9236	**-0.6536**	0.0696	**0.1576**	0.7651	**-0.4747**	0.0724	**-0.2317**	0.2962	0.0000
157.5872	**-0.5110**	0.1781	**-0.0722**	0.6635	**0.2178**	1.3905	**-0.4938**	0.2384	**-0.6619**	0.0670	**2.2122**	1.0200	**-0.3614**	0.2866	**-0.3485**	0.1512	0.0017
159.3317	**1.9556**	0.8377	**0.2577**	1.0047	**-0.3207**	0.3711	**-0.0986**	1.0937	**-0.7947**	0.0262	**-0.4599**	0.3895	**-0.4062**	0.4814	**-0.1813**	0.2250	0.0038
159.3739	**-0.4215**	0.1920	**-0.1472**	0.4849	**-0.4020**	0.1389	**-0.5269**	0.0763	**-0.5779**	0.0930	**2.1068**	0.3116	**-0.4074**	0.0234	**0.2650**	0.7196	0.0000
159.9327	**-0.0798**	0.5396	**-0.5124**	0.0295	**-0.4754**	0.1435	**-0.2151**	0.3224	**-0.2426**	0.7073	**1.8330**	0.8656	**-0.2721**	0.3540	**-0.3192**	0.1505	0.0004
162.0279	**0.5860**	0.3850	**-0.4955**	0.4953	**-0.7376**	0.1960	**-0.8475**	0.0396	**-0.3576**	0.2645	**2.0626**	0.9273	**-0.3224**	0.5872	**-0.0154**	0.2402	0.0000
162.5426	**0.0039**	0.9857	**-0.0904**	1.0939	**-0.6293**	0.2645	**1.8740**	0.9318	**-0.8230**	0.0177	**0.2731**	0.1906	**-0.5032**	0.3950	**-0.1667**	0.3597	0.0039
163.1173	**1.4010**	1.0039	**-0.4542**	0.0369	**0.8026**	0.8631	**0.4229**	0.5695	**-0.4186**	0.8697	**-0.8114**	0.4546	**0.1026**	0.6387	**-1.0504**	0.3776	0.0049
163.2308	**-0.5245**	0.1760	**-0.3439**	0.3826	**1.1252**	1.3054	**1.9347**	0.3346	**-0.6746**	0.0631	**-0.6160**	0.0954	**-0.5308**	0.0227	**-0.1945**	0.4015	0.0001
164.0713	**-0.3911**	0.3060	**-0.6567**	0.4246	**-0.3122**	0.0865	**0.1753**	0.5065	**1.1114**	0.4797	**0.2232**	1.1014	**-0.8265**	0.2403	**-0.0479**	0.4120	0.0096
165.1336	**-0.5252**	0.6667	**-0.3356**	0.5115	**-0.0544**	0.2377	**-0.2627**	0.5729	**-0.2661**	0.8233	**-0.4070**	1.0564	**2.6140**	1.5253	**-0.0254**	0.9637	0.0086
165.6308	**-0.2816**	0.2136	**-0.2970**	0.0486	**-0.2470**	0.1291	**-0.3872**	0.0959	**-0.4465**	0.1335	**-0.3818**	0.1416	**2.7106**	2.5676	**-0.0594**	0.1454	0.0093
166.8449	**-0.3277**	0.3842	**2.4618**	0.7529	**0.0462**	0.7517	**-0.7763**	0.0458	**-0.2072**	1.0656	**-0.1870**	1.0145	**-0.4513**	0.2877	**-0.1912**	0.5469	0.0011
167.0311	**-0.8833**	0.1206	**-0.7303**	0.3313	**-0.4123**	0.7960	**-0.2690**	0.0314	**0.3860**	0.6227	**0.9098**	1.2188	**-0.6948**	0.4773	**1.6561**	1.3076	0.0073
168.4252	**0.1885**	0.8383	**2.0794**	1.1792	**-0.0625**	1.0258	**0.2908**	1.1347	**-0.8061**	0.1525	**-0.5475**	0.4657	**-0.5348**	0.3543	**-0.4749**	0.0979	0.0082
169.0363	**-0.5385**	0.3009	**-0.4279**	0.0478	**0.0661**	0.5958	**0.1986**	0.3658	**1.2417**	0.5618	**-0.0334**	0.7533	**-0.5985**	0.1370	**-0.6011**	0.1988	0.0013
171.6706	**-0.0298**	1.2007	**-0.3558**	0.5451	**2.4816**	0.8164	**0.1768**	1.6083	**-0.7745**	0.0324	**-0.5100**	0.4116	**0.0591**	0.6527	**-0.3803**	0.0523	0.0063
171.7417	**-0.5646**	0.1698	**-0.5927**	0.0223	**-0.5606**	0.1489	**1.8903**	0.2889	**-0.3973**	0.5858	**0.0700**	0.7351	**-0.5789**	0.0225	**1.0955**	2.0447	0.0091
171.9542	**-0.2008**	0.5741	**-0.2980**	0.3857	**-0.6846**	0.2015	**1.0716**	1.3927	**-0.8385**	0.0131	**1.6172**	0.6238	**-0.5252**	0.3532	**-0.2402**	0.3407	0.0015
171.9878	**-0.2861**	0.4033	**-0.8819**	0.1200	**-0.3470**	0.3640	**-0.0069**	0.8275	**1.3047**	0.5863	**-0.2182**	0.3438	**0.5188**	0.4758	**-0.6368**	0.0480	0.0007
173.0439	**-0.6207**	0.2925	**-0.6343**	0.1263	**-0.2042**	0.3942	**0.1565**	0.2277	**1.0440**	0.5447	**0.3391**	1.1646	**-0.6179**	0.1995	**-0.1137**	0.1419	0.0095
174.8381	**-0.6358**	0.4994	**0.9808**	1.2002	**-0.3729**	0.3508	**1.6404**	0.5018	**-0.3523**	0.7951	**-1.0136**	0.2356	**0.3312**	0.7368	**-0.5928**	0.4973	0.0021
175.2613	**-0.6602**	0.1551	**-0.1721**	0.9003	**-0.5915**	0.2856	**-0.1439**	1.1018	**-0.8021**	0.0240	**1.7448**	0.6495	**1.1391**	1.3289	**-0.3631**	0.1448	0.0050
175.9266	**-0.4637**	0.1854	**0.7144**	1.8447	**2.1954**	0.8757	**-0.5690**	0.0707	**-0.3016**	0.5696	**-0.5573**	0.1070	**-0.4579**	0.0231	**-0.2888**	0.1499	0.0040
175.9658	**-0.2433**	0.9033	**-0.4164**	0.2668	**-0.6849**	0.1568	**-0.2409**	0.7142	**1.4312**	0.5954	**-0.5453**	0.4058	**0.9471**	0.8668	**-0.3864**	0.3962	0.0037
178.6731	**-0.6362**	0.1588	**-0.5158**	0.2772	**-0.1342**	0.2984	**2.0035**	1.1187	**-0.5219**	0.4180	**0.1974**	0.8980	**-0.1769**	0.3341	**-0.2938**	0.2135	0.0007
178.9535	**-0.7413**	0.1193	**-0.2808**	0.3512	**-0.0945**	0.5381	**0.6668**	0.8135	**1.0526**	0.6623	**-0.0488**	0.7213	**-0.5802**	0.2800	**-0.6154**	0.1489	0.0057
178.9988	**-0.4134**	0.4190	**-0.6249**	0.2015	**-0.5253**	0.4126	**0.1335**	0.2129	**1.5329**	0.2717	**-0.3219**	0.8458	**0.5457**	0.3717	**-0.8829**	0.1665	0.0001

179.4745	0.0312	1.0405	-0.4859	0.0318	-0.1896	0.5881	-0.5678	0.0708	-0.6163	0.0811	2.1608	1.6094	-0.4564	0.0231	0.2455	0.8008	0.0072
179.5705	-0.5519	0.2055	0.0960	0.4259	-0.6081	0.1519	0.0838	0.8180	-0.4273	0.6734	1.8267	0.3688	-0.3050	0.7416	-0.5669	0.1563	0.0004
179.7972	-0.6111	0.1627	-0.3329	0.5196	-0.3783	0.5571	2.3044	1.3772	-0.7559	0.0381	-0.6066	0.2051	0.9223	0.2731	-0.2256	0.3869	0.0001
180.8887	-0.9048	0.4183	0.9302	1.2049	-0.4100	0.5165	0.2637	0.4889	-0.4440	0.4312	1.5545	1.3326	0.0811	0.7198	-0.9880	0.1476	0.0094
181.0524	0.2982	0.5907	0.3210	0.4077	-0.7731	0.3045	0.7818	1.3641	-0.6737	0.8473	-0.3734	0.5193	-1.2333	0.4483	1.6724	1.1663	0.0074
181.9607	-0.5691	0.4044	-0.0806	0.1609	-0.2989	0.3353	1.1136	1.0561	-0.7140	0.2386	1.3960	0.3744	-0.4700	0.3679	-0.7505	0.1611	0.0001
183.0311	0.0105	0.4412	-0.3666	0.0492	-0.2876	0.1174	-0.4285	0.0314	1.4240	1.2168	-0.2873	0.3100	-0.3089	0.0568	-0.1668	0.1474	0.0039
183.1151	-0.4324	0.3157	-0.5450	0.2381	-0.3553	0.1517	0.2694	0.8258	1.4647	0.5019	-0.3209	0.4603	-0.4106	0.1098	-0.1557	0.3986	0.0006
183.8964	-0.5046	0.1791	-0.5300	0.0279	-0.3653	0.3677	-0.1411	0.8320	-0.6559	0.0689	1.6574	0.8029	0.6972	1.4206	-0.2282	0.3065	0.0086
184.8408	-0.7445	0.1285	-0.3489	0.4424	-0.4165	0.6636	2.0866	1.2704	-0.2827	0.6825	0.2171	0.7062	0.0784	0.8321	-0.3959	0.4062	0.0055
186.3574	-0.4455	0.1882	-0.3778	0.1880	-0.4286	0.1406	1.4746	1.3199	-0.6004	0.0860	0.5899	0.9918	-0.4361	0.0233	-0.0844	0.3338	0.0083
188.0015	0.2994	0.9464	-0.6315	0.3494	-0.7223	0.1293	-0.0812	0.8196	1.3907	0.0495	-0.6000	0.5990	0.3980	0.5040	-0.6444	0.1588	0.0021
188.9871	-0.3148	0.3409	-0.4022	0.3370	-0.4775	0.1215	0.2047	0.7078	1.5316	0.3634	-0.1712	0.2083	-0.4385	0.1543	-0.5447	0.0870	0.0000
189.0742	-0.2999	0.3545	-0.4698	0.3792	0.3812	0.8909	-0.0341	0.3428	-0.7305	0.2687	-0.6447	0.2754	-0.3731	0.9388	2.9297	2.0674	0.0027
189.6394	-0.0807	0.8440	-0.1359	0.7082	-0.1459	0.9488	-0.3171	0.6295	-0.7508	0.0397	2.0537	0.5119	-0.3540	0.4946	-0.4677	0.1539	0.0016
190.0457	-0.6041	0.2511	-0.5779	0.0581	-0.2678	0.3389	0.1286	0.2824	1.1785	0.4919	0.2530	1.1496	-0.5375	0.1908	-0.2339	0.1792	0.0056
190.6673	0.1439	0.7374	-0.4149	0.2762	-0.5268	0.1468	-0.6394	0.0615	-0.6837	0.0603	2.1204	0.9090	-0.4503	0.1804	0.4817	0.9983	0.0002
191.4248	-0.5280	0.3808	1.5603	1.5338	-0.4556	0.1385	1.0382	0.5092	-0.6902	0.4078	-0.3264	0.7572	0.0176	0.5865	-0.5727	0.0537	0.0070
191.9431	-0.6379	0.1585	0.7574	1.2802	-0.2151	0.5906	-0.7431	0.0492	-0.4505	0.3309	2.0768	1.0471	-0.1295	0.5592	-0.4929	0.1693	0.0013
192.5086	0.7426	1.3866	-0.5080	0.2978	-0.6600	0.1552	0.0506	0.2146	-0.7965	0.0257	1.6439	0.2059	-0.3814	0.2957	-0.3955	0.2765	0.0005
195.0011	-0.5455	0.3706	-0.6003	0.4933	0.0334	0.4886	-0.7873	0.3632	1.0270	0.9202	0.9040	0.5358	-0.1603	0.6727	-0.3134	0.6380	0.0096
195.8436	-0.2823	0.3940	-0.4959	0.1207	-0.1749	0.5619	0.0436	0.4741	-0.5990	0.2027	2.0391	1.3974	-0.3071	0.2373	-0.3687	0.1679	0.0012
196.4746	0.1583	1.3606	1.9753	0.4601	-0.5377	0.3994	0.0717	0.7070	-0.5592	0.6297	-0.3222	0.5419	-0.2998	0.4156	-0.6864	0.1618	0.0039
197.0263	-0.2050	0.1577	-0.1501	0.1205	-0.4743	0.6189	2.0925	0.8228	-0.5548	0.3812	-0.1123	0.4824	-0.2708	0.6563	-0.5650	0.1899	0.0001
198.1301	-0.4762	0.3632	-0.3844	0.2741	-0.2833	0.5231	-0.3507	0.5570	1.8040	0.2387	-0.2339	0.2084	-0.7206	0.2494	0.2212	0.3935	0.0000
198.1611	-0.3725	0.1995	-0.3920	0.0402	-0.3478	0.1354	-0.4780	0.0831	0.2332	1.3532	-0.4695	0.1243	2.5454	1.9985	-0.1740	0.1475	0.0077
198.7631	-0.6841	0.1514	0.2621	0.8314	-0.0064	1.1181	2.1092	1.0905	-0.8245	0.0173	-0.4572	0.5683	-0.1537	0.9846	0.0792	0.6644	0.0089
199.1203	-0.4233	0.7892	-0.9058	0.4340	-0.1230	0.5707	-0.1576	0.2407	-0.3317	0.6836	-0.2226	0.6033	-0.5131	0.8028	2.8666	0.8354	0.0001
199.6721	-0.4721	0.1841	-0.3651	0.2556	-0.4580	0.1424	-0.3551	0.4071	-0.6254	0.0783	0.1529	1.3067	2.9434	1.0062	-0.1887	0.2886	0.0001
200.9138	-0.4975	0.5724	-0.4641	0.2082	-0.3451	0.3901	0.2229	0.6323	0.5269	0.5059	0.9071	0.8378	-0.0913	0.5038	-1.0356	0.3139	0.0083
201.0555	-1.0009	0.4686	-1.0834	0.2093	-0.4184	0.8211	0.5228	1.3385	-0.1823	0.5687	0.1669	0.7756	0.5547	0.7419	1.8526	1.0335	0.0086
201.4747	-0.3661	0.2005	0.2141	1.0760	-0.3406	0.1350	-0.4715	0.0840	-0.5258	0.1090	1.7901	1.4052	-0.3410	0.0239	-0.0836	0.2407	0.0073
201.9771	-0.2871	0.6853	-0.8300	0.0014	-0.6926	0.1897	-0.2423	0.7403	1.7542	0.4603	-0.3074	0.3812	0.6334	0.5522	-0.1507	0.5596	0.0002
203.628	-0.0271	0.7784	0.1782	0.6326	-0.5059	0.1455	-0.6206	0.0639	-0.6659	0.0658	1.7356	0.7181	0.0807	1.0218	-0.3539	0.1513	0.0022
204.0698	-0.0529	0.1483	-0.6960	0.2155	0.1078	0.3428	0.2558	0.2979	0.9383	1.1362	-0.2922	0.3929	-0.9196	0.2187	0.0110	0.1532	0.0064
206.163	1.3632	0.2474	0.4417	1.2675	0.5385	0.7335	0.2696	0.6116	-0.4987	0.4320	-1.0301	0.6101	0.0655	0.9303	-1.3589	0.2718	0.0050
208.4263	-0.1403	0.8673	-0.6675	0.7402	0.0883	0.3786	1.1121	0.5614	-0.7645	0.6543	1.3462	0.2388	-0.8140	0.3066	-0.4591	0.6799	0.0014
208.6595	-0.7007	0.5722	0.2320	1.1414	-1.0193	0.1672	0.2297	0.3563	-0.3323	0.7677	1.4862	1.0315	1.1135	0.6715	-1.0355	0.1693	0.0023
211.4425	-0.4809	0.1828	-0.4004	0.2097	-0.4678	0.1430	-0.3561	0.2716	1.3296	0.7558	0.4416	0.3172	-0.3672	0.1909	-0.2773	0.1822	0.0001
212.2071	-0.5112	0.2493	0.0506	1.3812	-0.2601	0.9636	-0.3335	0.6396	-0.8084	0.0674	2.0002	0.4471	0.0514	0.7469	-0.2457	0.5052	0.0093
214.5476	-0.6887	0.1507	-0.2773	0.7806	-0.2470	0.7163	-0.1859	0.4068	-0.6936	0.2343	2.0762	1.3645	0.6831	1.2812	-0.3719	0.1723	0.0081
214.6907	-0.2772	0.3054	1.9704	1.5656	-0.6531	0.1891	0.0588	0.7024	-0.5443	0.3108	0.1117	1.0009	-0.4912	0.2021	-0.3131	0.2233	0.0097
214.9431	-0.9621	0.1199	-0.4605	0.8556	-0.5532	0.4348	0.4702	0.1347	0.4614	1.0396	1.4487	0.9108	0.2346	0.8544	-0.7515	0.3521	0.0080
215.0522	-0.4831	0.1824	-0.5075	0.0299	-0.4703	0.1432	-0.3614	0.4153	2.2756	1.4507	-0.0105	1.0418	-0.3010	0.3096	-0.1130	0.4506	0.0020
215.1167	-0.2621	0.6492	-0.5632	0.0250	2.5151	1.0555	0.1167	0.9808	-0.6857	0.0597	-0.0294	1.0779	-0.5450	0.0226	-0.1822	0.4464	0.0008
215.8145	0.5414	0.5810	2.0213	0.9371	-0.9091	0.1710	-0.3304	0.6747	-0.3962	1.0347	-0.5995	0.2540	0.3981	0.4186	-0.5486	0.4224	0.0008
215.9965	-0.3972	0.4237	-0.5404	0.2249	-0.7582	0.1536	0.2698	1.3220	1.5122	0.4791	-0.2612	0.4360	0.2820	0.5461	-0.5936	0.4352	0.0058
216.0734	-0.3360	0.3624	-0.7828	0.4079	-0.2756	0.5471	1.1564	0.3134	0.6731	0.6798	0.1051	0.6866	-0.7646	0.6453	-0.5276	0.5530	0.0039
216.8399	-0.2931	0.7037	-0.1760	0.1866	-0.6479	0.3526	0.2953	0.2248	-0.3731	0.5972	1.6813	0.6920	-0.4293	0.0677	-0.5866	0.4456	0.0003
220.1794	1.3573	0.9012	0.3130	1.1140	0.7666	0.3786	0.2553	0.2943	-0.5368	0.3435	-1.0230	0.4421	0.0794	0.6362	-1.4194	0.2420	0.0008
221.3771	-0.4567	0.1865	-0.4799	0.0324	-0.4410	0.1413	1.0723	1.4100	-0.6109	0.0828	-0.5506	0.1083	2.0087	1.4550	-0.2800	0.1497	0.0026
221.7008	-0.1343	0.7174	-0.4502	0.3301	-0.5589	0.2307	-0.5252	0.2937	1.2766	0.5589	0.2573	0.8268	0.0137	0.6841	-0.5172	0.2294	0.0093
221.9086	-0.6834	0.3253	-0.5444	0.7682	-0.5341	0.5840	0.6683	0.5339	0.7082	0.7589	0.7193	0.5535	-0.2047	0.5121	-0.7301	0.2331	0.0088
222.0845	-0.7330	0.6926	-0.6991	0.6746	-0.1781	0.2927	0.8540	1.0291	1.1309	0.2220	-0.2972	0.3940	-0.2727	0.1162	-0.3298	0.4452	0.0058
224.8516	0.4226	0.9141	1.4743	0.1192	-0.7140	0.1586	-0.8082	0.0427	0.3061	1.0668	-0.4397	0.6276	-0.6212	0.2330	0.0649	0.9783	0.0084
225.3992	-0.4251	0.1914	-0.4470	0.0353	-0.4060	0.1391	1.7700	0.7155	-0.5813	0.0919	0.3949	1.4710	-0.3125	0.1547	-0.2403	0.1489	0.0027
225.9107	-0.5814	0.1672	-0.3190	0.5025	-0.5792	0.1501	1.9511	0.7305	-0.2055	0.9141	-0.4942	0.3487	-0.0438	0.1203	0.4295	1.3482	0.0046

226.3893	-0.8115	0.2855	0.4593	0.4817	-0.6775	0.5057	1.3582	0.8912	0.9028	0.4810	-0.5071	0.5678	-0.5176	0.2001	-0.4266	0.5652	0.0006
230.3956	-0.6286	0.1599	-0.3804	0.4820	-0.4529	0.4628	1.5508	1.4574	-0.7724	0.0331	1.0353	0.6949	0.0616	0.7355	-0.4967	0.1546	0.0046
230.8866	-0.4664	0.5302	-0.1338	0.5450	-0.5957	0.0795	-0.0714	0.3049	-0.5030	0.1386	1.9797	0.9905	-0.2776	0.4218	-0.2119	0.4920	0.0003
230.9157	-0.6567	0.4886	-0.5928	0.7033	-0.5523	0.3092	0.5668	0.2865	0.9646	0.3887	0.4576	0.8093	0.1363	1.0098	-0.8660	0.2083	0.0098
232.0784	-0.1870	0.3596	-0.1331	0.4263	-0.5805	0.6575	0.3215	0.8698	1.5914	0.5326	-0.2870	0.8830	-0.6068	0.7228	-0.7772	0.4749	0.0086
232.1738	-0.6418	0.2182	1.9458	0.4055	-0.5946	0.3197	0.7138	1.3155	-0.3794	0.7649	0.0511	1.0003	-0.7172	0.0218	-0.3450	0.4713	0.0031
234.1967	1.4127	0.4095	0.1251	0.6513	0.9668	1.1621	0.0543	0.3125	-0.4597	0.6270	-1.1107	0.2610	0.3161	0.9372	-1.3605	0.1162	0.0010
234.7259	-0.2710	0.4320	-0.4296	0.0368	-0.3876	0.1380	2.1491	1.1577	-0.5657	0.0967	-0.5042	0.1174	-0.3918	0.0235	0.5814	1.2398	0.0008
235.1979	1.2364	0.8851	0.6290	1.2749	0.8566	0.0713	-0.4308	0.3798	-0.1963	1.0362	-1.0988	0.3617	0.1514	0.4352	-1.2409	0.1936	0.0042
236.0255	-0.9143	0.2869	0.1145	0.3216	-1.1212	0.3181	1.0036	1.0677	0.4435	1.3942	-0.1607	0.5287	1.3796	0.8261	-0.5414	0.4096	0.0082
238.1656	1.9194	1.7331	-0.2939	0.0489	-0.2437	0.1289	-0.3842	0.0964	-0.4437	0.1344	-0.3789	0.1421	-0.2362	0.0246	-0.0557	0.1453	0.0043
239.7184	0.0393	0.8489	-0.3498	0.0439	-0.3030	0.1326	-0.4376	0.0888	1.4427	0.7838	-0.4305	0.1320	-0.3003	0.0241	-0.0561	0.2176	0.0009
241.1243	-0.7288	0.1392	-0.6113	0.2693	-0.6530	0.1968	0.9636	0.6051	1.0553	0.7946	-0.7139	0.0525	0.2452	1.1121	0.2348	0.5699	0.0034
244.5218	-0.8175	0.4739	0.7023	1.1702	-0.9051	0.4978	0.1870	0.9718	0.2801	0.3864	0.2614	0.8041	1.2378	0.2599	-1.1768	0.1737	0.0048
246.0838	-0.6645	0.2147	-0.3976	0.3202	-0.2120	0.5082	-0.0312	0.8957	1.3434	0.4349	0.1874	0.4920	-0.5155	0.3128	-0.2337	0.5559	0.0053
246.2106	-0.6859	0.1511	-0.7195	0.0111	-0.6950	0.1574	-0.6745	0.2303	0.6269	1.4907	0.5008	0.7348	-0.2410	0.8559	2.4389	0.4938	0.0004
246.3355	-0.7097	0.3032	0.1275	0.7239	-0.7796	0.2442	-0.5144	0.8995	1.3963	0.4301	0.6571	0.8014	-0.1745	0.4236	-0.4557	0.5182	0.0039
247.6274	-0.6258	0.1604	2.1644	0.4464	-0.3373	0.4490	0.5735	1.4749	-0.7697	0.0339	-0.1036	0.7283	-0.6522	0.0221	-0.0881	0.3050	0.0007
251.1689	1.3638	0.7304	-0.0121	1.0058	-0.4661	0.8063	-0.3030	0.7820	0.7742	0.1785	-0.8394	0.5456	-0.2501	0.1984	-0.7329	0.0953	0.0070
252.9073	-0.8015	0.1624	-0.6120	0.0709	-0.3454	0.3464	-0.3091	0.2230	0.1156	0.3211	1.8379	0.9492	0.6226	1.5515	-0.5415	0.1950	0.0038
253.9454	0.2402	1.0339	-0.4303	0.0368	-0.3883	0.1380	2.1358	0.5475	-0.5663	0.0965	-0.5048	0.1173	-0.3926	0.0235	-0.2201	0.1485	0.0000
255.14	0.4506	0.3709	-0.7772	0.3588	-0.5046	0.6711	0.8390	1.5183	0.7574	0.6292	-1.1137	0.5652	-0.8194	1.0391	1.3525	0.5742	0.0098
257.1644	-0.3003	0.3396	0.0485	0.4931	-0.2787	0.3388	-0.6160	0.0645	1.3482	0.9004	-0.3047	0.2501	-0.2199	0.5075	-0.1990	0.2406	0.0031
261.8464	0.0445	0.4408	-0.8049	0.0035	-0.5458	0.3626	1.8896	0.4087	0.4190	1.2674	-0.2731	0.7976	-0.3941	0.3599	-0.5608	0.2899	0.0014
263.5799	-0.4544	0.6471	1.9635	0.5895	-0.5105	0.2988	-0.8512	0.0393	0.4310	0.7386	-0.5347	0.3520	-0.1739	0.7633	0.1956	1.2973	0.0035
263.7388	-0.5327	0.3808	0.1065	1.0441	-0.6699	0.1558	0.6371	1.2736	-0.8049	0.0231	1.8031	0.3696	-0.2286	0.1777	-0.3678	0.1434	0.0017
264.0082	-0.5280	0.1755	-0.1232	0.7707	-0.5201	0.1463	0.1938	1.3720	-0.2959	0.6033	-0.6194	0.0948	2.4329	0.8321	-0.3700	0.1517	0.0008
264.3277	-0.2320	0.4979	-0.1106	0.5470	-0.3865	0.1379	-0.1208	0.4151	-0.5647	0.0970	1.6913	1.4707	-0.2501	0.1520	-0.2181	0.1484	0.0095
265.864	-0.7463	0.2751	0.2111	0.9004	-0.6876	0.5266	1.4570	0.2638	0.7418	0.3936	0.3796	1.0964	-0.7528	0.5847	-0.9507	0.1668	0.0013
268.5202	-0.2531	0.2597	-0.4517	0.0349	-0.4110	0.1394	2.1572	1.1933	-0.5855	0.0906	-0.3809	0.3155	0.1174	0.4809	-0.2079	0.1856	0.0001
272.106	-0.4226	0.5447	-0.4950	0.4006	0.0407	0.4052	1.7019	1.2956	-0.3303	0.6664	0.3394	0.2869	-0.4099	0.3235	-0.5969	0.2291	0.0051
274.1958	-0.0601	0.1586	0.1915	0.9628	0.1866	0.3208	0.2318	0.9272	-0.5039	0.7109	-1.1099	0.6810	-0.9927	0.4867	2.4245	1.0057	0.0006
275.4247	0.0000	0.7237	2.1898	1.0322	-0.1146	0.9038	-0.7731	0.0461	-0.2576	0.9570	-0.3874	0.6635	-0.1910	0.4501	-0.2626	0.3354	0.0044
277.8494	-0.1518	0.1093	-0.3399	0.2786	-0.4645	0.1428	-0.3441	0.3486	1.4597	1.0952	-0.0801	0.4482	-0.4305	0.0770	-0.2613	0.0722	0.0016
278.5815	-0.7634	0.1391	0.8795	1.4583	-0.4695	0.4793	1.1569	0.6827	-0.8989	0.0078	1.0572	0.3646	-0.7081	0.1697	-0.3347	0.2090	0.0013
279.0845	-0.3549	0.2022	-0.3736	0.0418	-0.3283	0.1342	-0.4604	0.0856	-0.5154	0.1123	-0.4525	0.1276	-0.3276	0.0240	3.6720	0.8509	0.0000
279.2944	-0.1259	0.5518	2.0737	0.4940	-0.1220	0.8423	0.1095	1.2339	-0.7858	0.0290	-0.1957	0.9558	-0.6727	0.0220	-0.2288	0.5848	0.0051
280.0934	-0.6414	0.5511	0.0760	0.5692	-0.3407	0.7236	0.4842	1.2159	1.3870	0.2538	0.1522	0.7459	-0.5989	0.3019	-1.0087	0.1089	0.0087
282.9615	-0.4053	0.3202	-0.2795	0.6951	-0.2714	0.5850	-0.6681	0.1649	0.0300	0.8235	1.7531	0.2775	-0.0711	0.8313	-0.5126	0.1550	0.0018
283.4793	-0.3159	0.2083	-0.3328	0.0454	-0.0454	0.3711	-0.4214	0.0911	-0.4787	0.1236	1.7904	1.4001	-0.2808	0.0243	-0.1026	0.1462	0.0013
288.8611	-0.6914	0.5781	-0.0051	0.5494	-0.8172	0.4249	1.0033	0.5584	0.5044	0.7767	0.6373	0.6766	-0.4769	0.8162	-0.8026	0.0203	0.0070
289.9074	-0.3888	0.1970	0.4570	1.5359	-0.3658	0.1366	1.9869	1.3189	-0.5471	0.1024	-0.4851	0.1212	-0.3682	0.0237	-0.1945	0.1480	0.0083
292.0388	-0.5806	0.2462	-0.7291	0.1212	-0.3251	0.6515	0.1242	0.4619	1.7150	1.2119	0.1104	0.8364	-0.0005	0.5050	-0.5938	0.1451	0.0052
293.0938	-0.4704	0.5028	0.9901	0.6360	-0.6799	0.1565	-0.0980	1.1312	1.4972	1.1235	-0.3884	0.4343	-0.7078	0.0219	-0.5002	0.2160	0.0047
293.984	-0.8642	0.1405	0.2290	0.1405	0.3355	1.0030	-0.4348	0.7128	0.4329	0.7819	1.2934	0.5554	-0.6047	0.4369	-0.8246	0.0441	0.0033
294.077	-0.6358	0.2570	-0.5625	0.4223	-0.2760	0.3220	0.1408	0.2754	1.3299	0.5300	0.0118	0.7738	-0.6116	0.4246	0.0330	0.3751	0.0013
295.0893	-0.4408	0.0764	-0.4613	0.2858	-0.1614	0.5015	-0.0699	0.0966	1.2542	0.7806	-0.0678	0.7631	-0.3159	0.0600	-0.3898	0.2091	0.0046
295.2458	-0.4766	0.3398	0.1500	0.5910	0.0281	0.5737	0.9144	1.0254	1.0099	0.4274	-0.9938	0.6487	-0.4663	0.5228	-0.6748	0.5201	0.0076
296.9177	-0.4614	0.1858	2.5726	0.0888	-0.4462	0.1417	-0.1528	0.7362	-0.6154	0.0814	-0.5552	0.1074	0.1418	1.0161	-0.2860	0.1498	0.0000
299.3117	-0.0259	0.5609	-0.2820	0.3864	0.1800	0.4354	-0.2532	0.2806	0.4471	0.8325	0.9221	0.8876	-0.9236	0.3687	-0.9186	0.1739	0.0094
300.6665	-0.4727	0.5134	2.0175	1.1673	-0.3683	0.4717	-0.0306	0.6163	-0.2745	0.6782	-0.5445	0.5401	-0.1283	0.6676	-0.3990	0.6336	0.0064
300.7033	0.0680	1.4051	0.4345	0.8969	-0.7005	0.3367	1.5539	0.2836	-0.9153	0.0123	-0.3450	0.5547	0.5896	0.8451	-0.6885	0.1595	0.0087
303.23	-0.1208	0.4883	-0.8385	0.6048	-0.6228	1.4873	-0.0165	1.0187	-0.3506	0.4423	-0.8070	0.7356	1.6111	0.6035	1.7876	0.8202	0.0053
304.0249	-0.3648	0.4966	-0.3368	0.2798	-0.8089	0.4243	-0.4940	0.6696	0.3329	1.0588	0.8800	0.6633	1.3740	1.1363	-0.9832	0.0558	0.0069
305.0692	-0.7625	0.2815	-0.5005	0.6756	-0.3853	0.5621	0.7100	0.7516	0.5265	0.8492	1.4905	0.8137	-0.4283	0.5658	-0.9260	0.4447	0.0031
306.2786	-0.4119	0.1934	-0.4332	0.0365	-0.0061	0.8056	-0.5173	0.0777	-0.2156	0.6398	-0.5074	0.1168	-0.3959	0.0235	3.2547	1.6650	0.0001
307.6279	-0.6411	0.1580	-0.6726	0.0152	-0.6454	0.1543	0.4064	0.9826	0.6927	0.8317	1.0923	0.7530	-0.3303	0.3221	-0.5125	0.1550	0.0040

308.4413	**-0.7242**	0.1452	**-0.1679**	1.0314	**0.3294**	0.9546	**0.1023**	0.7972	**-0.0395**	0.7187	**1.7635**	0.2357	**-0.7701**	0.0216	**-0.6172**	0.1576	0.0035
308.504	**-0.5545**	0.1714	**-0.5821**	0.0233	**0.2277**	0.5253	**2.2680**	0.8409	**-0.3606**	0.6050	**-0.3806**	0.5155	**-0.0570**	0.8646	**-0.4033**	0.1524	0.0002
309.9344	**-0.4557**	0.2901	**-0.3980**	0.3564	**-0.0937**	0.3422	**-0.0055**	0.2677	**-0.0288**	1.3618	**1.7146**	0.7469	**-0.4223**	0.2971	**-0.4683**	0.0334	0.0068
310.0575	**1.9432**	1.7135	**-0.2971**	0.0486	**-0.2471**	0.1291	**-0.3872**	0.0959	**-0.4466**	0.1335	**-0.3818**	0.1416	**-0.2398**	0.0246	**-0.0595**	0.1454	0.0034
310.2803	**-0.5690**	0.3701	**-0.8111**	0.1341	**-0.3337**	0.2434	**-0.4609**	0.4891	**1.2892**	0.7921	**-0.1531**	0.2279	**1.6350**	1.4082	**-0.6334**	0.4904	0.0012
314.5788	**-0.5833**	0.1669	**2.1884**	0.6321	**-0.5813**	0.1502	**0.1650**	1.5005	**-0.6020**	0.1790	**-0.6727**	0.0843	**0.3379**	0.8061	**-0.0806**	0.4688	0.0014
317.5529	**-0.8057**	0.1326	**1.1455**	0.4041	**-0.8277**	0.1659	**-0.5807**	0.6027	**-0.4509**	0.8356	**1.5985**	1.5890	**0.4324**	0.7067	**-0.2366**	0.6767	0.0074
321.9199	**-0.2939**	0.2648	**-0.3812**	0.0663	**-0.2433**	0.3654	**-0.5015**	0.4705	**1.4483**	0.9675	**0.0409**	0.3962	**-0.2313**	0.5202	**-0.3179**	0.3379	0.0038
326.1151	**-0.5468**	0.2865	**-0.5763**	0.1135	**-0.5407**	0.2918	**1.9735**	0.4122	**-0.3607**	0.6643	**0.2195**	0.7761	**-0.2781**	0.1352	**-0.3511**	0.3397	0.0000
326.128	**-0.1665**	0.6571	**-0.2779**	0.3106	**-0.4185**	0.1399	**-0.2262**	0.5660	**1.9944**	0.5306	**-0.5310**	0.1121	**-0.4252**	0.0233	**-0.2545**	0.1492	0.0000
326.8479	**-0.6422**	0.1578	**-0.6738**	0.0151	**-0.0604**	0.5205	**1.4360**	1.1690	**-0.7851**	0.0292	**0.7004**	0.7237	**0.2696**	0.8591	**-0.3567**	0.1173	0.0039
327.1201	**-0.5713**	0.3081	**-0.3403**	0.3868	**-0.1515**	0.2933	**0.9558**	0.9722	**1.0326**	0.7158	**-0.5438**	0.0455	**-1.0058**	0.2332	**0.1481**	0.2990	0.0009
329.7983	**0.4652**	0.9616	**-0.8330**	0.0012	**-0.6561**	0.2404	**-0.7027**	0.3719	**-0.6575**	0.4605	**1.4500**	1.0144	**1.1423**	0.5524	**-0.1670**	0.7733	0.0015
331.6591	**-0.5201**	0.6012	**0.9767**	0.7335	**-0.4840**	0.3161	**-0.4653**	0.9069	**-0.5480**	0.4565	**1.7309**	0.5957	**-0.5017**	0.6929	**-0.5257**	0.3710	0.0012
332.1364	**-0.4565**	0.3423	**-0.3010**	0.0989	**-0.4516**	0.3586	**-0.2512**	0.6078	**1.2962**	0.9022	**-0.0020**	0.3185	**-0.2691**	0.1535	**0.0203**	0.4037	0.0057
332.4717	**-0.4501**	0.1875	**2.2210**	0.7117	**-0.4337**	0.1409	**-0.5555**	0.0725	**0.2974**	1.5828	**-0.2437**	0.5892	**-0.4416**	0.0232	**-0.2718**	0.1495	0.0014
333.5099	**-0.3388**	0.2047	**-0.3568**	0.0433	**-0.3104**	0.1331	**-0.4443**	0.0878	**-0.5002**	0.1169	**2.2912**	2.0020	**-0.3083**	0.0241	**0.0644**	0.4164	0.0039
335.8347	**-0.3864**	0.3965	**-0.4912**	0.2829	**0.4169**	0.6430	**-0.8311**	0.0768	**-0.0735**	0.9103	**1.8058**	0.4214	**-0.2990**	0.4447	**-0.1056**	0.8064	0.0011
339.3634	**1.8481**	1.7351	**-0.3609**	0.0429	**-0.3147**	0.1334	**0.1017**	0.5644	**-0.5039**	0.1158	**-0.4407**	0.1299	**-0.3130**	0.0241	**-0.1364**	0.1468	0.0079
342.9794	**-0.5828**	0.1670	**-0.5390**	0.1253	**-0.1679**	0.6548	**-0.6881**	0.0555	**0.6752**	0.9016	**0.0237**	0.6701	**1.4282**	1.1279	**-0.4310**	0.1600	0.0075
345.0689	**-0.3493**	0.4538	**-0.4320**	0.1264	**-0.3660**	0.3580	**0.3427**	0.2380	**1.0587**	0.7814	**-0.0810**	0.7792	**-0.0654**	0.1822	**-0.8661**	0.2955	0.0052
348.6108	**-0.0354**	1.0834	**-0.2230**	0.4828	**-0.2332**	0.2589	**-0.7008**	0.2119	**0.2385**	1.0111	**-0.6125**	0.3279	**2.1933**	0.3966	**-0.5881**	0.1569	0.0004
349.2653	**-0.5631**	0.3334	**1.0454**	1.7560	**-0.4005**	0.8619	**0.6363**	0.2983	**-0.4888**	0.7357	**-0.8659**	0.0470	**1.5489**	0.3249	**-0.6921**	0.1596	0.0078
350.6043	**-0.7175**	0.1462	**0.3611**	1.1564	**-0.4033**	0.5059	**0.7023**	1.3501	**-0.5862**	0.3003	**-0.6367**	0.3280	**1.9225**	0.9132	**-0.2057**	0.5405	0.0087
353.8741	**-0.4685**	0.3895	**-0.6280**	0.0192	**-0.5980**	0.1513	**2.1852**	0.6282	**-0.2590**	0.4192	**0.1703**	1.1207	**-0.4805**	0.2223	**0.0106**	0.9049	0.0005
354.1964	**-0.4602**	0.3969	**-0.0828**	0.7028	**-0.7458**	0.1607	**-0.2836**	0.0281	**-0.2618**	0.5837	**-0.2309**	0.5417	**-0.2440**	0.7061	**2.7336**	2.2241	0.0074
361.191	**-0.7907**	0.5236	**-0.4211**	0.2790	**-0.4839**	0.5077	**0.5204**	0.4677	**0.8443**	0.6027	**0.6064**	0.5276	**-0.3366**	0.4178	**-0.6811**	0.3375	0.0020
362.1934	**-0.7784**	0.4834	**-0.4508**	0.3011	**-0.5613**	0.4627	**0.8143**	0.2829	**1.2170**	0.5086	**-0.3741**	0.7754	**-0.3722**	0.2359	**0.0543**	0.2163	0.0004
362.4342	**-0.5428**	0.1732	**0.6194**	1.1861	**-0.5364**	0.1474	**-0.6481**	0.0604	**-0.1630**	0.5978	**2.0299**	0.2916	**-0.5527**	0.0226	**-0.2560**	0.3267	0.0001
363.9014	**-0.5608**	0.1704	**-0.3163**	0.4539	**-0.2433**	0.4835	**0.6606**	1.1411	**-0.7086**	0.0526	**1.6373**	0.9957	**-0.2456**	0.5510	**-0.4112**	0.1526	0.0045
364.2117	**0.2530**	0.4206	**0.4620**	0.7913	**-0.4219**	0.3211	**-0.1908**	0.7039	**-0.8688**	0.4153	**-0.1913**	0.8103	**-1.0385**	0.1190	**2.6104**	1.9586	0.0032
365.901	**-1.0066**	0.2662	**0.0944**	0.4463	**-0.6384**	0.7509	**0.4590**	0.4648	**0.5257**	0.3767	**1.1518**	0.9069	**-0.1622**	0.4916	**-0.8627**	0.8124	0.0050
371.7173	**-0.2056**	0.5475	**-0.4311**	0.0367	**0.4481**	1.3892	**-0.5154**	0.0779	**-0.5670**	0.0963	**1.7201**	1.0778	**-0.2986**	0.1474	**-0.2212**	0.1485	0.0094
372.9959	**-0.4704**	0.8307	**0.5545**	0.4290	**-0.7380**	0.3698	**1.7759**	1.1232	**0.3540**	0.9335	**-0.5744**	0.7779	**0.1382**	0.2307	**-1.0621**	0.3347	0.0033
373.9103	**0.0344**	0.8092	**-0.6925**	0.2194	**-0.4994**	0.2415	**0.5264**	0.6177	**1.1288**	0.9293	**-0.4713**	0.3714	**0.2278**	0.4422	**-0.7769**	0.1796	0.0060
376.149	**-0.1945**	0.7764	**-0.2558**	0.4619	**0.3189**	0.6985	**0.2873**	0.5847	**1.5645**	0.3997	**-0.7995**	0.6964	**-0.6094**	0.4451	**-0.8812**	0.0287	0.0013
377.1328	**-0.5215**	0.1958	**-1.0995**	0.3582	**0.1266**	0.5665	**0.7329**	0.3973	**0.9072**	0.4639	**0.3342**	0.7559	**-0.7150**	0.1980	**-0.4152**	0.8545	0.0021
378.2956	**-0.2334**	0.6602	**-0.0825**	1.0978	**-0.5886**	0.3731	**-0.2506**	0.8434	**-0.7924**	0.0504	**0.2533**	0.3839	**-0.6288**	0.2366	**2.7312**	1.3859	0.0008
378.6002	**-0.3582**	0.2017	**-0.3770**	0.0415	**-0.3319**	0.1344	**2.2377**	1.9728	**-0.3738**	0.3405	**-0.1223**	0.4805	**-0.3315**	0.0239	**-0.1559**	0.1472	0.0061
380.3181	**-0.6006**	0.2049	**-0.5582**	0.2329	**-0.2639**	0.1794	**0.6254**	0.5743	**-0.6135**	0.2239	**-0.3947**	0.5525	**-0.4910**	0.0912	**2.8373**	1.6734	0.0001
382.2181	**-0.0475**	0.7974	**-0.5736**	0.0240	**-0.2090**	0.7215	**-0.3703**	0.4286	**-0.6951**	0.0568	**-0.1614**	0.7330	**-0.5569**	0.0226	**3.0190**	1.0429	0.0000
383.3669	**-0.6447**	0.1963	**-0.8103**	0.0031	**-0.7573**	0.2227	**1.0035**	0.3714	**1.3824**	0.8993	**0.0420**	0.8594	**0.0119**	0.3818	**-0.6785**	0.1592	0.0002
383.747	**0.6440**	0.6977	**-0.3957**	0.9807	**0.3945**	1.0046	**0.0269**	1.0946	**-0.6030**	0.4369	**-0.6935**	0.2952	**2.0892**	1.1517	**-0.8687**	0.1644	0.0074
390.8688	**-0.4788**	0.1831	**-0.2536**	0.2263	**-0.4655**	0.1429	**-0.5841**	0.0686	**0.0311**	1.0755	**-0.5719**	0.1041	**2.4932**	0.8034	**-0.0223**	0.3453	0.0000
391.052	**-0.4217**	0.1919	**2.7391**	1.2257	**-0.4023**	0.1389	**-0.5271**	0.0763	**-0.5781**	0.0929	**-0.0707**	0.5546	**-0.4077**	0.0234	**-0.0390**	0.1140	0.0000
392.2834	**-0.7632**	0.9011	**-0.3543**	1.0360	**-0.0336**	0.5817	**1.1114**	0.3961	**-1.0980**	0.2429	**0.3203**	0.7861	**-0.5121**	1.0328	**1.7653**	0.8647	0.0052
394.2004	**1.2101**	0.8014	**-0.3885**	0.7519	**1.2838**	1.1819	**0.3465**	0.3650	**0.1560**	0.4367	**-0.8121**	0.5526	**-0.8926**	0.8344	**-1.1884**	0.7922	0.0049
396.3091	**-0.2196**	0.2147	**-0.0872**	0.0778	**-0.4586**	0.2838	**-0.3879**	0.1857	**-0.7809**	0.0929	**-0.0984**	0.1956	**-0.4223**	0.2530	**3.1878**	2.2835	0.0006
397.3124	**-0.2812**	0.2137	**-0.2966**	0.0487	**-0.2465**	0.1290	**-0.3867**	0.0960	**-0.4461**	0.1336	**-0.3813**	0.1417	**-0.2392**	0.0246	**2.9607**	2.7587	0.0082
406.306	**0.3650**	0.9927	**-0.2832**	0.4025	**-0.7380**	0.1309	**0.2074**	0.1576	**1.3145**	0.4304	**-0.5145**	0.5217	**-0.5512**	0.5876	**-0.3451**	0.3223	0.0030
409.1926	**-0.3448**	0.2038	**-0.3631**	0.0428	**-0.3170**	0.1335	**-0.4503**	0.0870	**-0.5059**	0.1152	**-0.4427**	0.1295	**3.2075**	1.0885	**-0.1391**	0.1469	0.0000
410.3386	**-0.2975**	1.1117	**-0.9414**	0.3087	**-0.6829**	0.1890	**1.1692**	0.9783	**0.8872**	0.7075	**-0.4567**	0.4407	**-0.8049**	0.3857	**1.1449**	1.0522	0.0054
410.374	**-0.3889**	0.4197	**-0.5667**	0.2661	**-0.1589**	0.3276	**-0.2463**	0.5975	**1.3955**	0.3954	**0.3109**	1.2278	**-0.5454**	0.1479	**-0.4255**	0.0228	0.0072
413.7702	**-0.1000**	0.3935	**-0.3747**	0.0417	**-0.3294**	0.1343	**-0.4614**	0.0854	**-0.3820**	0.2800	**1.7932**	1.5176	**-0.2193**	0.1729	**-0.1531**	0.1471	0.0033
416.2383	**-0.3370**	0.5217	**-0.5362**	0.1868	**-0.1638**	0.1135	**-0.4547**	0.4072	**-0.4146**	0.2376	**-0.0027**	0.4056	**-0.5273**	0.0975	**3.0370**	2.2579	0.0014
416.9961	**-0.4031**	0.4083	**-0.5531**	0.0870	**-0.3660**	0.4830	**-0.2026**	0.4624	**-0.7109**	0.0520	**1.8392**	0.2175	**-0.4977**	0.1197	**0.9556**	1.7379	0.0029
417.3446	**-0.3528**	0.2026	**-0.3714**	0.0420	**-0.3259**	0.1341	**-0.4583**	0.0858	**1.6966**	0.5716	**-0.1892**	0.5229	**-0.3251**	0.0240	**-0.0587**	0.2268	0.0000

423.023	-0.4152	0.1929	-0.4366	0.0362	0.3399	1.2069	1.9682	1.4587	-0.4048	0.2012	-0.5106	0.1162	-0.3998	0.0235	-0.1535	0.2320	0.0057
430.5121	-0.5853	0.1666	-0.6144	0.0204	-0.0282	0.8978	-0.3258	0.3762	-0.5504	0.2467	2.3094	0.6517	-0.0366	0.5457	-0.0805	0.7095	0.0001
431.3321	-0.9280	0.1810	-0.3117	0.4546	-0.1036	0.8511	0.5893	0.0991	0.5893	0.7703	0.6874	0.6570	-0.4996	0.0552	-0.6698	0.3913	0.0065
437.1275	-0.3486	0.7178	-0.6068	0.6944	-0.2122	0.9600	0.4525	0.3267	1.0455	0.4121	0.2467	0.6013	-0.2280	0.1176	-1.0902	0.1805	0.0099
441.1439	-0.8294	0.2653	-0.2059	0.9682	-0.5465	0.8060	0.0491	0.1965	1.4783	0.3445	0.3205	0.1017	-0.7009	0.3194	-0.0346	1.0404	0.0074
441.3041	-0.5180	0.0270	-0.0346	0.8498	-0.4611	0.1973	0.0244	0.5452	1.6636	0.0437	-0.8137	0.1803	0.0939	0.6779	-0.3040	0.3937	0.0003
442.3245	-0.1182	0.5210	0.0741	0.5860	-0.8033	0.2752	-0.6835	0.3688	-0.4856	0.2280	1.0130	1.2932	-0.7777	0.4564	1.9627	1.6928	0.0085
442.7371	-0.2477	0.8455	-0.6766	0.0149	-0.6496	0.1546	1.2908	1.2505	-0.1649	0.5984	1.3102	0.2246	-0.4877	0.3060	-0.5173	0.1551	0.0016
447.3429	-0.4661	0.2420	-0.5668	0.4064	-0.1991	0.4105	0.2192	0.1505	1.1824	0.5407	0.3452	0.7397	-1.0601	0.2179	-0.1159	0.3742	0.0004
448.6002	-0.3488	0.5859	0.0474	1.1737	0.5091	1.0871	-0.9260	0.0355	-0.6511	0.5104	0.6266	1.2011	2.0989	0.7348	-0.7391	0.1608	0.0054
449.0838	0.1256	0.7315	-0.3467	0.2506	-0.1007	0.5795	-0.3030	0.5412	1.3419	0.7093	-0.5358	0.1445	-0.3439	0.2303	-0.3101	0.1504	0.0049
451.2549	-0.3289	0.2063	-0.3465	0.0442	2.4363	2.3001	-0.4344	0.0892	-0.4909	0.1198	-0.0991	0.4363	-0.2964	0.0242	-0.1190	0.1465	0.0090
452.6687	-0.4065	0.5770	1.8673	1.1758	-0.1184	1.0894	-0.4084	0.4238	-0.7874	0.2031	0.9844	1.0717	-0.5504	0.2534	-0.5355	0.3497	0.0054
453.1472	-0.2784	0.2141	-0.2936	0.0489	-0.2434	0.1288	-0.3839	0.0964	-0.4435	0.1345	-0.3786	0.1422	-0.2358	0.0246	2.9252	2.7572	0.0091
458.3006	-0.2804	0.2138	-0.2957	0.0487	-0.2456	0.1290	-0.3859	0.0961	-0.4453	0.1339	-0.3806	0.1418	-0.2382	0.0246	2.9494	2.7543	0.0084
460.5529	-0.6431	0.0744	1.2471	1.1058	-0.7156	0.2709	1.3774	0.6427	0.3728	0.1771	-0.6824	0.2768	-0.3973	0.5629	-0.7979	0.1624	0.0001
461.5173	-0.5681	0.1693	-0.0424	0.9419	-0.5644	0.1492	-0.0224	0.4644	2.1175	0.9145	-0.3103	0.6574	-0.3866	0.1899	-0.3954	0.1766	0.0004
463.4338	-0.1198	1.0821	0.0780	0.8300	-0.7715	0.2105	0.1046	0.7207	1.7923	0.8075	-0.4851	0.3621	-0.5126	0.5409	-0.3873	0.4501	0.0088
466.8853	-0.2738	0.0471	-0.2625	0.3027	-0.3404	0.1436	-0.1796	0.5671	1.4399	1.2324	-0.4977	0.1525	-0.3150	0.1993	-0.0652	0.1916	0.0062
484.0249	-0.2817	0.2136	-0.2971	0.0486	-0.2471	0.1291	-0.3873	0.0959	-0.4466	0.1335	-0.3819	0.1415	-0.2399	0.0246	2.9761	2.7897	0.0087
484.3047	0.0914	0.5324	-0.3928	0.4641	-0.2043	0.3408	-0.5754	0.3200	1.6073	0.5380	-0.3543	0.1212	-0.5720	0.3319	-0.0534	0.4279	0.0001
485.0682	-0.5704	0.2255	-0.0893	1.2049	-0.2740	0.2652	1.9586	1.2725	-0.6496	0.4065	0.4538	0.5019	-0.5645	0.3926	-0.2527	0.4806	0.0060
485.6837	0.0723	1.1126	-0.6393	0.0182	0.2147	1.1631	-0.2590	0.8200	-0.3233	0.7768	-0.4949	0.4040	2.5440	1.0776	-0.4723	0.1540	0.0039
486.5339	-0.4646	0.1853	-0.4883	0.0316	-0.4498	0.1419	1.2768	2.1480	-0.6184	0.0805	-0.5583	0.1068	2.2114	0.7248	-0.2901	0.1499	0.0031
487.4374	0.2287	1.1851	-0.0169	0.8734	-0.4867	0.1442	1.9185	1.3158	-0.6496	0.0708	-0.3032	0.3973	-0.4989	0.0229	-0.3320	0.1508	0.0098
490.7504	-0.5361	0.5363	2.0057	0.1352	-0.2754	1.0139	0.8842	0.2732	-0.8975	0.1626	0.0459	0.4485	-0.7344	0.3545	-0.5679	0.2156	0.0000
494.2368	0.4911	1.3518	-0.5799	0.4488	1.2011	1.3864	-0.5220	0.4105	-0.8258	0.2043	-0.7910	0.1913	-0.6227	0.2871	2.3538	1.0212	0.0014
495.8764	-0.6385	0.2389	-0.5760	0.2652	-0.5212	0.3106	0.3317	0.4760	0.4938	0.1971	1.1251	1.2640	-0.1299	0.3364	-0.6451	0.4727	0.0076
497.3412	-0.7534	0.4115	-0.8407	0.1843	-0.6076	0.3057	0.4985	0.5946	0.8473	0.2103	0.7503	0.9783	-0.5172	0.6021	0.0484	0.1621	0.0019
501.4487	-0.5773	0.1679	-0.4343	0.1306	-0.5746	0.1498	2.0419	1.6129	0.7958	1.3914	-0.6669	0.0855	0.1602	0.2079	-0.4320	0.1531	0.0054
502.2543	0.9642	1.3415	-0.3139	0.2075	0.0618	0.2990	-0.9324	0.1784	-0.4609	0.2813	-0.0858	1.0855	-0.9928	0.0211	2.2663	1.7631	0.0064
502.5252	-0.3202	0.4436	-0.5887	0.0227	-0.5564	0.1486	1.6832	0.9719	-0.7087	0.0526	0.9543	1.5205	0.0793	0.7184	-0.4113	0.1526	0.0066
503.0417	-0.5555	0.4898	0.1886	0.4715	-0.6466	0.2680	-0.0427	0.3025	1.5264	0.7060	-0.3999	0.3431	0.2331	1.0636	-0.7248	0.1040	0.0023
510.3702	-0.5330	0.5716	-0.8620	0.1964	-0.5012	0.2157	1.2874	1.0814	0.5023	0.4932	0.4697	0.8965	-0.0336	0.0630	-0.8247	0.3307	0.0034
513.2779	-0.4659	0.5570	-0.0203	0.5741	-0.3094	0.2325	0.0664	0.1797	1.6720	0.2468	-0.3319	0.4721	-0.5757	0.0386	-0.5128	0.1337	0.0000
518.8592	-0.4323	0.2703	-0.2834	0.5113	0.1817	0.6789	0.3503	0.3398	1.1204	0.8350	-0.3318	0.3080	-0.7124	0.0570	-0.5336	0.0816	0.0035
524.3682	-0.5160	0.3100	-0.8005	0.1499	-0.5535	0.2661	0.2483	0.2761	1.1596	0.4653	0.6866	0.7433	-0.2982	0.5056	-0.5790	0.1617	0.0002
529.8578	0.3967	1.6768	-0.3017	0.3172	-0.4380	0.2619	1.8982	0.6889	-0.2932	0.3725	-0.6489	0.1407	-0.3235	0.2512	-0.4762	0.1541	0.0060
531.6614	1.8439	0.9482	-0.5596	0.0253	0.7712	1.0311	-0.6383	0.0616	-0.4330	0.4479	-0.0206	1.1025	-0.5409	0.0227	-0.3762	0.1518	0.0026
533.3397	-0.4049	0.2832	-0.5218	0.4222	-0.2907	0.5218	0.4904	0.6263	1.2808	0.4345	-0.2331	0.7741	-0.5391	0.4433	-0.4813	0.6211	0.0070
534.4741	-0.5949	0.1471	-0.7315	0.4684	0.2870	1.1662	0.1926	0.3008	1.5901	0.2172	-0.6740	0.6419	0.3794	0.5674	-0.6876	0.3418	0.0014
535.0794	-0.4238	0.1916	-0.4456	0.0354	0.0967	0.8050	-0.5292	0.0760	-0.5801	0.0923	0.0901	1.1228	2.6695	1.9279	-0.2387	0.1488	0.0041
535.433	0.0904	0.8635	-0.5604	0.1779	-0.5290	0.1704	1.6636	0.9651	-0.7761	0.0319	0.1809	0.7543	0.2085	0.9352	-0.5016	0.1547	0.0055
535.658	-0.2788	0.2140	-0.2940	0.0489	-0.2438	0.1289	-0.3843	0.0963	-0.4438	0.1343	-0.3790	0.1421	-0.2363	0.0246	2.9670	2.6204	0.0051
541.66	-0.4362	0.2322	0.2694	0.9941	-0.1373	0.7118	2.1138	1.2783	-0.7319	0.0455	-0.0699	1.0998	-0.4106	0.3552	-0.4425	0.1533	0.0078
541.9621	-0.2817	0.2136	-0.2972	0.0486	-0.2471	0.1291	-0.3873	0.0959	-0.4466	0.1335	-0.3819	0.1415	-0.2399	0.0246	2.9718	2.7731	0.0083
546.9641	-0.0048	0.8631	-0.5759	0.0238	-0.5428	0.1478	1.7670	0.3472	-0.0870	1.0688	0.0525	1.1076	-0.5596	0.0226	-0.3958	0.1523	0.0067
550.4282	-0.3622	0.2838	-0.5747	0.0239	-0.3922	0.2266	1.8567	1.1377	-0.5443	0.3078	-0.2092	0.7847	0.5170	1.0941	-0.2153	0.4013	0.0053
552.5012	-0.4036	0.0303	-0.4290	0.2727	-0.6164	0.2884	0.4470	1.0935	1.4434	0.2756	-0.2452	0.2912	-0.3363	0.3143	-0.4822	0.1225	0.0006
557.7551	-0.2816	0.2136	2.1353	2.0770	-0.2470	0.1291	-0.3872	0.0959	-0.4465	0.1335	-0.3818	0.1416	-0.2397	0.0246	-0.0594	0.1454	0.0097
560.4113	-0.4483	0.5461	-0.6121	0.3823	0.0494	0.7596	0.0495	1.1643	1.5819	0.3950	-0.0501	0.1537	-0.4034	0.4474	-0.6440	0.2306	0.0065
561.0916	-0.1655	0.3932	-0.3249	0.3048	-0.3959	0.4813	0.0618	0.3131	1.2256	0.8522	-0.3914	0.2918	-0.1705	0.3463	-0.5068	0.3257	0.0053
565.1933	0.0034	0.7909	0.3115	0.7062	-0.6235	0.1529	1.8604	1.5809	-0.7656	0.0352	0.0766	0.7489	-0.5621	0.1288	-0.4876	0.1544	0.0092
567.8766	-0.2815	0.2136	2.1354	2.0801	-0.2469	0.1291	-0.3871	0.0959	-0.4464	0.1335	-0.3817	0.1416	-0.2396	0.0246	-0.0593	0.1454	0.0098
569.8058	-0.6271	0.4150	0.6288	0.7186	-0.0839	0.6448	0.3218	0.4461	-0.7672	0.4836	1.8207	1.6883	-0.9332	0.0753	-0.4093	0.5664	0.0079
571.4997	-0.2978	0.2111	-0.1701	0.2472	2.5266	2.3264	-0.4034	0.0936	-0.4617	0.1288	-0.3974	0.1385	-0.2592	0.0244	-0.0799	0.1458	0.0071
579.6151	-0.4201	0.1922	-0.1987	0.2105	-0.4004	0.1388	-0.4748	0.1542	1.6237	1.3318	-0.3522	0.1677	-0.2679	0.2355	0.1675	0.2951	0.0019

582.7148	**-0.3329**	0.2057	**-0.3506**	0.0439	**-0.3038**	0.1327	**-0.4384**	0.0886	**-0.4947**	0.1186	**-0.4312**	0.1318	**2.7220**	2.2674	**0.2847**	0.5634	0.0031
584.4177	**-0.2753**	0.1262	**-0.7633**	0.2114	**-0.9411**	0.5920	**0.0913**	0.6078	**0.9145**	0.7899	**1.0356**	1.1691	**-0.4995**	0.0345	**-0.0639**	0.4868	0.0083
585.2049	**-0.3911**	0.3562	**-0.0848**	0.6140	**-0.5089**	0.4606	**-0.5024**	0.5099	**2.1820**	1.4501	**-0.4699**	0.4406	**0.2248**	0.6771	**-0.5567**	0.1561	0.0023
585.4431	**0.0949**	0.7508	**-0.5600**	0.2758	**-0.5654**	0.2061	**1.6254**	0.9161	**-0.1880**	0.8833	**0.4507**	0.4236	**-0.4945**	0.7301	**-0.5754**	0.5064	0.0075
585.6576	**-0.5179**	0.1771	**-0.5439**	0.0267	**-0.5088**	0.1456	**2.2024**	0.4699	**-0.1085**	1.0194	**0.2892**	0.7933	**-0.5228**	0.0228	**-0.3571**	0.1514	0.0001
587.5937	**-0.2800**	0.2138	**-0.2953**	0.0488	**2.5473**	2.3376	**-0.3855**	0.0962	**-0.4450**	0.1340	**-0.3802**	0.1419	**-0.2378**	0.0246	**-0.0573**	0.1454	0.0067
592.1613	**-0.5791**	0.2587	**-0.6941**	0.3366	**-0.6479**	0.3817	**0.7015**	0.5970	**0.8128**	0.1020	**0.6968**	0.9076	**-0.3638**	0.7196	**-0.5813**	0.3609	0.0030
594.375	**-0.3611**	0.4140	**-0.8314**	0.3253	**-0.3609**	0.4479	**0.9067**	0.9713	**1.4500**	0.7126	**-0.0172**	0.5730	**-0.8506**	0.7392	**-0.4203**	0.2890	0.0020
599.1903	**-0.6263**	0.3777	**-0.8012**	0.0038	**-0.7126**	0.2825	**1.3501**	0.7321	**0.1920**	0.5981	**1.0147**	1.2798	**-0.0179**	0.9670	**-0.6675**	0.1589	0.0061
602.1813	**-0.2729**	0.3014	**-0.0441**	0.3902	**-0.3771**	0.3598	**-0.5341**	0.5786	**-0.3331**	0.4857	**2.0361**	0.6499	**-0.2329**	0.7466	**-0.3346**	0.3636	0.0003
614.3189	**0.0728**	1.1146	**-0.6401**	0.0181	**2.3963**	0.8267	**-0.7152**	0.0523	**-0.7549**	0.0384	**-0.6984**	0.0793	**0.4286**	1.0546	**0.4587**	0.7704	0.0004
617.416	**-0.2787**	0.3091	**-0.9037**	0.4998	**-0.9884**	0.1770	**-0.0077**	0.4889	**1.2936**	0.6951	**0.8458**	0.3979	**-0.0038**	0.5295	**-0.4771**	0.5785	0.0003
619.398	**-0.4496**	0.5456	**-0.5833**	0.5293	**-0.6526**	0.2626	**0.2247**	0.5700	**0.5229**	1.3615	**1.6316**	0.4569	**-0.0346**	0.3723	**-0.7111**	0.4127	0.0056
621.3953	**-0.1340**	0.3765	**-0.6655**	0.0516	**-0.2670**	0.3577	**0.4874**	0.3223	**1.1452**	0.8298	**-0.2770**	0.4671	**-0.6505**	0.2726	**-0.3635**	0.2620	0.0011
625.263	**-0.3288**	0.2050	**-0.9060**	0.3102	**-0.0812**	0.1408	**1.0116**	1.0125	**0.9820**	0.4554	**0.1532**	0.2756	**-0.7653**	0.5182	**-0.6826**	0.5834	0.0009
632.0909	**-0.2816**	0.2136	**-0.2970**	0.0486	**-0.2469**	0.1291	**-0.3871**	0.0959	**-0.4465**	0.1335	**-0.3817**	0.1416	**-0.2397**	0.0246	**2.9674**	2.7653	0.0082
641.2776	**0.0943**	0.4527	**-1.0940**	0.1678	**-0.2460**	0.7152	**1.2404**	0.9116	**0.7526**	0.9522	**0.4625**	0.0876	**-0.7343**	0.5632	**-0.8943**	0.1393	0.0015
643.0737	**-0.2797**	0.2139	**-0.2950**	0.0488	**-0.2448**	0.1289	**-0.3852**	0.0962	**-0.4447**	0.1341	**-0.3799**	0.1419	**2.6568**	2.5381	**-0.0570**	0.1454	0.0099
643.1952	**0.3220**	1.2396	**-0.4686**	0.0334	**-0.3382**	0.2978	**2.1185**	0.1641	**-0.6007**	0.0859	**-0.5401**	0.1104	**-0.4365**	0.0233	**-0.1761**	0.2566	0.0000
643.4689	**1.9697**	1.3366	**-0.3864**	0.0407	**0.3835**	1.1976	**-0.4726**	0.0838	**-0.5269**	0.1087	**-0.4643**	0.1253	**-0.3423**	0.0239	**-0.1672**	0.1474	0.0029
644.3959	**-0.1600**	0.6171	**-0.4249**	0.0373	**-0.1174**	0.4115	**-0.5094**	0.0787	**2.1524**	0.9695	**-0.4998**	0.1183	**-0.3864**	0.0236	**-0.2137**	0.1483	0.0000
645.3157	**-0.0907**	0.7552	**-0.4870**	0.1401	**-0.8214**	0.6081	**0.1771**	0.7969	**1.1370**	0.1642	**0.4519**	0.6180	**-0.0523**	0.7133	**-0.9773**	0.5348	0.0092
653.4196	**0.0602**	0.9848	**-0.8007**	0.5320	**-0.9975**	0.2530	**-0.1378**	1.1496	**1.5573**	0.3361	**0.3450**	0.3188	**-0.3198**	0.4204	**-0.0534**	0.1434	0.0044
659.4174	**-0.5422**	0.4480	**-0.5555**	0.2266	**-0.7112**	0.3020	**-0.0926**	0.6195	**1.5075**	0.7368	**0.6658**	0.9700	**0.0105**	0.5517	**-0.7576**	0.4696	0.0023
665.4305	**-0.2920**	0.0297	**-0.4673**	0.2273	**-0.6766**	0.3094	**0.2246**	0.3017	**1.5153**	0.3172	**0.2139**	1.2421	**-0.4991**	0.5495	**-0.6103**	0.4135	0.0025
666.1286	**0.0063**	0.9668	**0.9747**	1.3107	**-0.6585**	0.3034	**-0.0018**	0.8043	**-0.8652**	0.0061	**-0.8117**	0.0573	**1.9304**	1.2546	**-0.1415**	0.5052	0.0077
667.1446	**0.4610**	1.1503	**1.8295**	0.8539	**-0.2745**	0.5043	**-0.1486**	0.8349	**-0.9804**	0.4214	**-0.0762**	0.5632	**-0.3598**	0.3822	**-0.5691**	0.3573	0.0054
667.2944	**-0.1364**	0.6266	**-1.0578**	0.4007	**-0.1594**	0.1374	**0.8665**	0.9987	**0.9778**	0.5922	**0.5313**	0.5651	**-0.2469**	0.8592	**-1.2482**	0.5244	0.0037
669.302	**0.3021**	0.4722	**-0.7398**	0.2517	**-0.3603**	0.8323	**0.0843**	0.8096	**1.2205**	0.0674	**0.4247**	0.4775	**-0.1061**	1.0129	**-1.4351**	0.4454	0.0038
673.42	**-0.4949**	0.3072	**-0.9944**	0.4288	**-0.6280**	0.3717	**0.7163**	0.7319	**1.2378**	0.7444	**0.9999**	0.7894	**-0.6030**	0.0921	**-0.6685**	0.6322	0.0005
677.3749	**-0.6352**	0.3009	**-0.1104**	0.3705	**-0.9467**	0.4235	**-0.7137**	0.4038	**1.2920**	1.0683	**0.8426**	0.8813	**0.2073**	0.6245	**-0.2591**	0.8455	0.0092
678.511	**-0.8171**	0.2618	**-0.4760**	0.6144	**-0.8586**	0.3935	**0.6940**	0.5509	**1.1370**	0.4987	**-0.5073**	0.8030	**0.1026**	0.9813	**0.6436**	0.2126	0.0037
682.3067	**0.1200**	0.4994	**-0.3572**	0.2054	**0.4270**	0.4296	**0.5007**	0.6794	**0.9447**	0.7351	**0.2594**	0.4454	**-0.2997**	1.0433	**-2.0706**	0.9291	0.0022
684.2902	**-0.2425**	0.6507	**-0.5449**	0.3416	**-0.4933**	0.8633	**0.3671**	0.4496	**0.8867**	0.5092	**0.4930**	0.4333	**-0.1296**	0.3294	**-1.0786**	0.5679	0.0086
687.1717	**-0.3741**	0.5320	**-0.0433**	0.6261	**0.0955**	0.4358	**-0.3146**	0.6420	**2.0263**	1.2822	**-0.4799**	0.2684	**-0.8332**	0.0923	**-0.2070**	0.5907	0.0024
687.5476	**-0.1599**	0.4751	**-0.3098**	0.5773	**-0.5739**	0.2028	**-0.7085**	0.0531	**1.8535**	0.2982	**-0.4787**	0.4079	**0.0954**	1.2296	**0.1577**	0.5759	0.0016
689.3403	**0.0218**	0.3072	**-0.3108**	0.3382	**-0.2900**	0.3577	**-0.6393**	0.0615	**-0.6835**	0.0604	**-0.6252**	0.0937	**0.4143**	1.6544	**2.8672**	1.6489	0.0018
691.1903	**-0.0600**	0.4326	**-0.4296**	0.2584	**-0.3632**	0.4546	**-0.0409**	0.1035	**1.1808**	1.0646	**-0.5644**	0.1381	**0.0207**	0.3203	**-0.3330**	0.2039	0.0095
691.4539	**-0.8717**	0.2671	**-0.8001**	0.0975	**-0.4894**	0.2762	**0.6244**	1.1174	**1.3912**	0.6763	**0.3404**	0.3158	**-0.2175**	0.7939	**-0.4209**	0.6242	0.0040
693.1016	**-0.2866**	0.2128	**-0.3022**	0.0482	**2.5148**	2.3600	**-0.3921**	0.0952	**-0.4512**	0.1321	**-0.3866**	0.1406	**-0.2457**	0.0245	**0.0253**	0.2744	0.0083
693.1858	**-0.4870**	0.1529	**2.2094**	0.6157	**-0.3912**	0.7811	**-0.3919**	0.7942	**-0.4919**	0.8639	**-0.2784**	0.4604	**-0.0291**	0.6304	**-0.0387**	1.0955	0.0050
694.0418	**-0.3677**	0.2003	**2.6854**	1.6324	**-0.3174**	0.1784	**-0.4731**	0.0838	**-0.5273**	0.1086	**-0.3245**	0.3195	**-0.3429**	0.0239	**-0.0444**	0.0706	0.0001
696.3293	**-0.0232**	0.6618	**-0.3223**	0.3070	**0.1221**	0.3333	**0.3821**	0.4157	**0.7530**	0.6492	**0.1417**	0.5435	**-0.2388**	0.3624	**-1.7012**	0.7080	0.0017
696.3978	**-0.4323**	0.2885	**-0.6897**	0.2368	**-0.6278**	0.1996	**-0.7617**	0.5404	**1.1339**	0.6108	**-0.2040**	0.4119	**-0.3597**	0.6461	**2.2095**	1.8612	0.0021
697.5144	**-0.4003**	0.2926	**-0.7243**	0.0106	**-0.0625**	0.2954	**-0.0387**	0.9323	**1.2636**	0.6417	**0.2361**	0.3544	**-0.4839**	0.4266	**-0.3909**	0.1620	0.0032
699.3123	**0.3660**	0.8089	**-1.0204**	0.3450	**0.4708**	1.4335	**0.5788**	0.7408	**0.6654**	0.4704	**0.4556**	0.3201	**-0.3210**	0.5232	**-1.6632**	0.5766	0.0087
704.3949	**-0.1442**	0.5578	**-0.6745**	0.1575	**-0.8784**	0.2260	**0.1240**	0.9010	**1.1053**	0.2183	**0.6282**	0.7152	**-0.1034**	0.6979	**-0.6981**	0.2776	0.0040
705.3961	**-0.1322**	0.4964	**-0.7041**	0.2331	**-0.7963**	0.0747	**0.1872**	0.7973	**1.1696**	0.2498	**0.5282**	0.6524	**-0.2118**	0.7683	**-0.7464**	0.1152	0.0020
705.7935	**-0.1931**	0.4168	**-0.4877**	0.0317	**-0.4493**	0.1419	**-0.5695**	0.0706	**-0.2665**	0.6306	**2.0558**	0.7550	**0.0190**	0.4321	**-0.2742**	0.1626	0.0000
710.2031	**-0.8132**	0.1315	**-0.0476**	0.8654	**0.2410**	0.4238	**0.2100**	1.0086	**1.6521**	0.4205	**0.0051**	1.1317	**-0.7389**	0.2562	**-0.7293**	0.1606	0.0063
710.6268	**-0.1739**	0.2868	**-0.1939**	0.8989	**-0.5057**	0.2643	**-0.1400**	0.7267	**1.4740**	0.3631	**-0.2601**	0.5187	**-0.3838**	0.5451	**-0.4004**	0.1089	0.0064
713.3117	**-0.2583**	0.1923	**-0.5698**	0.1104	**0.0010**	0.2997	**0.2093**	0.4021	**0.9305**	0.9413	**0.1159**	0.5595	**-0.3438**	0.1679	**-0.9092**	0.4914	0.0082
716.4066	**0.0349**	0.5905	**-0.9327**	0.2531	**-0.8354**	0.4591	**0.1678**	0.9796	**0.9806**	0.3060	**0.7798**	0.7573	**-0.1673**	0.6392	**-0.6823**	0.1622	0.0055
718.5759	**-0.3476**	0.3812	**-0.5555**	0.2202	**-0.3270**	0.6909	**-0.6986**	0.3125	**1.3302**	0.3717	**0.8913**	1.1513	**-0.6792**	0.1323	**-0.0292**	0.8300	0.0050
722.6047	**-0.4017**	0.1950	**-0.4225**	0.0375	**-0.3801**	0.1375	**-0.5071**	0.0791	**1.2531**	0.6255	**0.4624**	1.2121	**-0.3837**	0.0236	**-0.2108**	0.1483	0.0049
727.3557	**0.1730**	0.5298	**-0.7939**	0.1792	**-0.0505**	0.2706	**0.4875**	0.7292	**0.8467**	0.2730	**0.3680**	0.5181	**-0.1938**	0.6567	**-1.5920**	0.8886	0.0016
730.0596	**-0.5771**	0.1679	**-0.4751**	0.2247	**-0.0067**	0.9193	**-0.4271**	0.4659	**1.6048**	0.4607	**0.1215**	0.3986	**-0.2533**	0.3354	**-0.4318**	0.1531	0.0005

730.284	**2.1596**	0.6923	**-0.5583**	0.3318	**-0.9050**	0.8536	**-0.3902**	0.2972	**-0.1653**	0.9633	**0.3862**	0.6600	**-0.0146**	0.4706	**-0.3664**	0.7950	0.0016
731.2684	**0.0801**	0.6237	**-0.1050**	0.5505	**-0.3827**	0.1377	**2.0138**	1.3860	**-0.5615**	0.0980	**-0.4998**	0.1183	**-0.3864**	0.0236	**-0.2137**	0.1483	0.0011
736.319	**0.7848**	0.6018	**-0.7652**	0.4841	**1.1526**	1.0466	**-0.4666**	0.7949	**0.1702**	0.4421	**0.6640**	0.6002	**-1.4246**	0.9481	**-0.4327**	0.6007	0.0061
736.513	**-0.0253**	1.1564	**-0.7257**	0.5206	**-0.8422**	0.5044	**0.0939**	0.7057	**1.1878**	0.3067	**0.7904**	0.9366	**-0.9999**	0.1568	**0.0614**	0.0867	0.0072
742.2975	**0.4620**	1.5411	**-0.5038**	0.0302	**2.3006**	0.6261	**-0.5849**	0.0685	**-0.6324**	0.0761	**-0.5726**	0.1040	**-0.4769**	0.0230	**0.2887**	0.8529	0.0007
750.7743	**-0.2755**	0.2145	**-0.2906**	0.0492	**-0.2402**	0.1286	**-0.3810**	0.0968	**-0.4407**	0.1353	**-0.3758**	0.1427	**-0.2324**	0.0246	**3.0528**	2.7531	0.0064
751.5304	**0.6817**	0.7137	**-1.0576**	0.3550	**-0.1321**	0.5734	**1.4495**	0.6676	**-0.1362**	0.3465	**0.4571**	0.7661	**-0.7721**	0.0479	**-0.8316**	1.0145	0.0020
753.3364	**0.7768**	0.6919	**-0.8757**	0.1200	**0.8151**	1.4203	**0.6363**	0.2773	**0.5042**	0.4504	**-0.6139**	0.6177	**-0.0341**	0.5249	**-1.5138**	0.9211	0.0077
754.285	**1.7601**	0.9485	**-0.2223**	0.4439	**-0.7046**	0.1580	**-0.5668**	0.3603	**-0.0372**	0.7148	**-0.7800**	0.0634	**-0.2258**	0.8625	**0.5623**	0.9969	0.0044
757.1808	**-0.1924**	0.2361	**-0.6551**	0.1264	**-0.5478**	0.2189	**-0.4031**	0.3906	**1.5108**	0.7331	**-0.1170**	0.5919	**0.0072**	0.3753	**-0.0559**	0.6324	0.0010
758.1869	**-0.1265**	0.4065	**-0.1782**	0.4068	**-0.3457**	0.3177	**-0.2645**	0.3142	**1.3544**	0.7282	**-0.1618**	0.3079	**-0.4227**	0.3421	**-0.4916**	0.4174	0.0017
759.1159	**-0.7125**	0.1869	**-0.4153**	0.6154	**-0.3747**	0.5438	**0.8923**	0.5722	**-0.1784**	0.6544	**1.3516**	1.1355	**-0.5446**	0.3998	**-0.4164**	0.1707	0.0056
764.2759	**-0.1522**	0.5174	**1.9917**	0.6634	**-0.6511**	0.1547	**-0.7515**	0.0483	**-0.2326**	0.9382	**-0.3337**	0.6207	**-0.0969**	1.0237	**-0.0878**	0.5289	0.0028
772.4342	**0.3067**	1.1718	**-0.4357**	0.0363	**-0.3940**	0.1384	**-0.5197**	0.0773	**-0.5711**	0.0950	**2.0293**	0.5553	**-0.3988**	0.0235	**-0.2266**	0.1486	0.0001
782.974	**-0.0289**	0.8082	**1.6555**	0.6855	**-0.1459**	1.2879	**0.8235**	0.5526	**-1.0285**	0.0466	**-0.1813**	0.3337	**-0.5032**	0.4517	**-0.8044**	0.1161	0.0024
783.1169	**-0.2617**	0.5203	**0.0458**	0.8805	**-0.4383**	0.1412	**-0.5596**	0.0719	**1.4421**	0.4954	**-0.5482**	0.1088	**-0.4466**	0.0232	**0.5248**	1.2398	0.0093
799.2177	**-0.8954**	0.1188	**-0.5318**	0.6959	**0.0704**	0.2514	**0.0162**	0.9700	**0.9531**	0.6760	**0.8507**	0.5665	**-0.6531**	0.4204	**-0.3085**	0.4604	0.0090
800.4189	**-0.1838**	0.9920	**-0.8830**	0.4366	**-0.2918**	0.4015	**0.6018**	0.4286	**1.1234**	0.3721	**-0.8227**	0.3319	**0.4797**	0.7968	**-0.5215**	0.5063	0.0048
805.3087	**-0.1250**	0.4608	**-0.7750**	0.3219	**-0.1437**	0.2385	**-0.7667**	0.2504	**1.3079**	0.7845	**0.2840**	0.6574	**0.0550**	0.2990	**-0.1840**	0.7186	0.0037
815.3275	**-0.8362**	0.1811	**-0.2080**	0.2799	**1.0409**	1.3000	**-0.5145**	0.2004	**0.5396**	0.4819	**0.8593**	0.7862	**-0.8037**	0.2242	**-0.6125**	0.2007	0.0035
819.396	**0.5379**	0.5530	**-1.4108**	0.4592	**0.7997**	1.2525	**0.4829**	0.5556	**0.7947**	0.0571	**-0.5945**	0.6177	**-0.0273**	0.6833	**-0.8790**	0.9001	0.0088
821.4093	**0.6525**	0.4338	**-1.2454**	0.7141	**0.4481**	0.7338	**0.3017**	0.6959	**1.0167**	0.5681	**-0.7287**	0.8280	**-0.5699**	0.4255	**-0.0705**	0.0464	0.0041
825.5536	**-0.2419**	0.5455	**-0.4476**	0.2935	**0.0021**	0.7223	**-0.7016**	0.0539	**0.0209**	0.6286	**1.9636**	0.2114	**-0.6169**	0.0223	**-0.4561**	0.1537	0.0000
838.0642	**0.0291**	0.7889	**2.2276**	1.2487	**-0.0550**	0.5960	**-0.3687**	0.3647	**-0.6609**	0.0673	**-0.6019**	0.0982	**-0.1821**	0.5557	**-0.3471**	0.1512	0.0007
848.2675	**0.5103**	0.9955	**-0.9220**	0.3020	**1.7117**	0.6440	**0.4366**	0.2583	**-0.8831**	0.4519	**0.6292**	0.4797	**-0.6170**	0.6298	**-0.8788**	0.4015	0.0002
852.246	**1.6373**	0.7099	**-0.1633**	0.4120	**-0.0111**	0.2949	**-0.7885**	0.4027	**0.4988**	1.3999	**-0.4895**	0.4739	**-0.3081**	0.5960	**-0.5801**	0.1507	0.0085
863.4396	**-0.1961**	0.8633	**-0.8019**	0.0951	**0.2377**	0.4840	**0.5863**	0.8988	**1.1689**	0.3748	**0.1843**	0.4847	**-0.2875**	0.6193	**-1.4869**	0.7052	0.0027
863.8219	**-0.2809**	0.2137	**-0.2963**	0.0487	**-0.2463**	0.1290	**-0.3865**	0.0960	**-0.4459**	0.1337	**-0.3811**	0.1417	**2.6622**	2.5032	**-0.0586**	0.1454	0.0086
870.6955	**-0.4021**	0.0851	**-0.2694**	0.7486	**-0.3401**	0.5084	**1.8063**	1.1921	**-0.2944**	0.8844	**0.2526**	0.4411	**-0.3630**	0.3784	**-0.5343**	0.1600	0.0081
871.4632	**-0.4851**	0.1821	**-0.5096**	0.0297	**-0.4725**	0.1433	**1.3570**	1.6442	**-0.6376**	0.0745	**-0.2754**	0.4216	**-0.4836**	0.0230	**1.5923**	1.0934	0.0044
878.4179	**-0.2859**	0.2129	**2.1708**	1.8596	**-0.2518**	0.1294	**-0.3915**	0.0953	**-0.4506**	0.1323	**-0.3859**	0.1407	**-0.2449**	0.0245	**-0.0649**	0.1455	0.0031
882.5406	**1.9430**	1.7133	**-0.2971**	0.0486	**-0.2471**	0.1291	**-0.3872**	0.0959	**-0.4466**	0.1335	**-0.3818**	0.1416	**-0.2398**	0.0246	**-0.0595**	0.1454	0.0034
888.4012	**0.1723**	0.7530	**0.0744**	0.8128	**1.4817**	0.4461	**-0.2756**	0.6288	**0.4522**	0.5613	**-0.5331**	1.0224	**-0.8774**	0.6167	**-1.0230**	0.2720	0.0074
888.5279	**-0.3584**	0.2017	**-0.3773**	0.0415	**-0.3321**	0.1345	**-0.4639**	0.0851	**-0.5186**	0.1112	**-0.4558**	0.1270	**3.3288**	0.2102	**-0.1562**	0.1472	0.0000
891.6714	**-0.3377**	0.2355	**-0.5217**	0.0286	**-0.1118**	0.5859	**-0.6020**	0.0663	**-0.0634**	1.0301	**2.3267**	1.1482	**-0.4974**	0.0229	**-0.1674**	0.3724	0.0004
892.339	**-0.5266**	0.5570	**-1.0164**	0.1530	**0.0290**	1.0239	**-0.0998**	1.0098	**-0.0786**	0.5544	**-0.4394**	0.4449	**2.2824**	1.5451	**0.0970**	0.3035	0.0076
895.8239	**-0.2816**	0.2136	**2.1354**	2.0795	**-0.2469**	0.1291	**-0.3871**	0.0959	**-0.4465**	0.1335	**-0.3817**	0.1416	**-0.2397**	0.0246	**-0.0594**	0.1454	0.0098
897.6964	**0.4213**	1.3079	**-0.3996**	0.0395	**-0.3558**	0.1360	**-0.4852**	0.0821	**1.3793**	0.4740	**-0.4764**	0.1229	**-0.3574**	0.0238	**-0.1831**	0.1477	0.0034
901.1875	**-0.5417**	0.1734	**0.9165**	1.5882	**-0.5352**	0.1473	**1.7810**	0.4140	**-0.6908**	0.0581	**-0.4355**	0.2498	**-0.1555**	0.7064	**-0.3872**	0.1521	0.0021
908.4374	**0.7224**	0.5196	**-0.5823**	0.4391	**0.6366**	1.2293	**0.3068**	0.6175	**0.3869**	0.7257	**-1.3010**	0.2325	**0.7152**	0.8418	**-1.1365**	0.3169	0.0056
909.3646	**-0.3613**	1.2605	**-0.2560**	0.6825	**1.8027**	0.7894	**-0.6501**	0.8038	**0.8853**	0.5599	**-0.7070**	0.6806	**-0.1894**	0.4178	**-0.4439**	0.4794	0.0093
913.2725	**0.3110**	0.8685	**-0.5677**	0.5005	**-0.5941**	0.7749	**-0.6038**	0.3097	**1.6429**	0.2863	**0.2800**	0.5511	**-0.5116**	0.5664	**-0.3907**	0.5677	0.0025
918.3019	**-0.6135**	0.4279	**-0.8197**	0.0022	**-0.3352**	0.2426	**0.1757**	0.9851	**1.0692**	0.3297	**-0.4783**	0.4163	**-0.6223**	0.3564	**1.6893**	1.2905	0.0014
918.8246	**-0.5112**	0.1781	**-0.0562**	0.4637	**-0.4285**	0.1349	**-0.3825**	0.4523	**-0.3071**	0.5519	**1.7556**	1.3076	**-0.3046**	0.3463	**-0.1501**	0.1937	0.0036
922.1373	**-0.2753**	0.2146	**-0.2904**	0.0492	**2.5112**	2.4127	**-0.3808**	0.0968	**-0.4406**	0.1354	**-0.3757**	0.1428	**-0.2321**	0.0246	**-0.0514**	0.1453	0.0100
930.1249	**-0.4348**	0.1899	**-0.4571**	0.0344	**0.5716**	0.6053	**-0.1145**	0.1072	**1.2504**	1.0793	**-0.5295**	0.1125	**-0.4233**	0.0233	**-0.2525**	0.1491	0.0015
937.2856	**-0.1903**	0.8354	**-0.9447**	0.3873	**1.1938**	1.0601	**-0.8652**	0.4511	**0.3173**	0.7041	**0.3434**	0.5901	**0.8114**	0.9787	**-1.0681**	0.3994	0.0081
944.4324	**-0.2918**	0.8584	**0.8880**	0.5986	**-0.8436**	0.1669	**1.0945**	1.4051	**-0.4572**	0.4416	**-0.9010**	0.0404	**0.8742**	0.6555	**-0.4171**	0.3949	0.0084
951.3893	**-0.4965**	0.1804	**1.8514**	1.3616	**-0.4851**	0.1441	**0.9456**	1.3070	**-0.6483**	0.0712	**-0.5889**	0.1008	**-0.4972**	0.0229	**-0.0414**	0.5805	0.0036
951.4323	**1.3965**	0.2652	**-0.4639**	0.5203	**1.2152**	0.9183	**-0.4997**	1.1481	**-0.2808**	1.2575	**-0.9039**	0.2419	**0.2879**	0.4531	**-0.7720**	0.3345	0.0083
954.1889	**-0.2344**	0.6203	**-0.3913**	0.9137	**-0.5043**	0.4307	**-0.7761**	0.3438	**1.6701**	1.0736	**-0.7729**	0.3377	**1.5271**	0.1910	**-0.3029**	0.2881	0.0003
957.5587	**-0.5702**	0.1690	**-0.5985**	0.0218	**-0.1691**	0.8380	**-0.6754**	0.0570	**1.4352**	0.3450	**0.4504**	0.9795	**-0.5855**	0.0224	**0.2272**	0.7349	0.0022
970.435	**1.8889**	0.2770	**-0.1555**	0.1213	**-0.3268**	0.5248	**-0.5040**	0.2016	**0.0874**	1.0726	**-0.6526**	0.2103	**-0.2798**	0.7306	**-0.5003**	0.1898	0.0005
972.2235	**-0.7090**	0.1475	**-0.7436**	0.0089	**1.7206**	1.4221	**-0.1730**	0.7013	**-0.5274**	0.2831	**-0.4775**	0.4884	**-0.4938**	0.2450	**1.7673**	1.3743	0.0020
974.4009	**-0.4963**	0.1804	**2.0249**	1.3379	**-0.4849**	0.1441	**-0.6017**	0.0663	**0.3527**	0.8442	**-0.5888**	0.1008	**-0.1307**	0.6319	**-0.2339**	0.2340	0.0012
977.4003	**1.8823**	1.1365	**0.2575**	0.5504	**-0.8071**	0.1645	**-0.0784**	0.5228	**-0.2400**	0.5969	**-0.5486**	0.2730	**-0.4403**	0.7038	**-0.4026**	0.3493	0.0018
980.0231	**-0.3599**	0.2015	**-0.3788**	0.0414	**-0.3337**	0.1346	**-0.2877**	0.2314	**-0.2711**	0.3267	**1.6642**	1.1569	**-0.3335**	0.0239	**-0.1580**	0.1472	0.0005

987.6351	**-0.6478**	0.1570	**-0.6797**	0.0146	**0.8875**	0.7654	**-0.7530**	0.0481	**-0.7904**	0.0276	**0.1914**	1.6329	**-0.0071**	0.6785	**2.7504**	0.8960	0.0003
992.4166	**-0.2804**	0.2138	**2.1167**	2.0531	**-0.2456**	0.1290	**-0.3859**	0.0961	**-0.4454**	0.1339	**-0.3806**	0.1418	**-0.2383**	0.0246	**-0.0579**	0.1454	0.0094
992.4309	**1.6373**	0.8711	**0.1167**	0.5963	**1.0003**	1.3277	**-0.7798**	0.3129	**-0.1740**	0.1587	**-0.5723**	0.4970	**-0.6275**	0.2766	**-0.7866**	0.1621	0.0018
992.9971	**-0.1743**	0.3401	**-0.4275**	0.0370	**2.4794**	2.2778	**-0.1759**	0.3812	**-0.5637**	0.0973	**-0.1985**	0.3637	**-0.3301**	0.0862	**-0.2167**	0.1484	0.0083
995.3013	**-0.6753**	0.1527	**1.4793**	1.0818	**-0.6833**	0.1567	**1.2942**	0.1607	**-0.3291**	0.8264	**-0.7615**	0.0670	**-0.7116**	0.0219	**0.1945**	0.6128	0.0002
1001.3447	**0.9603**	0.6269	**-0.6255**	0.2354	**-0.3786**	0.7109	**0.4277**	1.0453	**0.9226**	0.4334	**-0.8920**	0.0819	**-0.3314**	0.5955	**-0.5569**	0.1512	0.0044
1002.4066	**-0.3512**	0.2028	**2.7242**	0.5188	**-0.3241**	0.1339	**-0.4567**	0.0861	**-0.5119**	0.1133	**-0.4489**	0.1283	**-0.3231**	0.0240	**-0.1471**	0.1470	0.0000
1007.9415	**-0.2522**	0.2247	**2.0746**	1.7766	**0.1687**	0.8638	**-0.4916**	0.0812	**-0.5447**	0.1032	**-0.4826**	0.1217	**-0.1387**	0.4146	**-0.1814**	0.1552	0.0076
1012.4009	**0.4765**	1.5318	**1.8487**	0.8400	**0.1716**	1.0076	**-0.5658**	0.0711	**-0.6145**	0.0817	**-0.5543**	0.1076	**-0.4541**	0.0231	**-0.2848**	0.1498	0.0089
1021.0196	**-0.6131**	0.1745	**-0.0676**	0.9975	**-0.6228**	0.1529	**-0.7259**	0.0511	**1.5755**	0.3868	**1.0898**	0.3736	**-0.5586**	0.1335	**-0.4868**	0.1544	0.0000
1023.5445	**-0.2797**	0.2139	**-0.2951**	0.0488	**-0.2449**	0.1289	**-0.3853**	0.0962	**-0.4448**	0.1341	**-0.3800**	0.1419	**2.6574**	2.5367	**-0.0571**	0.1454	0.0098
1032.5598	**-0.2781**	0.2141	**-0.2934**	0.0489	**2.5274**	2.3453	**-0.3837**	0.0964	**-0.4432**	0.1345	**-0.3784**	0.1422	**-0.2356**	0.0246	**-0.0550**	0.1453	0.0074
1034.7511	**-0.2816**	0.2136	**-0.2970**	0.0486	**-0.2470**	0.1291	**-0.3871**	0.0959	**-0.4465**	0.1335	**-0.3818**	0.1416	**-0.2397**	0.0246	**2.9770**	2.7980	0.0089
1040.3141	**-0.3320**	0.3451	**-0.2538**	0.4031	**0.0067**	0.4800	**0.0949**	0.2526	**1.4494**	0.8165	**-0.5773**	0.1099	**-0.3359**	0.3332	**-0.4981**	0.1714	0.0005
1040.4396	**-0.4492**	0.1877	**-0.4721**	0.0331	**-0.4327**	0.1408	**-0.5546**	0.0726	**2.0052**	0.3814	**-0.5434**	0.1097	**-0.0283**	0.6963	**0.4057**	1.0227	0.0001
1040.4763	**1.3730**	0.8881	**1.0667**	0.3517	**0.8661**	0.7449	**-0.9137**	0.3789	**-0.4727**	0.8499	**-0.9795**	0.2495	**-0.9745**	0.3238	**-0.0955**	0.9734	0.0008
1045.9756	**-0.0972**	0.5702	**-0.1367**	0.1383	**-0.4153**	0.3807	**-0.6691**	0.1789	**1.2739**	0.7608	**-0.0433**	0.3710	**0.0346**	0.8716	**-0.5184**	0.0811	0.0076
1046.3186	**-0.4012**	0.1951	**2.3788**	1.2158	**-0.3796**	0.1375	**-0.5067**	0.0791	**-0.5588**	0.0988	**-0.4971**	0.1188	**0.2032**	1.0378	**0.0155**	0.4944	0.0003
1052.2194	**-0.3961**	0.5277	**0.2023**	1.0489	**0.0405**	0.9584	**-0.0761**	0.1812	**1.9582**	1.0611	**-0.8474**	0.2922	**-0.5154**	0.1759	**-0.4074**	0.5377	0.0054
1054.4554	**1.6007**	0.1778	**0.4493**	0.6149	**1.3464**	0.8434	**-0.5804**	0.6412	**-0.5568**	0.7620	**-0.9283**	0.0353	**-0.9189**	0.0212	**-0.5063**	0.2141	0.0000
1060.7209	**-0.2704**	0.4146	**-0.4173**	0.0379	**-0.3746**	0.1371	**-0.5022**	0.0797	**1.9922**	1.1391	**-0.4928**	0.1197	**-0.3777**	0.0236	**0.2716**	0.8930	0.0004
1069.3189	**-0.5317**	0.5333	**1.7327**	1.3094	**-0.4540**	0.2569	**0.2350**	0.5414	**0.4449**	1.1455	**-0.6427**	0.3755	**-0.2845**	0.1634	**-0.7818**	0.1804	0.0076
1072.9744	**-0.2772**	0.2143	**-0.2925**	0.0490	**-0.2422**	0.1288	**-0.3828**	0.0966	**-0.4424**	0.1348	**1.6746**	1.3448	**-0.2345**	0.0246	**-0.0539**	0.1453	0.0014
1073.0407	**0.5996**	1.7010	**1.8459**	0.7876	**-0.3958**	0.1535	**-0.6033**	0.0661	**-0.5808**	0.0567	**-0.4543**	0.1360	**-0.1964**	0.0827	**-0.3321**	0.1508	0.0047
1073.0846	**-0.7357**	0.2231	**1.6939**	1.0024	**0.1671**	0.7364	**-0.3288**	0.5132	**0.4711**	1.2150	**-0.7413**	0.0833	**-0.3701**	0.3212	**-0.3247**	0.4823	0.0079
1076.4698	**1.4440**	0.8886	**-0.6608**	0.2516	**1.1243**	0.7571	**-0.6609**	0.2814	**0.1412**	0.4427	**-0.0052**	0.4446	**-0.3111**	0.4314	**-1.3131**	1.1031	0.0012
1080.2382	**-0.4260**	0.1913	**1.9565**	1.8888	**0.6286**	0.6140	**-0.5314**	0.0757	**-0.5821**	0.0916	**-0.5210**	0.1141	**-0.4128**	0.0234	**0.0840**	0.1997	0.0053
1094.4504	**1.5734**	0.4036	**-0.0763**	0.8224	**0.3648**	0.5941	**-0.6719**	0.7685	**0.1222**	0.6964	**-0.1632**	0.4992	**-1.1217**	0.4229	**-0.5566**	0.4715	0.0024
1101.5262	**-0.2805**	0.2138	**-0.2959**	0.0487	**-0.2458**	0.1290	**-0.3861**	0.0961	**-0.4455**	0.1338	**-0.3807**	0.1418	**-0.2384**	0.0246	**2.9513**	2.7547	0.0083
1102.4014	**0.1483**	0.3616	**2.1207**	1.2512	**0.4724**	0.8664	**-0.8077**	0.0428	**-0.6341**	0.3500	**-0.4678**	0.2767	**-0.5235**	0.2123	**-0.4587**	0.3125	0.0003
1104.1653	**0.1340**	0.9691	**1.8548**	1.3184	**-0.3698**	0.2067	**-0.6552**	0.2864	**-0.1359**	0.3499	**-0.0926**	0.8518	**-0.3365**	0.4590	**-0.7228**	0.0783	0.0088
1113.2778	**0.4768**	1.3792	**0.7582**	0.6274	**2.0778**	1.6696	**-0.6591**	0.3135	**-0.8642**	0.0063	**-0.8107**	0.0575	**-0.3391**	0.3615	**-0.1607**	0.3157	0.0062
1121.644	**-0.2769**	0.2143	**-0.2921**	0.0491	**-0.2418**	0.1287	**-0.3825**	0.0966	**-0.4421**	0.1349	**-0.3772**	0.1425	**-0.2341**	0.0246	**2.9092**	2.7638	0.0097
1137.8738	**-0.3898**	0.4060	**-0.1854**	0.6793	**-0.6643**	0.1555	**-0.6674**	0.1949	**-0.5509**	0.4097	**1.6004**	0.5850	**1.2825**	1.6915	**-0.5340**	0.1555	0.0040
1143.2193	**-0.1655**	0.3900	**2.5025**	0.9990	**-0.3999**	0.1387	**-0.5250**	0.0766	**-0.3201**	0.3551	**-0.5148**	0.1153	**-0.4051**	0.0234	**-0.2333**	0.1487	0.0000
1153.016	**-0.3767**	0.1989	**2.1488**	1.8877	**-0.3523**	0.1357	**-0.1644**	0.4443	**-0.2726**	0.1525	**-0.4734**	0.1235	**-0.3536**	0.0238	**-0.1792**	0.1476	0.0048
1169.0057	**-0.4907**	0.1813	**2.1584**	0.8316	**-0.4786**	0.1437	**-0.4693**	0.2453	**0.1140**	1.3276	**-0.5834**	0.1019	**0.2443**	1.1733	**-0.3183**	0.1541	0.0035
1170.0434	**0.4242**	0.6981	**2.3835**	0.6519	**-0.5656**	0.1492	**-0.6253**	0.1287	**-0.5169**	0.2988	**-0.4180**	0.2393	**-0.4284**	0.1585	**-0.4217**	0.1528	0.0000
1174.5409	**-0.3766**	0.2378	**-0.5643**	0.0249	**0.0914**	0.6146	**-0.6427**	0.0611	**-0.6867**	0.0594	**0.4064**	1.0335	**-0.5463**	0.0226	**2.9355**	1.9156	0.0007
1180.3243	**-0.6320**	0.1594	**-0.0037**	0.6649	**0.1130**	0.6567	**0.1746**	1.0428	**-0.7756**	0.0321	**-0.2660**	0.8337	**-0.6596**	0.0221	**2.8978**	1.7525	0.0016
1181.0603	**-0.7030**	0.1485	**1.7710**	0.6611	**-0.7139**	0.1586	**0.6091**	1.0178	**-0.5294**	0.3264	**0.7168**	0.3567	**-0.7447**	0.0217	**-0.5867**	0.1602	0.0000
1185.4291	**1.3834**	1.1081	**0.8745**	0.3086	**-0.4719**	0.7963	**-0.0345**	0.7554	**-0.0531**	0.7840	**-0.8940**	0.0417	**-0.8763**	0.0213	**-0.3469**	0.5009	0.0054
1187.2002	**1.9448**	1.7180	**-0.2972**	0.0486	**-0.2472**	0.1291	**-0.3873**	0.0959	**-0.4466**	0.1335	**-0.3819**	0.1415	**-0.2399**	0.0246	**-0.0596**	0.1454	0.0035
1191.5877	**-0.2803**	0.2138	**-0.2957**	0.0487	**-0.2456**	0.1290	**-0.3859**	0.0961	**-0.4453**	0.1339	**-0.3805**	0.1418	**-0.2382**	0.0246	**2.9487**	2.7542	0.0084
1192.1633	**-0.0269**	0.5129	**2.3438**	1.7436	**-0.2636**	0.2735	**-0.4478**	0.1228	**-0.5283**	0.1083	**-0.4657**	0.1250	**-0.3441**	0.0238	**-0.1553**	0.1581	0.0011
1192.9147	**-0.3965**	0.1958	**-0.2363**	0.1335	**-0.2431**	0.2014	**-0.4733**	0.1006	**1.7240**	1.3094	**-0.2610**	0.4778	**-0.3774**	0.0236	**-0.0162**	0.1795	0.0014
1194.4971	**2.0904**	1.0983	**-0.5946**	0.3952	**-0.2423**	1.1760	**-0.7917**	0.4061	**-0.3828**	1.1488	**-0.6068**	0.3799	**0.2139**	0.2259	**0.8868**	0.9301	0.0071
1197.7959	**-0.5366**	0.1546	**-0.6580**	0.0165	**-0.6299**	0.1533	**1.7350**	1.4297	**-0.7710**	0.0335	**0.2058**	1.0731	**0.1078**	0.5759	**0.6839**	0.5642	0.0054
1199.8164	**-0.4190**	0.1923	**-0.4405**	0.0359	**-0.1147**	0.4372	**-0.4168**	0.2496	**1.3355**	1.1452	**-0.0860**	0.5787	**-0.2992**	0.1650	**0.1361**	0.3363	0.0099

Ions obtained in negative ionisation mode:

m/z	**CHM mean**	CHM std.dev.	**CBM mean**	CBM std.dev.	**CHP mean**	CHP std.dev.	**CBP mean**	CBP std.dev.	**IHM mean**	IHM std.dev.	**IBM mean**	IBM std.dev.	**IHP mean**	IHP std.dev.	**IBP mean**	IBP std.dev.	Raw p value
50.5919	-0.2837	0.6841	-0.8194	0.0526	0.3541	1.1454	1.6655	0.1907	0.2878	0.7526	-0.8722	0.0159	-0.6587	0.4282	0.1305	0.5441	0.0016
52.0274	-0.2089	0.4769	-0.6040	0.1940	-0.2212	0.4477	-0.3462	0.4293	1.4311	0.2299	-0.1077	0.7876	0.3701	0.6936	-0.7924	0.0351	0.0010
52.6537	-0.4003	0.0853	-0.2684	0.3391	-0.3927	0.0837	-0.4580	0.1111	1.2096	0.5936	-0.1581	0.4026	0.1663	0.7711	-0.3860	0.2975	0.0020
54.4609	-0.6975	0.0922	-0.4426	0.3827	-0.2634	0.8662	-0.1547	0.1236	-0.4912	0.6079	1.6031	0.9300	0.3395	0.7151	-0.0331	0.4043	0.0062
56.3655	-0.6064	0.0899	-0.6818	0.0492	2.5678	2.0467	-0.0671	1.1124	0.3099	0.3773	-0.7628	0.0090	-0.4283	0.3764	0.1394	0.9273	0.0073
57.8855	-0.7510	0.0936	0.1939	1.1037	-0.7900	0.0564	-0.8473	0.0504	0.9549	0.5678	0.4140	1.5351	1.4358	0.3443	-0.5729	0.5056	0.0072
61.4221	-0.4678	0.0867	-0.5301	0.0459	0.4817	1.7238	2.0823	0.7847	-0.7134	0.1036	-0.0811	0.9361	-0.0347	0.5547	-0.6065	0.0468	0.0078
61.9877	0.5316	0.6029	0.2381	0.5027	-0.2849	0.5204	0.4656	1.1120	1.0522	0.4294	-0.5102	0.3575	-0.8500	0.3853	-1.1544	0.5845	0.0049
62.9586	0.1532	0.8750	2.0226	0.5795	-0.4137	0.7395	-0.9112	0.0543	0.0365	0.9287	-0.1302	0.8839	-0.7360	0.4472	-0.0555	0.7795	0.0047
65.1868	-0.8480	0.0963	0.6381	0.1128	-0.8999	0.0518	-0.7441	0.2881	0.2804	0.4461	-0.7336	0.3931	1.4902	1.4943	0.8417	0.6625	0.0009
65.2483	-0.3195	0.5705	-0.0823	1.2637	2.3600	0.7787	0.0817	1.0001	-0.1829	0.7030	-0.8459	0.0102	0.4954	1.2991	-0.8319	0.0335	0.0068
65.6679	-0.1539	0.2226	-0.3798	0.0432	-0.3136	0.0902	-0.4345	0.0327	2.0920	1.8562	-0.2315	0.5378	-0.1726	0.5330	-0.4743	0.0575	0.0073
67.1614	-0.1805	0.0816	-0.2157	0.0409	2.7123	2.3749	-0.2872	0.0318	-0.4835	0.1728	-0.3919	0.0907	-0.3119	0.0381	-0.3300	0.0703	0.0042
68.2516	-0.7714	0.0942	0.3607	1.1177	-0.8131	0.0553	1.0169	1.6040	-0.5792	0.6687	-0.9064	0.0234	1.5061	0.3369	0.7439	0.8310	0.0076
75.9648	-0.3717	0.5656	0.4413	1.0713	-0.7911	0.0564	0.0603	0.7718	-0.0399	0.8113	-0.6534	0.3964	2.0969	1.2864	-0.4824	0.6638	0.0088
78.8902	-0.5934	0.0896	-0.4945	0.3087	-0.1790	0.7176	-0.6925	0.0420	1.1607	0.4068	-0.3705	0.6673	0.2154	0.1970	0.5915	1.0838	0.0086
78.9883	-0.4662	0.0867	-0.5284	0.0459	-0.2547	0.3463	-0.1464	0.6998	-0.7122	0.1040	0.6030	1.1756	-0.4127	0.3705	1.8302	1.2055	0.0048
79.5315	-0.4510	0.0864	0.2424	1.3404	0.0888	0.8865	-0.5528	0.0360	-0.3562	0.6472	-0.6274	0.0386	-0.3151	0.5090	2.2385	1.3158	0.0079
89.3277	-0.3784	0.0849	-0.4323	0.0441	-0.3679	0.0857	-0.4815	0.0338	0.5066	1.1409	-0.1264	0.7058	1.2443	0.3095	-0.5205	0.0536	0.0040
90.3998	-0.3835	0.0850	-0.4379	0.0442	-0.3737	0.0852	-0.1341	0.6038	1.7642	1.3017	-0.5687	0.0516	0.3610	0.8025	-0.5254	0.0532	0.0024
94.4117	-0.4149	0.3882	0.2604	1.0606	0.0943	0.5057	-0.2394	0.6389	1.7330	0.7770	-0.6183	0.3727	-0.1538	0.6487	-0.8223	0.0339	0.0043
98.0887	-0.5647	0.0889	2.0008	0.2760	0.1033	0.6539	-0.4304	0.4392	-0.5237	0.5129	0.1828	1.5581	-0.3587	0.6167	-0.3450	0.5752	0.0080
101.4842	-0.7786	0.0943	-0.7284	0.2939	-0.1461	1.1303	-0.8744	0.0520	1.2511	0.7672	0.4761	0.5763	0.4308	0.6396	-0.0910	0.1848	0.0042
104.9294	-0.1942	0.7879	-0.5660	0.1812	-0.4837	0.2438	-0.6557	0.0692	-0.6998	0.1666	-0.3823	0.4474	1.7746	2.0206	1.5028	0.4611	0.0054
108.6145	0.0107	0.7061	-0.1824	0.5947	-0.6204	0.3628	1.9760	0.7229	-0.1975	0.7260	-0.3944	0.9419	-0.1486	0.7806	-0.7634	0.2594	0.0042
111.2208	-0.4202	0.0857	-0.0803	0.7220	-0.4152	0.0819	0.9052	0.6989	1.0938	0.9156	-0.6006	0.0445	-0.4094	0.2926	-0.5607	0.0504	0.0021
111.8636	-0.6772	0.0916	-0.3522	0.3360	2.3112	0.8009	-0.7749	0.0463	0.4518	1.2277	-0.3895	0.7535	0.5681	1.6370	-0.4950	0.5118	0.0068
111.9187	-0.3722	0.9319	-1.0761	0.0596	-0.5410	0.8090	-0.7809	0.4183	0.9963	0.1682	0.7735	1.0022	-0.1552	0.8498	0.7444	0.3688	0.0088
117.1423	-0.5790	0.0892	-0.6518	0.0485	-0.5951	0.0683	0.6075	1.1778	0.4092	1.0086	-0.3479	0.6633	-0.7403	0.0170	1.3354	0.5858	0.0071
119.6420	-0.0043	0.6018	0.3050	0.6039	-0.7571	0.0582	-0.1477	0.5810	1.8714	1.4669	-0.7580	0.1960	-0.0273	0.8347	-0.5701	0.2886	0.0073
119.8124	-0.6083	0.0899	-0.6839	0.0493	1.7887	1.1415	-0.1242	1.0169	-0.5173	0.5683	-0.0989	1.1588	1.5428	0.7760	-0.7418	0.0377	0.0026
122.8954	-0.5549	0.0054	-0.6501	0.0816	-0.5594	0.1039	-0.4146	0.4691	-0.4728	0.4261	0.0628	0.5916	2.2784	1.2097	0.6217	1.3937	0.0018
126.9994	-0.1560	0.1314	-0.0951	0.4124	-0.2554	0.0951	-0.3840	0.0320	1.4478	0.9279	-0.4778	0.0717	-0.4179	0.0328	-0.1800	0.4506	0.0005
139.9431	0.2147	0.7992	0.5701	0.7218	0.1626	0.4199	-0.3924	0.2750	0.9681	0.7531	-0.4669	0.3572	-0.9071	0.1295	-0.5762	0.2728	0.0065
141.8676	-0.4079	0.3349	-0.4361	0.3114	-0.4977	0.2846	-0.7518	0.0451	-0.4916	0.6703	-0.0444	0.4622	2.7293	0.7152	0.3575	0.7800	0.0000
141.9420	0.8910	1.2196	0.1803	0.5580	-0.5593	0.3866	0.1358	0.3707	1.0016	0.5799	-0.8734	0.1867	-0.6604	0.4621	-0.4880	0.5531	0.0090
143.8694	0.2715	1.3398	-0.6785	0.0492	0.0418	0.8310	-0.2220	0.4651	-0.3061	0.8226	-0.6655	0.1706	2.1858	0.6788	-0.5201	0.3863	0.0029
144.5235	-0.3142	0.0837	-0.3620	0.0429	-0.2952	0.0917	-0.4185	0.0325	1.3960	0.7033	0.1669	1.1047	-0.4557	0.0310	-0.4587	0.0588	0.0022
145.9307	-0.5218	0.0879	-0.5893	0.0472	-0.2899	0.3908	0.0752	0.5797	-0.7567	0.0906	-0.0040	0.6304	0.2116	0.8058	2.1926	1.7526	0.0058
149.9152	-0.7677	0.0940	-0.7101	0.2105	-0.2456	0.6768	0.1436	1.7612	-0.3884	0.2548	-0.2378	0.6875	2.2414	0.3406	0.1669	0.5761	0.0045
149.9710	-0.2896	0.5599	-0.1002	0.5149	-0.4503	0.5596	0.0508	0.5467	1.1832	0.5339	-0.0729	0.3916	-0.8365	0.1517	-0.2895	0.3327	0.0051
150.9686	-0.4477	0.4403	-0.3766	0.1690	-0.6234	0.1295	0.1150	0.0896	1.0480	0.8603	-0.0283	0.1743	-0.2112	0.4359	-0.2576	0.3040	0.0039
158.7280	-0.0359	0.7170	-0.4783	0.0449	-0.4155	0.0818	-0.1372	0.3616	1.4246	0.9755	-0.3041	0.5381	-0.3280	0.3937	-0.3066	0.3901	0.0082
159.2989	-0.5618	0.0888	-0.4106	0.3413	0.2236	1.0576	-0.0327	0.6017	1.4085	0.4367	-0.3847	0.2992	-0.4672	0.2194	-0.2977	0.7193	0.0091
160.1598	-0.1756	0.2208	-0.0235	0.6146	-0.3373	0.0882	-0.4550	0.0332	1.1434	0.8695	-0.2150	0.5064	-0.1306	0.3758	-0.4945	0.0558	0.0091
160.9485	2.7887	1.6557	-0.2637	0.0415	-0.1934	0.1005	-0.3303	0.0317	-0.5186	0.1623	-0.4301	0.0822	-0.3591	0.0358	-0.3722	0.0664	0.0001
161.2457	-0.1618	0.4950	-0.4747	0.0449	-0.4118	0.0821	0.1917	0.9744	1.3134	0.4495	-0.5980	0.0451	-0.0823	0.4299	-0.3759	0.2645	0.0024
163.2212	-0.3201	0.0838	-0.3685	0.0430	-0.3018	0.0912	-0.4243	0.0326	-0.5952	0.1392	1.7415	1.2007	0.3973	0.3026	-0.3612	0.1208	0.0001
166.9614	1.7468	0.9896	0.7043	1.0157	-0.0826	0.4655	-0.2168	0.8262	0.5616	0.1084	-0.6229	0.0975	-1.2741	0.2188	-0.9976	0.1459	0.0003
167.5000	-0.3502	0.0844	-0.4014	0.0435	1.6773	2.0567	1.7606	1.3522	-0.6193	0.1320	-0.5396	0.0580	-0.4943	0.0290	-0.4933	0.0559	0.0090
167.9576	0.7905	0.9122	0.7929	0.5632	-0.3359	0.6012	-0.4955	0.3487	1.1154	0.2284	-0.4764	0.3997	-1.1048	0.2939	-0.7740	0.2936	0.0002
168.8892	-0.5544	0.4585	-0.9787	0.0055	-0.7120	0.3684	-0.6753	0.1407	-0.1993	0.2117	0.3528	0.8889	2.2467	0.4810	0.5756	0.9582	0.0000

168.9595	**1.6359**	0.7574	**0.7118**	0.2322	**0.0329**	1.2017	**-0.3071**	0.6498	**0.7362**	0.1572	**-0.6880**	0.2418	**-1.0907**	0.3640	**-1.0818**	0.1197	0.0002
172.3071	**-0.3282**	0.0840	**-0.3774**	0.0431	**0.4543**	1.3033	**-0.4322**	0.0327	**-0.6017**	0.1372	**-0.5205**	0.0622	**2.9440**	0.6331	**-0.4722**	0.0577	0.0000
175.5747	**-0.2559**	0.6020	**-0.7360**	0.0505	**-0.6823**	0.0625	**-0.7540**	0.0452	**0.8108**	0.4541	**1.0656**	0.7337	**0.8009**	0.1715	**-0.7877**	0.0354	0.0000
175.7076	**-0.9665**	0.0998	**-0.7845**	0.1984	**0.7949**	0.7366	**1.4010**	0.7392	**0.0335**	1.0874	**-0.6273**	0.4791	**-0.3424**	0.3586	**0.2018**	0.2842	0.0017
177.9694	**-1.1501**	0.1904	**-0.2483**	0.2277	**-0.7547**	0.6948	**0.2569**	0.3717	**0.7404**	0.6343	**0.0551**	0.7451	**-0.2793**	0.0404	**0.5638**	0.2166	0.0018
178.9140	**-0.4726**	0.0868	**-0.5354**	0.0460	**-0.1568**	0.5255	**-0.5740**	0.0368	**-0.4894**	0.4470	**-0.2252**	0.4566	**2.2352**	1.7395	**0.5967**	1.0362	0.0053
180.9203	**-0.3138**	0.4626	**-0.6634**	0.3401	**-0.7201**	0.1754	**-0.6338**	0.6310	**-0.7465**	0.3193	**0.3455**	0.5003	**2.4464**	0.2869	**0.3083**	0.8505	0.0000
182.9321	**0.9515**	1.2928	**0.6723**	0.4181	**-0.1633**	0.4299	**-0.2512**	0.8641	**1.1286**	0.5394	**-0.6188**	0.2396	**-0.8593**	0.4543	**-1.0698**	0.5053	0.0047
182.9784	**-0.5247**	0.2169	**-0.3006**	0.3364	**-0.6432**	0.2680	**-0.2368**	0.2818	**-0.7097**	0.1836	**0.6621**	1.1726	**-0.0874**	0.5704	**1.6007**	0.8023	0.0022
184.9297	**0.7750**	1.3586	**0.7610**	0.4678	**-0.2494**	0.2686	**-0.2741**	0.8027	**1.1297**	0.5204	**-0.5524**	0.3181	**-0.7025**	0.4643	**-1.1152**	0.5528	0.0073
184.9993	**-0.6016**	0.5568	**-0.6855**	0.2774	**-0.7339**	0.5736	**-0.2578**	1.0616	**-0.2755**	0.5821	**0.2503**	1.0194	**0.0041**	0.4374	**2.0143**	0.3566	0.0024
185.4527	**-0.4603**	0.0866	**-0.5220**	0.0458	**0.0847**	0.9182	**-0.0453**	0.8651	**-0.0620**	1.0131	**-0.6356**	0.0368	**-0.4762**	0.2502	**1.8828**	0.4352	0.0022
187.8614	**-0.4776**	0.1057	**-0.6641**	0.0488	**-0.1081**	0.8340	**-0.5756**	0.1029	**-0.2569**	0.2755	**-0.2463**	0.3401	**2.5654**	1.2850	**-0.0803**	0.9051	0.0003
193.0527	**2.6320**	1.5502	**-0.3598**	0.0429	**0.0530**	0.5387	**-0.4165**	0.0324	**-0.5889**	0.1411	**-0.5066**	0.0653	**-0.0825**	0.6118	**-0.4567**	0.0590	0.0002
193.2479	**-0.4972**	0.5251	**-0.6579**	0.4673	**-0.6961**	0.3666	**1.9598**	1.1296	**-0.3324**	0.5972	**0.6628**	0.1808	**-0.8214**	0.2077	**-0.0263**	0.3886	0.0002
195.9080	**-0.7199**	0.0842	**-0.8592**	0.0362	**-0.5642**	0.5827	**-0.3116**	0.2209	**-0.3491**	0.7230	**0.3648**	0.9133	**2.0496**	0.9539	**0.4007**	0.9362	0.0014
196.2371	**-0.3131**	0.0837	**-0.3608**	0.0429	**-0.2939**	0.0919	**-0.4174**	0.0324	**-0.3596**	0.4729	**0.0328**	0.9694	**-0.4544**	0.0310	**2.3359**	1.6382	0.0022
197.4447	**2.1732**	1.2554	**-0.4032**	0.7520	**-0.8147**	0.0553	**-0.3643**	0.5064	**-0.3795**	0.5496	**-0.4297**	0.4796	**1.1479**	0.8793	**-0.4463**	0.5318	0.0010
199.9719	**-0.4516**	0.3140	**0.3774**	0.5288	**-1.1076**	0.6607	**-0.6290**	0.3872	**-0.2194**	0.4430	**1.0983**	0.5003	**-1.0919**	0.0898	**1.6182**	0.1935	0.0000
200.9774	**-0.1338**	0.1399	**-0.3806**	1.1378	**-0.9563**	0.3206	**-0.5275**	0.4858	**-0.1190**	0.5407	**0.5591**	0.4968	**-0.6676**	0.5911	**1.7366**	0.7011	0.0018
201.7443	**-0.5382**	0.0883	**0.2095**	0.6786	**-0.5490**	0.0716	**2.2665**	0.9627	**-0.0319**	0.6122	**-0.7034**	0.0219	**-0.5048**	0.3130	**-0.2832**	0.3882	0.0000
201.9836	**-0.4771**	0.5994	**-0.3294**	1.0989	**-1.0546**	0.7803	**-0.1886**	0.6547	**0.2918**	0.5231	**0.6119**	0.7181	**-0.8320**	0.0939	**1.2824**	0.3833	0.0095
203.9711	**-1.1530**	0.3121	**-0.7624**	1.0914	**-0.9022**	0.1448	**-0.1501**	0.6236	**0.1864**	0.2768	**0.4684**	0.4266	**0.4415**	0.4965	**1.5020**	0.6282	0.0006
204.6321	**-0.4342**	0.0860	**-0.4933**	0.0452	**0.9905**	1.1692	**-0.5363**	0.0354	**1.1479**	0.7054	**-0.6128**	0.0418	**-0.5846**	0.0246	**0.1379**	0.6310	0.0021
209.9518	**0.7892**	0.9892	**0.6859**	0.5283	**-0.1397**	0.7081	**0.3213**	1.0864	**1.0220**	0.6051	**-0.8079**	0.2331	**-0.7926**	0.4811	**-1.2203**	0.6351	0.0073
210.0775	**-0.7279**	1.1507	**1.2287**	0.9674	**0.0058**	0.0870	**1.5024**	1.0822	**-0.1492**	0.1693	**-0.5517**	1.1152	**-1.1338**	0.3937	**-0.2900**	0.3300	0.0090
211.4266	**-0.7471**	0.0935	**1.3920**	0.5378	**-0.7856**	0.0567	**-0.8435**	0.0502	**0.5791**	0.6700	**-0.0487**	0.7743	**0.0315**	0.8238	**-0.0314**	0.8353	0.0030
214.8840	**-0.3142**	0.1817	**-0.8872**	0.1369	**-0.2364**	0.7617	**-0.6570**	0.3891	**-0.4854**	0.4891	**0.2244**	1.0484	**2.4530**	0.7783	**0.1790**	0.9236	0.0005
216.6379	**-0.3458**	0.0843	**2.4448**	1.3766	**-0.3309**	0.0887	**-0.4494**	0.0330	**-0.6157**	0.1330	**0.4548**	1.0423	**-0.4896**	0.0293	**-0.4890**	0.0563	0.0002
219.2329	**-0.4713**	0.0868	**-0.5339**	0.0460	**0.2822**	1.2804	**-0.1557**	0.7553	**-0.2164**	0.9293	**-0.3604**	0.4584	**2.5498**	1.3196	**-0.6099**	0.0466	0.0031
220.1643	**-0.6874**	0.0919	**-0.7705**	0.0514	**-0.2590**	0.7597	**-0.7849**	0.0469	**0.7889**	0.9110	**0.1521**	1.0087	**1.5571**	0.7391	**-0.2144**	1.0744	0.0100
222.8800	**-0.3959**	0.0166	**-0.2754**	0.1991	**-0.1094**	0.6092	**-0.1720**	0.0594	**1.1819**	0.7225	**-0.1173**	0.4274	**-0.2981**	0.1835	**-0.5191**	0.0825	0.0017
224.9119	**-0.5280**	0.0880	**-0.5960**	0.0473	**-0.5373**	0.0724	**-0.2730**	0.3436	**-0.5107**	0.3340	**-0.1823**	0.7475	**0.9185**	0.2071	**1.8870**	1.5107	0.0012
225.0095	**-0.1492**	0.0675	**-1.0447**	0.3234	**-0.3453**	0.1468	**-0.7698**	0.6559	**-0.2281**	1.0079	**0.4153**	0.4434	**1.8269**	1.4850	**0.4648**	0.7144	0.0076
225.9297	**1.8622**	0.7845	**-0.2658**	0.8470	**0.2956**	0.9917	**0.5794**	0.4708	**0.5686**	0.5174	**-1.1032**	0.3437	**-0.4169**	0.7390	**-1.4184**	0.3395	0.0005
226.5896	**-0.2025**	0.0819	**-0.2397**	0.0412	**-0.1685**	0.1027	**-0.3088**	0.0317	**-0.5011**	0.1675	**-0.4110**	0.0864	**-0.3355**	0.0370	**2.3675**	1.9402	0.0019
229.1860	**0.5053**	1.3162	**-0.3491**	0.0427	**2.8870**	1.6665	**-0.4068**	0.0323	**-0.5810**	0.1435	**-0.4980**	0.0672	**-0.4429**	0.0316	**-0.4473**	0.0598	0.0005
231.0138	**-1.2164**	0.1553	**-0.5373**	0.2098	**-0.1794**	0.7365	**0.0185**	0.2072	**0.6750**	0.8736	**0.3582**	0.2751	**-0.0786**	0.1560	**0.2022**	0.3802	0.0042
231.9246	**-0.3858**	0.4824	**-0.5951**	0.0639	**-0.6560**	0.0642	**-0.6163**	0.0780	**-0.8171**	0.0962	**0.3546**	1.4705	**1.2483**	1.2726	**1.7144**	0.4710	0.0028
233.2332	**-0.4157**	0.0856	**0.3412**	0.6825	**-0.4101**	0.0823	**-0.5181**	0.0349	**-0.6717**	0.1162	**-0.5967**	0.0454	**2.4434**	1.0772	**0.3225**	1.4716	0.0008
234.0821	**-0.6454**	0.6406	**-0.7532**	0.0990	**-0.5741**	0.3191	**-0.5212**	0.4754	**0.7978**	0.1516	**0.9646**	1.0226	**0.4518**	0.2794	**-0.3477**	0.4918	0.0023
235.8774	**0.6774**	1.4198	**-0.3095**	0.4775	**-0.0761**	0.5381	**-0.6414**	0.0396	**-0.7722**	0.0860	**-0.1796**	0.9212	**-0.4932**	0.3676	**2.2013**	1.0347	0.0038
235.9490	**-0.2321**	0.7591	**1.1088**	0.5622	**-1.0513**	0.6371	**-0.8615**	0.9281	**0.0655**	0.0774	**0.8348**	1.1016	**-1.0715**	0.3185	**1.0683**	0.6149	0.0024
238.4963	**-0.5293**	0.0881	**-0.5974**	0.0473	**0.0793**	0.4716	**0.0568**	1.1545	**1.7562**	0.5782	**-0.3610**	0.5902	**-0.3972**	0.4821	**-0.3376**	0.5956	0.0038
240.0023	**0.6846**	1.1441	**-0.7261**	0.1724	**-0.7696**	0.0575	**-0.8296**	0.0494	**1.1340**	0.4803	**0.1116**	0.6438	**-0.3110**	0.8124	**0.1461**	0.4389	0.0067
241.9009	**-0.9181**	0.0510	**-1.0543**	0.0590	**-0.3851**	0.5096	**-0.2909**	0.5517	**-0.0514**	0.4377	**0.1766**	0.8207	**2.0675**	0.5022	**0.4772**	1.1988	0.0005
245.0458	**-0.7662**	0.0763	**-0.4480**	0.1539	**-0.4067**	0.5294	**-0.5193**	0.1484	**-0.2099**	0.7054	**0.5879**	1.0999	**-0.1766**	0.0899	**1.3063**	0.7666	0.0075
245.9785	**-0.4640**	0.5112	**-0.3008**	0.7645	**-1.1067**	0.2944	**-0.2570**	0.1795	**-0.3377**	0.5250	**0.9478**	0.3651	**-0.7238**	0.2109	**1.7410**	0.1348	0.0000
246.9787	**0.0584**	0.8516	**-0.5199**	0.4127	**-1.1090**	0.2410	**0.2295**	0.3373	**-0.9734**	0.3166	**1.3689**	0.1594	**-0.3730**	0.4819	**1.1764**	1.0865	0.0004
247.9826	**-0.7188**	0.1419	**-0.3465**	0.4662	**-0.8609**	0.1042	**-0.3012**	0.2766	**0.0970**	0.4373	**0.4271**	0.8026	**-0.5256**	0.3996	**1.7772**	1.0607	0.0006
249.9146	**-0.5650**	0.3686	**-0.4967**	0.2570	**-0.6510**	0.6033	**-0.7992**	0.1866	**0.8126**	0.8997	**0.4995**	0.2541	**0.4677**	0.6409	**0.0983**	0.2571	0.0046
252.9847	**-0.5046**	0.4640	**0.1600**	0.2228	**-0.8220**	0.0549	**-0.2023**	0.2867	**-0.6180**	0.3185	**0.1012**	0.5049	**-0.2889**	0.5728	**2.2605**	1.7763	0.0026
253.0073	**-0.3091**	0.3293	**-0.5331**	0.0460	**0.0997**	0.6072	**-0.5720**	0.0367	**1.0387**	0.2498	**0.6707**	1.2196	**-0.4779**	0.2636	**-0.6092**	0.0466	0.0058
255.0281	**0.0337**	1.0278	**-0.6180**	0.0782	**-0.6321**	0.0658	**-0.3308**	0.2781	**-0.5082**	0.2433	**-0.5953**	0.2902	**0.4601**	0.8924	**2.1518**	0.8677	0.0004
260.9035	**0.5989**	0.7757	**-0.6451**	0.2353	**0.6491**	0.6659	**0.1274**	0.7697	**1.1854**	1.1452	**-1.1603**	0.2166	**0.4308**	1.1222	**-1.0995**	0.1954	0.0081
260.9503	**1.7358**	0.9890	**0.5857**	0.6637	**0.0138**	0.5222	**-0.2727**	0.8579	**0.5151**	0.3562	**-0.6109**	0.1002	**-1.2465**	0.1727	**-0.9632**	0.2391	0.0002
261.3794	**-0.2125**	0.0821	**0.1646**	0.6782	**-0.1799**	0.1017	**-0.3186**	0.0317	**-0.5091**	0.1651	**1.4689**	1.2990	**-0.3463**	0.0364	**-0.3608**	0.0675	0.0063

261.9522	**1.9164**	1.4027	**0.8515**	1.1799	**-0.3614**	0.6680	**-0.2647**	0.5831	**0.2568**	0.3173	**-0.2097**	0.3131	**-1.2516**	0.0522	**-1.0381**	0.3676	0.0021
262.9679	**-0.1399**	0.6167	**0.8440**	0.8001	**-1.0790**	0.5960	**-0.4659**	0.8978	**-0.1132**	0.2949	**0.9473**	1.2359	**-1.2140**	0.2282	**1.0464**	0.9361	0.0097
264.9673	**-0.5932**	0.8503	**0.6905**	0.6370	**-1.0625**	0.6565	**-0.4573**	0.6339	**0.0972**	0.2296	**0.9018**	1.1882	**-1.0290**	0.2381	**1.1475**	0.7258	0.0056
265.4286	**-0.3402**	0.0842	**-0.3905**	0.0434	**1.8885**	1.8382	**1.3984**	1.5648	**-0.6113**	0.1343	**-0.5310**	0.0599	**-0.4836**	0.0296	**-0.4837**	0.0567	0.0100
265.9788	**-0.6170**	0.4361	**0.9483**	0.4481	**-0.3986**	0.2493	**-1.0327**	0.2124	**0.5899**	0.3433	**0.3400**	1.3138	**-0.8641**	0.1655	**0.8621**	1.0896	0.0075
268.9065	**0.3429**	1.1873	**0.6566**	0.0559	**-0.6973**	0.6719	**-0.7521**	0.1782	**1.2542**	0.2493	**-0.3751**	0.5480	**-0.6533**	0.4606	**-0.0960**	0.7342	0.0078
269.9060	**-0.6756**	0.4797	**-0.6831**	0.3981	**-0.5857**	0.5495	**-0.0958**	0.4952	**-0.4966**	0.3737	**0.2515**	0.4130	**2.6465**	0.7586	**-0.2074**	0.9409	0.0000
271.0015	**-0.9056**	0.0907	**-0.1764**	0.5745	**-0.3682**	0.5539	**-0.4716**	0.3818	**-0.3813**	0.3939	**0.3679**	0.3373	**-0.4487**	0.5639	**2.3775**	0.6845	0.0000
271.9937	**-0.5660**	0.1422	**-0.0866**	0.5273	**-0.2819**	0.5873	**-0.1855**	0.9534	**-0.3334**	0.3304	**-0.2698**	0.7442	**-0.5071**	0.1859	**2.1185**	1.3470	0.0059
274.9989	**-0.3712**	0.6055	**-0.7538**	0.5311	**-0.6067**	0.2453	**-0.6411**	0.1186	**0.6858**	0.7810	**0.7339**	0.4624	**0.1178**	0.1344	**0.0221**	0.4854	0.0054
276.6910	**0.7212**	2.0434	**-0.4894**	0.0451	**2.5145**	1.2704	**-0.5328**	0.0353	**-0.6836**	0.1126	**-0.2745**	0.6034	**0.0051**	0.6551	**-0.5707**	0.0496	0.0079
276.9306	**2.5117**	1.2977	**-0.3405**	0.4947	**0.0913**	0.7878	**-0.2201**	0.3204	**0.1488**	0.6654	**-0.5337**	0.0758	**-0.6253**	0.4173	**-0.8896**	0.3985	0.0003
280.1596	**-0.2942**	0.0834	**-0.3401**	0.0426	**-0.2724**	0.0937	**-0.3988**	0.0322	**-0.5745**	0.1454	**0.0153**	0.8079	**2.5545**	1.7811	**-0.4394**	0.0605	0.0009
284.9415	**-0.0790**	0.8042	**0.0338**	0.5934	**-0.6133**	0.0670	**-0.6941**	0.0421	**-0.7441**	0.1681	**0.7510**	0.7618	**-0.4298**	0.5515	**1.7394**	1.4532	0.0066
286.8182	**-0.4232**	0.0858	**-0.4813**	0.0450	**-0.4187**	0.0816	**-0.1708**	0.6103	**-0.6777**	0.1144	**-0.0707**	0.9446	**2.8312**	1.2699	**-0.0457**	0.5166	0.0001
290.6422	**-0.3115**	0.0837	**-0.3591**	0.0429	**2.6938**	2.0978	**-0.4158**	0.0324	**-0.5883**	0.1413	**0.3459**	1.5095	**-0.2507**	0.3698	**-0.4561**	0.0591	0.0085
290.9127	**-0.8259**	0.1428	**-0.2414**	0.4850	**-0.6183**	0.3238	**-0.4876**	0.5322	**0.5113**	0.6798	**0.2337**	0.9983	**-0.2947**	0.0888	**1.3321**	0.4052	0.0028
293.1750	**-0.1814**	0.0816	**-0.2167**	0.0409	**2.7334**	2.4190	**-0.2881**	0.0318	**-0.4842**	0.1726	**-0.3926**	0.0905	**-0.3129**	0.0381	**-0.3308**	0.0702	0.0047
295.0183	**-0.4835**	0.4419	**-0.6740**	0.5235	**-0.9180**	0.4317	**0.2320**	0.6498	**-0.4181**	0.9157	**-0.1515**	0.7154	**0.5994**	1.3444	**1.8817**	0.5140	0.0072
295.2751	**-0.4277**	0.0859	**-0.4862**	0.0451	**2.9944**	0.7295	**0.3722**	1.5349	**-0.6813**	0.1133	**-0.6071**	0.0431	**0.2231**	0.7912	**-0.5679**	0.0498	0.0001
298.9826	**-0.5667**	0.2609	**-0.2323**	0.3775	**-0.4440**	0.4524	**-0.3710**	0.0730	**-0.2743**	0.3921	**-0.3994**	0.1734	**-0.3909**	0.1924	**2.5658**	0.3453	0.0000
307.9455	**-0.6584**	0.2075	**-0.7096**	0.4729	**-0.3415**	0.4058	**-0.2414**	0.7441	**1.3147**	0.6048	**-0.0587**	0.4595	**-0.3928**	0.7363	**0.3950**	0.6646	0.0086
309.0410	**-0.6442**	0.5535	**-0.3064**	0.6853	**-0.5045**	0.4465	**-0.3759**	0.8910	**-0.1090**	0.8574	**-0.4335**	1.0200	**2.1149**	0.6241	**0.4143**	0.5902	0.0055
309.0761	**-0.0663**	0.4997	**-0.3198**	0.1206	**-0.2537**	0.1471	**-0.2981**	0.2835	**1.3309**	0.7883	**-0.1583**	0.4100	**-0.5096**	0.0562	**-0.4592**	0.0383	0.0005
309.1007	**-0.3665**	0.0847	**-0.4193**	0.0439	**-0.3544**	0.0868	**-0.1603**	0.5130	**-0.6324**	0.1280	**-0.5539**	0.0549	**0.2736**	1.3719	**2.4623**	0.4018	0.0001
310.0734	**-0.2664**	0.2797	**-0.3230**	0.1746	**-0.2911**	0.1409	**-0.2637**	0.4380	**1.3902**	0.6559	**-0.1290**	0.3988	**-0.4761**	0.0965	**-0.3942**	0.1333	0.0001
310.9937	**-0.1607**	0.1461	**-0.5262**	0.3014	**-0.3346**	0.3564	**-0.2909**	0.4201	**0.5374**	0.9883	**-0.7488**	0.1864	**-0.2252**	0.7011	**1.2486**	0.5052	0.0054
314.6542	**-0.2872**	0.0832	**-0.3325**	0.0424	**-0.2645**	0.0943	**-0.3920**	0.0321	**1.5890**	0.8650	**-0.3559**	0.1532	**-0.3151**	0.2073	**-0.2041**	0.4363	0.0001
315.9836	**-0.6466**	0.2717	**-0.1371**	0.5476	**-0.7639**	0.3351	**-0.5576**	0.3795	**-0.4568**	0.3601	**0.9586**	0.7226	**-0.6128**	0.3499	**2.1072**	0.4221	0.0000
316.9809	**-0.7318**	0.2860	**-0.2669**	0.2225	**-0.6568**	0.5684	**-0.0379**	1.0220	**-0.0995**	0.6462	**1.0074**	1.4339	**-0.9795**	0.2169	**1.4534**	0.4569	0.0087
317.9907	**-0.8035**	0.2051	**-0.5665**	0.4898	**-0.6206**	0.3557	**-0.6453**	0.2710	**-0.5369**	0.3975	**1.1535**	0.6445	**0.2360**	0.8175	**1.4884**	0.9128	0.0004
319.3237	**2.3294**	2.2426	**-0.1039**	0.2151	**-0.1810**	0.1016	**-0.1444**	0.2914	**-0.5099**	0.1649	**-0.4206**	0.0843	**-0.3474**	0.0364	**-0.3617**	0.0674	0.0096
325.2368	**2.0399**	1.5958	**-0.6817**	0.5179	**0.2284**	0.4731	**-0.4442**	0.4468	**-0.8245**	0.3776	**-0.5336**	0.8531	**-0.1637**	0.3036	**0.5474**	0.8454	0.0075
326.9540	**-0.3192**	0.0838	**-0.3675**	0.0430	**-0.3008**	0.0913	**-0.1395**	0.5238	**1.7515**	0.5310	**-0.5127**	0.0640	**-0.1140**	0.6202	**-0.4635**	0.0584	0.0000
326.9739	**-0.6081**	0.0188	**0.0731**	0.3204	**-0.1765**	0.2952	**-0.4869**	0.2157	**0.2196**	1.0264	**-0.3487**	0.3905	**-0.6075**	0.2769	**1.5416**	1.0666	0.0057
330.8950	**0.0394**	1.0073	**0.2131**	0.3670	**1.0605**	0.2792	**-0.4177**	0.6782	**1.1107**	0.9149	**-1.0852**	0.1720	**-0.2762**	0.7860	**-0.7064**	0.6366	0.0087
332.8968	**-0.7250**	0.2369	**1.7079**	0.3386	**0.0241**	1.1454	**0.0649**	0.7748	**0.9567**	0.4773	**-0.7590**	0.2808	**-0.2171**	0.3594	**-1.0554**	0.0955	0.0003
332.9600	**-0.9049**	0.2335	**-0.3937**	0.2039	**-0.7661**	0.3368	**-0.0461**	0.6464	**0.0581**	0.3278	**0.3766**	0.5759	**-0.4713**	0.3785	**1.5915**	1.2141	0.0020
333.0705	**-0.6124**	0.7154	**-0.9789**	0.4123	**-0.8306**	0.4519	**-0.4255**	0.5299	**0.4470**	0.6121	**0.4912**	0.3076	**-0.2173**	0.5961	**1.4574**	0.2586	0.0003
335.8998	**-0.9066**	0.2916	**0.1061**	0.4747	**-0.5597**	0.6094	**-0.5045**	0.5267	**-0.4965**	0.6668	**1.1445**	1.4405	**-0.5106**	0.6853	**1.5828**	0.2489	0.0041
336.0135	**-0.4328**	0.7344	**-1.0170**	0.0579	**-0.9731**	0.0499	**-0.2435**	0.7596	**0.2694**	0.7594	**0.7329**	1.1705	**-0.1399**	0.3281	**1.4525**	0.5043	0.0039
339.0225	**-0.4934**	0.2775	**-0.7824**	0.1604	**-0.7437**	0.1980	**-0.4196**	0.3108	**-0.5728**	0.2670	**0.1373**	0.2384	**0.9750**	1.1640	**1.9732**	1.2166	0.0005
339.2641	**0.1375**	0.5788	**-0.9528**	0.1037	**1.6469**	0.8928	**-0.8024**	0.2749	**0.7110**	1.1949	**0.1767**	0.9077	**-0.1270**	0.7797	**-0.9697**	0.1354	0.0044
340.8385	**-0.2824**	0.9625	**0.4017**	0.5300	**-0.3378**	0.5892	**-0.7167**	0.0830	**1.4610**	0.3047	**-0.2993**	0.4720	**-0.5191**	0.2102	**-0.2820**	0.6040	0.0034
342.9242	**0.1636**	0.9691	**0.1577**	0.5785	**0.1264**	0.9592	**-0.8018**	0.3184	**1.3544**	0.4214	**0.0497**	0.3057	**-0.8368**	0.3917	**-0.7188**	0.4709	0.0071
345.8699	**1.8835**	1.6764	**-0.5865**	0.3869	**0.2178**	0.4868	**0.1433**	0.3316	**0.4544**	1.1613	**-1.0391**	0.2111	**0.3540**	0.6403	**-1.0224**	0.3720	0.0085
346.2046	**-0.4239**	0.0858	**-0.4820**	0.0450	**-0.4194**	0.0815	**0.1121**	1.0781	**-0.6782**	0.1142	**0.3560**	0.8896	**-0.5735**	0.0251	**2.1554**	1.3180	0.0020
348.0698	**-0.3901**	0.1794	**-0.3466**	0.0443	**-0.5338**	0.1897	**-0.4252**	0.2728	**1.3807**	0.4727	**-0.1866**	0.0246	**0.0102**	0.2766	**-0.1629**	0.3196	0.0000
354.0392	**-0.8842**	0.1768	**-0.9657**	0.1307	**-0.9587**	0.5494	**-0.2787**	0.6301	**0.1568**	0.5566	**0.2654**	0.7871	**0.9607**	0.7294	**1.3923**	0.2759	0.0002
355.9524	**-0.7107**	0.4351	**-0.8923**	0.2625	**-0.5740**	0.6938	**-0.3199**	0.3426	**0.0067**	0.2034	**0.7488**	0.5607	**-0.2419**	0.2838	**1.6154**	1.4839	0.0043
357.8116	**-0.4320**	0.7869	**-0.1770**	0.2647	**-0.4422**	0.3347	**-0.3019**	0.4818	**1.2309**	0.4826	**0.3065**	0.5460	**-0.4637**	0.3662	**-0.3640**	0.4384	0.0070
365.0044	**-0.3093**	0.4489	**-0.4840**	0.2540	**-0.3291**	0.2315	**0.0738**	0.3582	**1.5005**	0.3429	**-0.5154**	0.3051	**-0.2211**	0.2214	**-0.3802**	0.2453	0.0000
369.0011	**-0.4869**	0.1902	**-0.4633**	0.1893	**-0.4451**	0.2533	**-0.6072**	0.1288	**0.0944**	0.5535	**-0.2447**	0.0857	**-0.4002**	0.2085	**2.2135**	0.9719	0.0000
371.7941	**0.0581**	1.0405	**-0.7384**	0.2260	**0.8383**	0.1422	**-0.7527**	0.3709	**1.2052**	0.4986	**-0.4161**	0.3474	**-0.1912**	0.1946	**-0.3501**	0.6979	0.0021
376.0436	**-0.4532**	0.2477	**-0.1815**	0.2469	**-0.8196**	0.1113	**-0.3785**	0.3949	**0.9032**	0.8842	**0.4478**	0.3590	**-0.1293**	0.3627	**-0.1343**	0.1521	0.0032
376.9474	**-0.5454**	0.5629	**-0.6992**	0.3601	**-0.5276**	0.8407	**-0.3173**	0.1659	**0.2488**	0.7672	**0.3327**	0.3744	**-0.6692**	0.3903	**1.4818**	0.4532	0.0016
378.8678	**-0.3891**	0.0851	**-0.4440**	0.0443	**-0.2190**	0.3624	**0.1072**	0.6713	**1.2666**	0.5498	**-0.1026**	0.8418	**-0.5362**	0.0270	**-0.2282**	0.4713	0.0070

382.9433	-0.0147	0.4704	-0.2960	0.0419	0.2357	0.4417	-0.3593	0.0318	1.1330	0.8415	-0.4558	0.0765	-0.3908	0.0342	-0.4006	0.0639	0.0013
384.6367	-0.4258	0.0858	2.5703	0.5411	-0.4215	0.0814	0.5460	1.8929	-0.6798	0.1138	-0.6054	0.0435	-0.4249	0.2760	-0.0592	0.4156	0.0008
385.7164	-0.4320	0.0860	-0.4909	0.0452	-0.4286	0.0808	-0.5341	0.0354	0.3867	1.1598	2.0149	1.1500	-0.2255	0.6323	-0.5720	0.0495	0.0014
385.8330	-0.2463	0.2008	0.1365	0.6214	-0.1932	0.5704	-0.2854	0.8123	1.2714	0.4866	-0.3565	0.0035	-0.2616	0.3913	-0.6647	0.3412	0.0074
385.9050	-0.5229	0.4599	1.7199	0.1275	-1.1452	0.4691	-0.6352	0.3658	-0.5404	0.0907	1.3785	1.1616	-0.5923	0.7544	0.3318	0.5937	0.0001
388.0508	-0.4584	0.5671	0.2510	0.2325	-0.9390	0.1960	0.0210	0.3676	1.0985	0.7103	0.2360	0.4692	-0.4461	0.0942	-0.3903	0.0554	0.0005
389.7048	2.4915	2.3838	-0.2096	0.0408	-0.1373	0.1054	-0.2817	0.0319	-0.4790	0.1742	-0.3870	0.0917	-0.3059	0.0384	-0.3246	0.0708	0.0090
406.1983	2.3256	1.1409	0.2242	1.0139	-0.5687	0.4338	-0.4575	0.7626	-0.7034	0.4239	-0.3331	0.4955	0.2973	0.6935	-0.5654	0.6247	0.0024
406.9734	-0.4538	0.3315	-0.2433	0.4800	-0.3785	0.4157	-0.0981	0.5730	-0.5759	0.4840	0.7099	1.4201	-0.7220	0.1064	1.9812	1.0452	0.0052
408.0668	-0.7055	0.2072	-0.0908	0.5308	-0.7603	0.2766	-0.3875	0.3361	0.1160	0.6811	1.4964	1.2668	-0.7780	0.3737	0.5896	0.4378	0.0031
409.9844	-0.2787	0.0831	2.5576	2.4691	0.6726	0.8269	-0.3836	0.0320	-0.5621	0.1492	-0.4774	0.0717	-0.4175	0.0329	-0.4245	0.0618	0.0097
410.8435	-0.1941	0.8721	0.2673	1.0099	-0.7904	0.0564	-0.8476	0.0505	0.9330	0.5146	-0.3428	0.9396	-0.7259	0.3468	1.4990	0.4050	0.0022
419.0562	0.2076	1.0957	-0.3010	0.4250	-0.3380	0.1541	-0.3785	0.3629	1.6115	0.2540	-0.6343	0.1208	-0.3574	0.4391	-0.2847	0.3713	0.0012
420.0525	-0.7662	0.4642	-0.0274	0.6719	-0.6817	0.3332	-0.1023	0.2466	0.9245	0.6134	0.6685	0.2285	-0.7133	0.4451	-0.0750	0.2210	0.0011
420.8809	-0.4017	0.2405	-0.3431	0.4342	-0.5293	0.3058	-0.7992	0.1077	0.2278	0.5175	1.2478	0.7167	-0.4605	0.3991	0.3297	1.1216	0.0091
423.0848	-0.1294	0.6668	-0.2677	0.6308	-0.0066	0.7559	-0.3616	0.6284	-0.8607	0.1399	0.3329	0.9661	2.2147	1.0994	-0.6827	0.1870	0.0023
425.1185	0.1036	0.4484	-0.5003	0.0453	-0.4383	0.0800	-0.5425	0.0357	1.3469	0.3120	0.2260	0.4426	-0.5611	0.0285	-0.3907	0.3548	0.0000
428.1147	-0.4545	0.1251	-0.4978	0.0889	-0.6511	0.3653	-0.2436	0.3023	1.0230	0.5963	0.3987	0.6172	0.1357	0.3192	-0.5549	0.2623	0.0006
429.1150	-0.6803	0.2498	-0.5175	0.2613	-0.7409	0.5326	-0.2176	0.6434	0.9815	0.4739	0.7470	0.5353	0.3482	0.2809	-0.6633	0.5937	0.0010
429.9799	-0.6616	0.3961	-0.8373	0.2011	-0.6519	0.4349	-0.1851	0.4651	-0.0845	0.8143	0.6728	0.1985	-0.6017	0.3931	1.9007	0.8609	0.0001
430.1211	-0.4974	0.3422	-0.7684	0.3068	-0.6809	0.1333	-0.0039	0.3587	1.1292	0.4910	0.5925	0.6237	-0.1660	0.0924	-0.4389	0.2567	0.0001
430.7417	-0.6404	0.2848	-0.3266	0.4984	-0.7251	0.1557	-0.2259	0.6562	1.1195	0.5858	0.1752	0.6723	-0.2666	0.5248	0.2007	0.3083	0.0077
433.9079	-0.6285	0.3926	-0.4783	0.5430	-0.2286	1.0594	-0.7812	0.4000	0.9227	0.3348	0.8979	0.4899	-0.9327	0.2587	0.6963	1.1443	0.0081
434.0351	-0.3348	0.2657	-0.2528	0.0547	-0.3700	0.3762	-0.0769	0.2962	0.9019	0.9494	0.2237	0.1975	-0.7644	0.2645	-0.1494	0.1127	0.0067
435.0377	-0.3331	0.2233	-0.1677	0.1823	-0.5953	0.1704	-0.3839	0.2864	0.9349	0.9518	0.3044	0.1980	-0.3304	0.3267	-0.1954	0.4008	0.0093
437.7807	-0.2427	0.2658	-0.2437	0.2784	0.0565	0.5847	-0.4270	0.3617	1.0834	0.8073	0.0377	0.3272	-0.3110	0.1459	-0.5981	0.1271	0.0058
439.7785	-0.3771	0.0770	-0.2405	0.2766	-0.1024	0.1958	-0.2987	0.1804	1.4166	0.5798	-0.1644	0.4611	-0.5810	0.0904	-0.2927	0.1567	0.0000
441.8906	-0.5251	0.0880	0.3008	0.0972	0.1249	1.1118	-0.6255	0.0389	1.5826	0.9868	-0.3605	0.5879	-0.5974	0.1274	-0.1948	0.3976	0.0048
443.0511	-0.4008	0.8069	-0.0400	0.8115	-0.8046	0.4743	-0.5086	0.4085	-0.1425	0.7236	0.0069	0.3997	-0.3780	0.2892	2.0204	0.7539	0.0015
445.0521	-0.6734	0.5257	-0.9283	0.3080	-0.1994	0.9450	0.1845	0.5954	-0.1540	0.1548	1.4668	0.1394	-1.0671	0.3206	1.0641	1.1711	0.0011
446.9412	-0.4120	0.0856	-0.4690	0.0448	-0.4059	0.0826	-0.4267	0.1849	-0.3708	0.3998	-0.2434	0.6302	-0.0749	0.5079	2.2655	1.1749	0.0001
447.0628	-0.4743	0.3190	-0.7361	0.0131	-0.6481	0.2001	0.1044	0.0849	0.0435	0.6971	0.7929	1.0699	-0.8700	0.0677	0.9889	0.5805	0.0021
447.9335	-0.1897	0.1143	-0.3500	0.0427	-0.2827	0.0928	-0.4077	0.0323	-0.5817	0.1433	-0.0863	0.7505	-0.4438	0.0315	2.6129	1.6546	0.0003
448.0654	-0.4723	0.3265	-0.7025	0.1730	-0.6968	0.3601	0.2797	0.1051	0.3220	0.7204	0.6364	0.8650	-1.0376	0.0410	0.8082	0.4074	0.0007
448.1071	0.3795	1.2576	0.2788	1.4233	-0.5085	0.0746	-0.2844	0.4406	-0.7412	0.0953	-0.4525	0.1750	2.5263	0.3711	-0.6400	0.0443	0.0007
449.0647	-0.7121	0.5413	-0.7312	0.1334	-0.5384	0.2942	-0.0889	0.3316	0.1060	0.7768	0.9010	0.5156	-0.8634	0.4611	1.1425	0.3108	0.0002
451.3268	-0.4210	0.0857	0.0433	0.9375	-0.4161	0.0818	0.0245	0.9218	-0.6759	0.1149	-0.6013	0.0444	2.3680	1.5087	0.1532	1.1876	0.0077
452.1187	-0.5909	0.2275	-0.4522	0.7457	-0.5452	0.5552	-1.1013	0.0138	0.6016	0.2492	1.2455	0.5154	0.2542	0.9153	0.0756	0.6538	0.0023
454.0439	-0.3474	0.0913	0.0363	0.1563	-0.8544	0.1322	-0.5216	0.4821	0.8180	0.9732	0.2873	0.2196	-0.3641	0.1286	0.2097	0.4524	0.0058
455.9926	-0.6670	0.0914	0.0379	0.6821	-0.4722	0.3532	-0.7648	0.0458	-0.6089	0.4899	-0.1071	0.3618	1.3152	1.4202	1.6678	1.3622	0.0072
456.9195	-0.8289	0.0958	0.0746	0.2173	-0.7699	0.1874	-0.3421	0.3768	0.0101	0.7857	0.1774	0.3679	-0.5868	0.2456	1.9155	1.0855	0.0002
457.0368	-0.1860	0.2791	-0.2689	0.3048	-0.6411	0.2249	-0.3200	0.6070	1.3011	0.6202	0.3423	0.5067	-0.8225	0.0284	-0.1706	0.4950	0.0006
457.7681	-0.0122	0.6885	-0.1827	0.4808	-0.1747	0.1792	-0.3328	0.1636	1.4050	0.9338	-0.4180	0.2178	-0.2953	0.3355	-0.5353	0.2489	0.0040
457.9100	0.0436	0.5978	-0.7648	0.0512	-0.7122	0.0607	-0.7798	0.0466	0.0042	0.8449	0.6179	1.2472	-0.3623	0.5210	2.1300	0.3990	0.0004
459.0307	-0.5291	0.5376	-0.2147	0.7372	-0.8276	0.3312	-0.9623	0.2590	0.8462	0.2282	0.8782	0.6262	-0.4608	0.3668	0.6687	1.1946	0.0055
462.0936	-0.9012	0.4559	-0.1479	0.1729	-0.3117	0.3094	-0.2805	0.3186	1.0000	0.4733	0.7107	0.3662	-0.4604	0.0611	-0.3282	0.6373	0.0003
462.1487	2.1439	0.6183	-0.4603	0.0446	-0.3969	0.0833	0.3156	1.4182	0.2262	1.5913	-0.5865	0.0476	-0.5522	0.0262	-0.5451	0.0516	0.0083
463.0739	-0.4961	0.4309	-0.1086	0.5789	-0.7259	0.7335	-0.4586	0.1810	1.1213	0.5342	0.2740	0.3503	0.4563	0.4827	-0.6883	0.4149	0.0027
463.9369	-0.6163	0.0901	0.1828	0.8473	-0.5759	0.0911	-0.3141	0.2328	-0.6385	0.3779	0.4441	0.8311	-0.4174	0.3499	2.0845	1.3832	0.0021
474.1097	-0.6957	0.6901	0.3879	0.9594	-0.5311	0.1622	-0.7652	0.2911	1.1270	0.4675	-0.3677	0.2785	0.7315	0.8464	-0.2678	0.4997	0.0073
476.0987	-0.5447	0.3172	-0.4050	0.1689	-0.8501	0.2006	-0.1374	0.4002	1.2164	0.3376	0.3429	0.4941	-0.0815	0.1838	-0.3775	0.2839	0.0000
476.1353	-0.3347	0.0841	2.8356	0.7838	-0.3184	0.0898	-0.4386	0.0328	-0.6069	0.1357	0.4088	1.6510	-0.4777	0.0299	-0.4784	0.0572	0.0001
478.0967	-0.2649	0.2212	-0.2311	0.0492	-0.4924	0.3193	-0.1936	0.1149	0.8736	0.9420	0.3619	0.3170	-0.4025	0.1655	-0.4871	0.1711	0.0057
478.5867	-0.1802	0.0816	2.6297	2.4811	-0.1434	0.1049	-0.2870	0.0318	-0.4833	0.1729	-0.3917	0.0907	-0.3116	0.0382	-0.3297	0.0703	0.0083
480.0770	-0.3103	0.3363	-0.3347	0.2481	-0.5345	0.3487	-0.2082	0.1650	0.8781	0.9113	0.3908	0.3733	-0.2368	0.1533	-0.4799	0.2708	0.0093
481.0795	-0.4586	0.2018	-0.3393	0.2220	-0.6291	0.4388	-0.2377	0.0800	0.8994	0.9058	0.3367	0.2870	-0.0603	0.2335	-0.3294	0.2617	0.0060
485.0903	-0.2152	0.1864	2.9124	1.6577	-0.1317	0.2387	-0.0406	0.6030	-0.5749	0.1453	-0.4914	0.0687	-0.4347	0.0320	-0.4399	0.0605	0.0001

485.1298	**-0.5121**	0.3203	**-0.7201**	0.1262	**-0.3079**	0.2727	**-0.0912**	0.4355	**1.2050**	0.7990	**-0.5450**	0.2258	**1.2296**	1.0120	**-0.5847**	0.6321	0.0013
490.0252	**-0.5588**	0.1369	**-0.4646**	0.2592	**-0.5013**	0.1411	**-0.3047**	0.5463	**1.1302**	0.5609	**0.3586**	0.9026	**-0.5875**	0.4763	**0.3130**	0.3791	0.0044
490.0998	**-0.4643**	0.2785	**-0.2858**	0.2202	**-0.7741**	0.2282	**-0.0197**	0.1437	**1.2362**	0.6511	**0.0982**	0.1448	**-0.2063**	0.1015	**-0.3596**	0.3423	0.0000
491.9104	**0.1652**	0.6184	**-0.5813**	0.5580	**-0.4789**	0.4291	**-0.2447**	0.1454	**-0.7476**	0.4701	**0.5376**	1.0339	**-0.5666**	0.4042	**2.2377**	1.4983	0.0034
492.1133	**-0.3479**	0.1938	**-0.4670**	0.1152	**-0.4892**	0.1660	**-0.2647**	0.1187	**0.9991**	0.8022	**0.3161**	0.3675	**-0.1775**	0.2009	**-0.3984**	0.2080	0.0009
493.1163	**-0.3737**	0.1995	**-0.4009**	0.0695	**-0.5625**	0.1478	**-0.2218**	0.1216	**1.0350**	0.7832	**0.2576**	0.3581	**-0.1938**	0.1898	**-0.3712**	0.2439	0.0006
496.1317	**-0.3603**	0.0846	**2.6294**	0.7721	**-0.0917**	0.4249	**0.5946**	1.8654	**-0.6274**	0.1295	**-0.5485**	0.0560	**-0.5052**	0.0285	**-0.5031**	0.0551	0.0007
506.0945	**-0.1069**	0.4402	**-0.5745**	0.5653	**-0.7883**	0.3083	**-0.5094**	0.3610	**0.9355**	0.7716	**0.5415**	0.4987	**-0.3076**	0.4257	**0.0903**	0.2968	0.0061
506.2547	**-0.4476**	0.0781	**-0.3366**	0.4880	**-0.5551**	0.0711	**1.7260**	0.9528	**-0.7741**	0.0854	**0.4199**	1.0618	**-0.5112**	0.3122	**0.2740**	1.1696	0.0075
509.1135	**-0.5399**	0.3747	**-0.4303**	0.3644	**-0.6525**	0.2071	**-0.2022**	0.1319	**0.9701**	0.7141	**0.4583**	0.3176	**-0.0660**	0.2699	**-0.3146**	0.5615	0.0024
512.1618	**-0.7216**	0.7606	**0.0954**	0.9525	**0.5667**	0.7437	**2.0698**	0.8319	**-0.4277**	0.1878	**-0.1767**	1.0355	**-1.0266**	0.3170	**-0.4238**	0.3674	0.0022
524.1442	**-0.7081**	0.0925	**-0.6435**	0.2149	**-0.3051**	0.8140	**-0.6711**	0.2490	**0.4064**	1.1150	**0.6381**	0.5422	**1.3701**	0.7718	**-0.4892**	0.5756	0.0070
528.7934	**0.9836**	1.6114	**-0.0611**	0.7265	**0.2110**	0.3604	**0.0760**	0.3730	**1.2532**	0.2943	**-0.7420**	0.2921	**-1.0136**	0.2101	**-0.8219**	0.4006	0.0067
529.1870	**-0.2279**	0.3486	**-1.0887**	0.3629	**-0.1078**	1.1003	**-0.1017**	0.4994	**-0.0997**	1.0640	**0.3049**	0.7079	**2.0509**	0.9688	**-0.5312**	0.7502	0.0089
531.1653	**-0.4654**	0.9489	**-0.7127**	0.7389	**-0.5409**	0.5348	**0.8636**	0.4784	**0.9540**	0.7056	**0.2989**	0.5618	**0.2897**	0.6506	**-1.1990**	0.6084	0.0094
531.2348	**-0.1812**	0.0816	**-0.2165**	0.0409	**-0.1445**	0.1048	**-0.2879**	0.0318	**-0.4841**	0.1727	**-0.3925**	0.0905	**-0.3127**	0.0381	**2.0728**	2.0359	0.0098
532.1277	**0.0759**	0.7713	**-0.4680**	0.4815	**-0.8358**	0.8366	**0.3551**	0.3168	**1.2163**	0.2541	**-0.7529**	0.3413	**0.9448**	0.4907	**-0.9190**	0.1107	0.0003
533.0234	**-0.4373**	0.0861	**-0.4967**	0.0453	**-0.2190**	0.3287	**-0.4019**	0.2364	**-0.1646**	0.4135	**-0.1083**	0.8372	**-0.5879**	0.0244	**1.9918**	1.2227	0.0008
535.1420	**-0.4235**	0.0999	**-0.3880**	0.2517	**-0.2812**	0.2767	**-0.7266**	0.0056	**0.9834**	0.5878	**0.6271**	1.0616	**0.2349**	0.3416	**-0.6532**	0.0911	0.0026
537.1410	**-0.4805**	0.1738	**-0.1079**	0.4866	**-0.5798**	0.2678	**-0.4788**	0.1786	**0.3505**	0.9762	**0.9374**	0.1938	**0.6528**	0.9162	**-0.8889**	0.0158	0.0052
544.2074	**-0.5539**	0.6447	**-0.1679**	1.1396	**0.5298**	0.6863	**-0.0599**	0.4397	**1.3751**	0.5490	**-1.1524**	0.2053	**0.0698**	0.1778	**-0.2920**	0.6020	0.0074
544.8995	**-0.2913**	0.2195	**-0.4547**	0.0888	**-0.4695**	0.0776	**-0.5695**	0.0366	**0.3520**	0.8884	**-0.4546**	0.3433	**-0.4686**	0.2414	**1.9327**	0.2612	0.0000
544.9815	**0.1017**	0.5196	**-0.4480**	0.0444	**-0.3841**	0.0844	**-0.4956**	0.0342	**0.0333**	1.0683	**1.6145**	0.5991	**-0.5401**	0.0268	**-0.5343**	0.0525	0.0006
545.0081	**-0.6080**	0.0899	**2.0887**	1.1073	**-0.0394**	0.5455	**-0.7069**	0.0427	**-0.5575**	0.5070	**0.1077**	1.0242	**-0.5460**	0.3750	**0.3641**	1.1322	0.0044
550.0224	**-0.6251**	0.3059	**-0.6841**	0.1526	**-0.4603**	0.5850	**-0.0846**	0.0537	**0.0201**	0.0847	**1.3570**	1.2296	**-0.8849**	0.0872	**0.8420**	1.1992	0.0069
550.0944	**0.0934**	1.0861	**0.3037**	0.2050	**-0.7561**	0.1715	**-0.4270**	0.5450	**1.1547**	0.5932	**-0.0997**	0.4338	**0.1668**	0.4239	**-0.9186**	0.5426	0.0095
557.2139	**-0.5732**	0.0891	**0.7045**	1.5002	**2.5999**	1.3405	**0.5617**	1.1044	**-0.7977**	0.0783	**-0.6738**	0.1107	**-0.5112**	0.3945	**-0.4625**	0.2704	0.0017
558.5616	**-0.3510**	0.0844	**-0.4023**	0.0436	**-0.3368**	0.0883	**-0.4546**	0.0332	**-0.1110**	0.7507	**-0.5403**	0.0578	**-0.2584**	0.4282	**2.1580**	0.6677	0.0000
561.2010	**0.2809**	1.1106	**0.1921**	0.7594	**-0.2882**	0.4959	**1.3491**	1.1544	**0.7844**	0.4268	**-0.5175**	0.7229	**-0.8511**	0.3683	**-1.2074**	0.2561	0.0097
562.0624	**-0.9985**	0.3019	**-0.2530**	0.3153	**-1.0033**	0.3722	**-0.1991**	0.4298	**0.8791**	0.6498	**0.2808**	0.4091	**-0.1523**	0.1977	**0.7724**	0.4357	0.0001
562.2112	**0.0104**	0.7277	**-0.0392**	0.1805	**-0.3025**	0.1210	**1.5721**	1.2058	**0.7238**	0.4497	**-0.6052**	0.9441	**-0.3370**	0.2202	**-1.3295**	0.2270	0.0020
563.2143	**-0.3273**	0.2149	**-0.0736**	0.7931	**-0.0566**	0.7397	**1.4522**	0.9864	**0.7417**	0.4975	**-0.7127**	0.4615	**-0.2764**	0.5519	**-1.0640**	0.2629	0.0035
567.0481	**0.0688**	0.4085	**-0.5727**	0.2294	**-0.6707**	0.3486	**-0.2976**	0.4881	**1.0903**	0.7898	**0.1174**	0.5416	**-0.5026**	0.2377	**0.0473**	0.2429	0.0044
567.4667	**-0.1682**	0.0814	**-0.2022**	0.0407	**2.5760**	2.3841	**-0.2751**	0.0319	**-0.4737**	0.1758	**-0.3812**	0.0930	**-0.2987**	0.0388	**-0.3181**	0.0713	0.0069
568.0473	**-0.3064**	0.4033	**0.1515**	0.7875	**-0.9507**	0.3979	**-0.9398**	0.0149	**1.0941**	0.6681	**0.4355**	0.1292	**0.0327**	0.6898	**-0.1711**	0.6750	0.0045
569.9833	**-0.1076**	0.3549	**-0.4134**	0.0438	**-0.3483**	0.0873	**-0.4645**	0.0334	**-0.6280**	0.1293	**0.5616**	1.9524	**-0.5061**	0.0284	**2.0506**	0.4131	0.0049
570.1033	**-0.4197**	0.0857	**-0.4774**	0.0449	**-0.4146**	0.0819	**-0.1441**	0.6278	**-0.6749**	0.1152	**1.5449**	0.8181	**0.7365**	1.2270	**-0.2990**	0.4801	0.0040
570.7593	**-0.4404**	0.3534	**0.2886**	0.6676	**0.0490**	0.7860	**-0.6091**	0.3365	**1.2103**	0.5020	**0.1067**	0.2016	**-0.5458**	0.3649	**-0.7097**	0.0910	0.0020
572.2154	**1.3074**	0.4552	**-1.0232**	0.0581	**0.5739**	1.3887	**-0.3318**	0.7369	**-0.4289**	1.1199	**-1.0344**	0.0516	**1.7027**	0.7402	**-0.2573**	0.7072	0.0034
573.1493	**-0.6519**	0.2144	**-0.4036**	0.4860	**-0.6461**	0.2672	**-0.5174**	0.4550	**1.1983**	1.1525	**1.1219**	0.4765	**0.7486**	0.6548	**-1.1289**	0.1334	0.0004
576.1302	**-0.5563**	0.4923	**-1.0138**	0.3911	**-0.5721**	0.6808	**1.2482**	0.4511	**0.5550**	0.8461	**0.4894**	0.5135	**-0.4576**	0.3806	**-0.3883**	0.4700	0.0018
577.0040	**-0.4684**	0.2491	**-0.6292**	0.1840	**-0.7809**	0.1555	**-0.7490**	0.1728	**1.3279**	0.6446	**0.6142**	0.2004	**-0.0110**	0.4386	**0.0603**	0.7988	0.0001
577.3969	**-0.0637**	0.2740	**-0.3127**	0.0422	**-0.2441**	0.0961	**2.6687**	1.1084	**-0.5544**	0.1515	**-0.4690**	0.0736	**-0.4072**	0.0334	**-0.4153**	0.0626	0.0000
585.2725	**-0.4913**	0.0872	**1.2338**	1.5170	**-0.4958**	0.0756	**2.0942**	1.3259	**-0.7322**	0.0980	**-0.6625**	0.0309	**-0.3728**	0.4516	**-0.0777**	0.5826	0.0022
587.2621	**-0.0349**	1.0631	**-0.6996**	0.0496	**-0.6446**	0.0649	**2.1599**	0.0253	**-0.2089**	1.1270	**0.4455**	1.1935	**-0.3205**	0.7937	**-0.4877**	0.4291	0.0058
588.1570	**0.0243**	0.4031	**-0.3872**	0.3261	**-0.4434**	0.0900	**-0.3442**	0.3959	**1.6309**	0.6589	**-0.4300**	0.4159	**-0.4539**	0.3084	**-0.1329**	0.1522	0.0001
592.0438	**-0.2566**	0.9632	**-0.6277**	0.2032	**-0.4506**	0.3874	**-0.2313**	0.6839	**0.1701**	0.8259	**-0.3012**	0.6120	**-0.5105**	0.5950	**1.7281**	0.2824	0.0060
594.0717	**-0.3686**	0.3010	**-0.2539**	0.1391	**-0.3226**	0.4468	**-0.3232**	0.1680	**1.1376**	0.7799	**0.1318**	0.2512	**-0.3619**	0.0892	**-0.3472**	0.2155	0.0013
594.1309	**0.0057**	0.3601	**-0.2909**	0.0418	**-0.1750**	0.0679	**-0.3547**	0.0318	**-0.3834**	0.3225	**-0.4517**	0.0774	**-0.3858**	0.0344	**2.2319**	2.0591	0.0070
598.0747	**-0.5590**	0.6744	**0.2176**	0.2979	**-0.5670**	0.8386	**-1.0358**	0.1520	**0.7247**	0.5977	**0.4670**	0.6570	**-0.5155**	0.6673	**0.8056**	0.5792	0.0094
606.7122	**-0.2384**	0.0824	**3.1163**	1.5433	**-0.2092**	0.0991	**-0.3440**	0.0317	**-0.5298**	0.1589	**-0.4423**	0.0795	**-0.3741**	0.0350	**-0.3857**	0.0652	0.0000
609.1451	**-0.9761**	0.4141	**-0.4356**	0.8458	**-0.5078**	0.4587	**-0.9642**	0.2548	**-0.2827**	0.1352	**1.1576**	0.3133	**0.9471**	1.1423	**0.8111**	0.9326	0.0029
610.1687	**0.5119**	0.4175	**-0.6518**	0.4411	**-0.7345**	0.1622	**-0.6340**	0.3783	**-0.5975**	0.3508	**-0.3232**	0.7839	**1.6414**	1.2674	**1.2025**	1.5125	0.0091
611.0422	**-0.4669**	0.0867	**-0.1244**	0.3209	**-0.1553**	0.3595	**-0.5684**	0.0366	**1.0619**	0.7481	**-0.6413**	0.0356	**0.4648**	0.2372	**-0.0250**	0.7177	0.0018
611.1474	**-0.4657**	0.2858	**-1.0325**	0.1357	**-0.3560**	0.7502	**-0.8934**	0.3128	**0.4907**	0.4148	**-0.1996**	0.4582	**0.5783**	1.1736	**1.6677**	1.0503	0.0028
614.2383	**-0.2024**	0.3718	**-0.7864**	0.3018	**-0.6545**	0.3404	**0.2345**	1.0425	**-0.4311**	0.6325	**1.2880**	0.9411	**0.8620**	0.5736	**-0.4678**	0.1423	0.0066
616.9189	**-0.4629**	0.0866	**-0.5248**	0.0458	**-0.4636**	0.0780	**-0.5644**	0.0364	**0.0503**	1.3796	**-0.6378**	0.0363	**0.9282**	1.6172	**1.9907**	0.6986	0.0078

623.2070	**-0.2791**	0.6704	**0.1293**	0.5641	**-0.6765**	0.1672	**0.4113**	0.3641	**1.5192**	0.6393	**-0.5134**	0.3378	**-0.3318**	0.4593	**-0.7465**	0.4736	0.0006
627.7161	**-0.1704**	0.6709	**-0.1991**	0.5046	**0.2955**	0.4297	**-0.1308**	0.5727	**1.6922**	1.0496	**-0.7655**	0.0084	**-0.2481**	0.4527	**-0.7430**	0.0377	0.0019
628.0507	**-0.2344**	0.4340	**-0.3266**	0.3137	**-0.2844**	0.3455	**-0.1690**	0.4150	**1.1180**	0.7722	**-0.1348**	0.1701	**-0.3604**	0.1913	**-0.3702**	0.3218	0.0066
629.0442	**-0.8276**	0.7636	**-0.4003**	0.5178	**-0.7056**	0.0797	**-0.3067**	0.2458	**0.4700**	0.7001	**1.4991**	1.4299	**-0.9435**	0.5738	**1.0139**	0.3494	0.0033
629.8658	**2.1463**	0.5501	**-0.3428**	0.7899	**-0.8957**	0.2302	**-0.7380**	0.2578	**0.0257**	1.1395	**0.0880**	0.4641	**-0.7560**	0.3580	**0.2199**	0.9779	0.0011
630.1649	**-0.3421**	0.0842	**-0.3925**	0.0434	**-0.1240**	0.4383	**-0.4458**	0.0330	**0.8392**	1.2467	**-0.4375**	0.2011	**1.0360**	0.4177	**-0.4855**	0.0566	0.0052
633.0492	**0.1073**	0.6982	**-0.3772**	0.7874	**-0.5984**	0.1130	**-0.2479**	0.4773	**-0.8363**	0.1896	**1.9166**	0.5024	**-0.4641**	0.7834	**0.5010**	1.2305	0.0040
636.0253	**-0.6041**	0.0898	**1.9238**	1.5756	**-0.3945**	0.3691	**1.1263**	0.9548	**-0.8225**	0.0708	**0.2710**	1.1734	**-0.3652**	0.7034	**-0.7377**	0.0380	0.0070
636.6679	**2.1581**	0.7037	**-0.4081**	0.0437	**-0.3428**	0.0878	**-0.4598**	0.0333	**0.5401**	2.0729	**-0.3000**	0.4553	**-0.5009**	0.0287	**-0.4992**	0.0554	0.0090
638.2284	**0.0706**	0.8821	**-0.5592**	0.2508	**0.2556**	0.5379	**2.3579**	1.1161	**-0.4935**	0.6685	**-0.4045**	0.6861	**-0.4269**	0.6804	**-0.5866**	0.3059	0.0013
639.3050	**-0.6274**	0.2459	**-0.3633**	0.4703	**-0.0619**	0.5268	**1.5855**	1.3009	**-0.6088**	0.3251	**-0.8717**	0.0158	**1.6257**	1.6580	**-0.1636**	0.1258	0.0057
639.6861	**-0.2721**	0.0857	**-0.1716**	0.0548	**-0.0191**	0.4268	**-0.2183**	0.2793	**1.0378**	0.9480	**-0.1757**	0.1422	**-0.3657**	0.1129	**-0.4673**	0.1695	0.0072
641.3706	**-0.5371**	0.8053	**-1.2648**	0.2208	**0.8755**	0.4229	**0.0801**	0.5910	**0.6259**	1.0986	**-0.6746**	0.1178	**1.6565**	1.2555	**-0.7211**	0.6476	0.0030
644.0530	**-0.4129**	0.3585	**0.2527**	0.0880	**-0.5928**	0.2169	**-0.1630**	0.5732	**1.1675**	0.6324	**-0.5046**	0.1363	**-0.4432**	0.4741	**0.0669**	0.3452	0.0010
646.0971	**-0.2776**	0.8163	**-1.0289**	0.0582	**-0.5345**	0.4896	**0.1132**	1.3345	**-0.0837**	1.0433	**-0.4315**	0.1060	**0.3965**	0.7603	**1.9751**	0.2617	0.0068
650.0575	**-0.2307**	0.2294	**-0.5844**	0.0471	**-0.1463**	0.4828	**-0.4847**	0.0978	**0.8060**	0.9182	**-0.0475**	0.8543	**-0.6740**	0.0202	**0.7318**	0.1948	0.0100
654.4031	**-0.0768**	0.6631	**-0.6013**	0.0474	**0.2979**	1.4173	**-0.6331**	0.0392	**1.5462**	0.4716	**-0.6987**	0.0229	**-0.6079**	0.1239	**0.4146**	0.8261	0.0072
654.9577	**-0.7800**	0.2450	**-0.2723**	0.8264	**-0.6375**	0.7197	**0.7729**	0.5175	**-0.3294**	0.6684	**0.5081**	0.3382	**-1.0361**	0.2218	**1.7341**	1.2212	0.0018
655.4131	**-0.5962**	0.0896	**-0.6707**	0.0490	**0.2987**	0.8785	**1.9816**	0.8206	**-0.3053**	0.8124	**-0.1641**	0.7371	**-0.5654**	0.3441	**-0.3979**	0.3028	0.0010
656.2225	**0.8528**	1.3904	**-0.9537**	0.0561	**0.5901**	0.2478	**1.7493**	0.7147	**-1.0231**	0.0113	**-0.3423**	1.0799	**0.6565**	0.2736	**-0.9791**	0.0315	0.0007
657.0496	**-0.5766**	0.0892	**-0.6492**	0.0485	**-0.2362**	0.6856	**-0.1448**	0.4315	**1.1478**	0.4954	**-0.7368**	0.0146	**-0.3619**	0.3253	**1.1024**	1.0375	0.0009
659.0736	**1.6556**	1.9462	**-0.0075**	0.8772	**-0.4783**	0.0769	**1.4784**	0.7261	**-0.7199**	0.1017	**-0.6491**	0.0338	**-0.6295**	0.0224	**-0.2875**	0.5882	0.0090
661.6713	**-0.1950**	0.6109	**0.2747**	1.3732	**-0.2078**	0.5188	**-0.2951**	0.5301	**1.5829**	0.3256	**-0.3980**	0.1747	**-0.6769**	0.0200	**-0.4970**	0.2341	0.0092
663.7793	**-0.6186**	0.0902	**1.2051**	0.1036	**0.0058**	0.5903	**-0.7173**	0.0433	**0.6353**	1.1529	**-0.3832**	0.4688	**-0.7257**	0.1082	**0.3030**	0.5388	0.0025
669.8580	**-0.8559**	0.0817	**0.5820**	1.2457	**-0.3955**	0.6574	**-0.3308**	0.9150	**-0.8881**	0.1909	**1.1665**	0.3273	**-0.6120**	0.3656	**1.2324**	0.3960	0.0020
671.3014	**-0.5657**	0.0889	**-0.6372**	0.0482	**-0.5800**	0.0694	**0.7153**	0.5301	**0.0360**	0.9704	**-0.7273**	0.0166	**2.4155**	1.1841	**-0.2200**	0.5158	0.0001
671.9705	**-0.3739**	0.2647	**-0.6509**	0.0485	**-0.0889**	0.6058	**-0.3719**	0.2337	**-0.2833**	0.6914	**0.4730**	0.6967	**-0.6203**	0.2147	**1.9704**	1.4687	0.0039
675.0634	**-0.3244**	0.5194	**0.0995**	0.8710	**-0.5433**	0.2506	**-0.6704**	0.2168	**-0.0656**	0.6783	**-0.2317**	0.7072	**-0.5332**	0.5294	**1.9810**	0.9359	0.0026
676.2130	**-0.2180**	0.6707	**-0.6325**	0.2489	**-0.7951**	0.2892	**1.0242**	0.8824	**1.0178**	0.6174	**-0.1873**	0.1726	**-0.2438**	0.3989	**-0.5210**	0.1912	0.0014
685.2208	**-1.0376**	0.1020	**-0.6416**	0.5623	**-0.6011**	0.4263	**1.7869**	1.3069	**0.7339**	0.5633	**-0.0121**	0.3965	**0.0028**	0.6364	**-0.5526**	0.0591	0.0010
686.3200	**-0.4066**	0.0854	**-0.3235**	0.2565	**-0.0528**	0.5787	**-0.0567**	0.8164	**-0.4713**	0.3979	**-0.4341**	0.2940	**2.6475**	1.6872	**-0.3430**	0.3839	0.0013
699.1996	**-0.1254**	0.5839	**-0.1142**	0.6879	**-0.4295**	0.0807	**-0.5349**	0.0354	**1.2889**	0.4192	**0.3693**	0.8670	**-0.5831**	0.0246	**-0.5728**	0.0494	0.0020
703.3790	**-0.1779**	0.5936	**-0.5906**	0.1342	**0.1395**	0.6677	**2.3956**	1.4896	**-0.2912**	0.4511	**-0.6491**	0.2137	**-0.1278**	0.6893	**-0.3643**	0.6765	0.0023
703.9184	**-0.3535**	0.3418	**-0.5883**	0.0471	**-0.0297**	0.3691	**-0.6214**	0.0387	**-0.5474**	0.4078	**-0.3302**	0.6320	**0.6099**	1.5661	**2.1140**	0.8164	0.0028
704.0891	**-0.3668**	0.0847	**-0.4195**	0.0439	**0.2493**	1.1312	**-0.4701**	0.0335	**-0.1428**	0.9078	**2.1239**	1.0693	**-0.5121**	0.0281	**-0.5093**	0.0546	0.0016
705.7411	**1.2045**	1.5255	**1.6582**	0.7407	**-0.6136**	0.0670	**-0.3969**	0.4767	**0.5819**	1.4683	**-0.5938**	0.2796	**-0.7578**	0.0162	**-0.6672**	0.1229	0.0091
708.1274	**0.1760**	0.9136	**-0.3064**	0.5104	**-0.5371**	0.0725	**-0.3215**	0.5338	**-0.0580**	0.7148	**-0.6199**	0.1050	**-0.4183**	0.4723	**2.1376**	1.2392	0.0028
724.6603	**0.2536**	0.6736	**-0.5141**	0.0928	**0.1011**	0.0884	**-0.3568**	0.3669	**1.0886**	0.9289	**-0.4588**	0.3410	**-0.2176**	0.2098	**-0.4447**	0.1571	0.0075
726.6689	**-0.2667**	0.2287	**-0.0643**	0.5790	**-0.0698**	0.6898	**-0.2829**	0.3929	**1.4282**	0.4159	**-0.4910**	0.1605	**-0.2489**	0.1506	**-0.5242**	0.1407	0.0004
727.0780	**0.0135**	0.5978	**-0.3572**	0.0428	**-0.1935**	0.1683	**-0.0413**	0.3063	**-0.5870**	0.1417	**-0.5045**	0.0658	**-0.4509**	0.0312	**2.1986**	1.6936	0.0016
727.9036	**-0.3658**	0.0847	**-0.4184**	0.0438	**-0.3536**	0.0869	**-0.1564**	0.5185	**-0.1691**	0.4971	**-0.5532**	0.0550	**-0.1976**	0.5149	**2.1545**	1.8713	0.0070
729.6666	**0.4258**	1.0507	**0.8413**	0.9104	**-0.4532**	0.5835	**-0.8187**	0.0488	**1.2980**	0.6008	**-0.3969**	0.5022	**-0.4729**	0.1933	**-0.7706**	0.1695	0.0033
731.3210	**-0.6735**	0.3134	**-0.6147**	0.2288	**-0.3517**	0.6685	**0.6060**	0.3565	**1.5207**	0.7154	**-0.9584**	0.3342	**0.3053**	1.0173	**-0.0226**	0.9319	0.0041
731.8458	**-0.3665**	0.1047	**-0.3556**	0.2766	**-0.4796**	0.0768	**-0.0377**	0.2114	**-0.3813**	0.4872	**-0.1110**	0.6716	**-0.5569**	0.1393	**2.0095**	1.4968	0.0022
733.1294	**-0.5151**	0.0877	**-0.5818**	0.0470	**-0.1608**	0.7001	**-0.6157**	0.0385	**-0.6056**	0.3027	**0.0594**	1.2984	**2.4347**	0.5647	**0.4237**	0.9802	0.0006
736.2508	**0.6175**	0.7234	**2.5320**	0.8941	**-0.4187**	0.8182	**-0.6985**	0.4369	**-0.3750**	0.5712	**0.0680**	0.3974	**-1.0235**	0.0062	**-0.3177**	1.1071	0.0004
738.0834	**-0.2843**	0.6210	**2.4448**	1.8399	**-0.6364**	0.0655	**0.0141**	0.8590	**-0.5346**	0.3402	**0.3667**	0.7898	**-0.5758**	0.3377	**-0.3840**	0.4531	0.0060
738.6129	**-0.2654**	0.0829	**-0.3086**	0.0421	**0.1303**	0.6249	**-0.3705**	0.0319	**1.6990**	1.2620	**-0.4658**	0.0743	**-0.4031**	0.0336	**-0.4116**	0.0629	0.0011
739.4598	**-0.4517**	0.0864	**-0.5124**	0.0456	**2.5665**	0.7332	**-0.5534**	0.0360	**-0.7005**	0.1075	**0.3891**	1.7820	**0.5317**	0.9756	**-0.5910**	0.0480	0.0012
745.3016	**-0.3656**	0.0847	**-0.4183**	0.0438	**2.6510**	1.9173	**-0.3109**	0.2670	**-0.1476**	0.7110	**-0.5531**	0.0550	**-0.5109**	0.0282	**-0.1639**	0.6222	0.0016
746.3697	**-0.4028**	0.0854	**-0.4590**	0.0446	**0.2495**	0.8187	**-0.5055**	0.0345	**-0.6614**	0.1193	**2.1386**	1.2800	**0.2776**	0.7860	**-0.5440**	0.0517	0.0006
749.1235	**0.4128**	0.1047	**0.0663**	1.0296	**-0.5941**	0.1914	**-1.0045**	0.0601	**-0.7650**	0.4311	**-0.1090**	0.6953	**0.0943**	0.6956	**2.0364**	0.9806	0.0008
753.1469	**-0.5941**	0.3053	**-0.8212**	0.0526	**-0.4158**	0.2510	**0.2099**	1.7539	**-0.0144**	0.8189	**-0.3787**	0.4547	**-0.1579**	0.5097	**2.0147**	0.3542	0.0086
755.5467	**2.8024**	1.4091	**-0.3976**	0.0435	**-0.3319**	0.0887	**-0.4503**	0.0331	**-0.6165**	0.1328	**-0.4266**	0.2256	**-0.3025**	0.3382	**0.0753**	0.5467	0.0000
756.3926	**-0.3603**	0.0846	**-0.4125**	0.0437	**-0.3474**	0.0874	**-0.4637**	0.0334	**-0.2959**	0.4456	**0.1764**	1.2844	**2.3901**	1.5575	**-0.5030**	0.0551	0.0026
759.8179	**-0.0407**	0.4687	**-0.4119**	0.0437	**-0.3468**	0.0874	**0.1261**	1.0531	**-0.6270**	0.1296	**2.2438**	1.1128	**-0.5047**	0.0285	**-0.5026**	0.0551	0.0003
759.9892	**-0.8415**	0.0961	**1.1806**	1.4655	**-0.6295**	0.4523	**-0.9362**	0.0558	**-0.5168**	0.4433	**-0.2300**	0.6662	**0.9678**	0.2794	**1.4378**	0.8815	0.0016

760.3933	0.0654	1.1886	-0.9148	0.8039	0.7652	0.6135	-0.5840	0.6197	-0.2439	0.4095	-0.1407	0.5187	2.2683	1.5290	-0.6316	0.5562	0.0077
760.7706	-0.3945	0.0852	-0.4499	0.0444	-0.3861	0.0842	-0.2149	0.4642	1.5287	0.8413	-0.5782	0.0495	-0.5420	0.0267	0.6577	1.3054	0.0034
761.2132	2.3322	1.3339	0.3202	0.9809	-0.3562	0.2801	0.5884	1.0268	-0.7618	0.0891	-0.6947	0.0238	-0.6857	0.0196	-0.3332	0.5912	0.0013
763.9246	-0.3326	0.4101	-0.0674	0.6503	0.2397	0.2881	-0.7748	0.0463	-0.1271	0.8885	-0.6412	0.3174	-0.5312	0.4106	2.0945	0.7325	0.0002
768.0455	-0.1859	0.5826	0.2256	0.8373	-0.6914	0.0620	-0.3921	0.6514	-0.0593	0.4446	-0.8128	0.0037	-0.2447	0.5264	2.1288	1.0617	0.0008
768.3607	-0.0500	0.5455	-0.6377	0.1418	-0.2867	0.3312	0.1243	0.3926	-0.6889	0.1878	-0.1912	1.0283	2.4791	1.4833	-0.5345	0.3073	0.0010
768.5202	-0.2361	0.0824	-0.2766	0.0417	-0.2067	0.0993	-0.3418	0.0317	-0.5280	0.1594	-0.4403	0.0799	2.9914	1.6579	-0.3835	0.0654	0.0000
769.1115	-0.2734	0.5687	1.3580	0.8079	0.8101	1.3588	-0.7523	0.0451	-0.8626	0.0588	-0.8044	0.0028	-0.3549	0.4646	1.3327	1.1660	0.0037
770.3953	-0.2444	0.3861	-0.1381	0.4311	-0.5260	0.0733	0.5179	0.6700	-0.6722	0.0504	-0.6858	0.0257	2.5286	1.6891	-0.2539	0.7122	0.0009
772.2962	-0.0871	0.8334	-0.0832	0.6184	-0.5173	0.5005	1.1279	0.5727	0.8929	0.8884	-0.2393	0.6899	-0.3597	0.3252	-1.2217	0.3629	0.0071
780.5276	-0.3925	0.0852	-0.3190	0.1806	-0.3839	0.0844	1.8272	1.6400	-0.6532	0.1218	0.6702	1.1462	-0.5399	0.0268	-0.3341	0.2939	0.0088
796.1842	0.9970	0.5668	-0.7376	0.4815	-0.7771	0.3373	-1.0846	0.1008	-0.2202	0.8989	-0.4552	0.3094	0.9639	1.2262	1.2938	1.3843	0.0080
799.8946	-0.1663	0.0814	-0.2002	0.0407	-0.1276	0.1063	-0.2249	0.0674	1.1065	0.9334	-0.3796	0.0934	-0.2967	0.0389	-0.3163	0.0715	0.0012
808.7070	-0.5415	0.0883	2.5123	0.8144	0.7498	1.7006	-0.6416	0.0396	-0.1915	0.5056	-0.3453	0.3882	-0.4661	0.4148	-0.6775	0.0417	0.0007
809.1386	-0.6991	0.0922	0.3973	1.3367	0.0503	0.1070	-0.3517	0.3553	-0.6059	0.5307	0.2089	1.2240	-0.7891	0.1470	2.1448	1.1779	0.0076
813.6490	-0.4623	0.0866	-0.2172	0.4883	0.0304	0.8089	-0.4579	0.1548	1.6719	0.2853	-0.4109	0.4123	0.0424	0.9737	-0.6013	0.0472	0.0014
816.3832	0.0354	0.8733	0.8418	0.7562	-0.6409	0.6902	-1.0588	0.0636	-0.3315	0.6978	-0.3415	0.3017	2.1767	0.7025	-0.4851	1.0750	0.0011
832.0246	-0.6763	0.0916	0.2759	0.1019	-0.5451	0.3384	-0.0300	0.8694	0.9856	0.3983	-0.7752	0.0886	-0.5053	0.5922	0.8714	1.0742	0.0058
838.2096	-0.1807	0.5799	2.1555	0.9030	-0.5814	0.0693	-0.6665	0.0408	0.0715	1.5437	-0.7283	0.0164	-0.3547	0.3218	0.8389	1.3735	0.0082
848.2692	2.3591	1.5595	-0.0939	0.9922	-0.1613	0.8065	-0.5838	0.1160	-0.6055	0.3718	-0.0258	0.6317	-0.7328	0.0174	0.0694	0.7790	0.0061
853.4333	0.4604	1.1233	-0.8986	0.2410	0.0178	0.4162	-1.0482	0.0629	-0.0762	0.5865	-0.6451	0.4869	1.8451	1.5226	0.4771	0.4521	0.0055
855.2474	0.1527	1.0708	1.6714	1.0160	-0.6453	0.4326	-0.2725	0.6208	0.8419	0.2978	-0.2708	0.5849	-1.0264	0.0062	-0.5506	0.4205	0.0022
865.0644	-0.7641	0.3000	1.1843	0.9831	-0.9341	0.0292	-0.9286	0.0684	0.7108	0.3126	0.5906	0.9913	-0.1374	1.0343	-0.0280	0.5042	0.0059
869.4473	0.1920	1.2463	-0.4241	0.2017	2.3765	1.5805	-0.6010	0.0379	-0.7393	0.0959	-0.4157	0.2499	-0.2841	0.6223	0.4394	0.9534	0.0060
869.4846	-0.8829	0.0493	-0.9184	0.0895	-0.5627	0.6660	0.8173	1.0182	-0.6131	0.7243	0.1020	1.0923	1.7423	0.5335	0.7950	1.2882	0.0066
873.4519	-0.7667	0.9465	-0.9876	0.4894	-0.0154	0.2932	0.2599	0.6743	-0.1304	1.2472	-0.2366	0.4492	2.2762	0.8538	-0.2631	0.4321	0.0023
874.6331	0.0352	0.5179	-0.5682	0.2970	-0.1119	0.5748	1.1939	1.4493	1.0493	0.2528	-0.6242	0.1883	-0.5796	0.3035	-0.7040	0.2192	0.0054
875.4447	-0.7523	0.0968	-0.5145	0.6149	0.0560	1.1335	-1.3385	0.1801	0.9298	0.1461	0.3588	0.3307	1.1868	0.5415	-0.2649	0.2204	0.0003
884.0657	0.1250	1.0432	-0.5961	0.0473	0.0326	0.9486	-0.1804	0.6697	-0.7617	0.0891	0.1117	0.4416	-0.6856	0.0196	1.9809	1.1772	0.0055
884.4091	-0.4297	0.8216	-0.3390	0.7982	-0.2888	0.8381	2.1266	0.5019	0.1810	0.5787	-0.8985	0.5440	-0.1084	1.1000	-0.1525	0.6183	0.0066
885.9091	0.0911	0.5957	-0.4801	0.0928	0.1436	0.7141	-0.3123	0.2977	1.2849	0.8256	-0.4683	0.2412	-0.4690	0.4141	-0.2442	0.4715	0.0096
888.2072	-0.3647	0.3099	0.3124	1.0321	-0.6137	0.0670	-0.6945	0.0421	-0.1348	1.2123	-0.1950	0.9559	-0.4101	0.2924	2.0497	0.3228	0.0035
888.3979	-0.7433	0.0934	-0.8316	0.0529	0.8998	1.0594	0.3256	1.0797	-0.9339	0.0374	0.4306	0.8395	1.6974	0.5725	-0.6396	0.4319	0.0011
890.5884	1.8865	0.7198	-0.0683	0.3033	-0.8709	0.0576	-0.0282	1.1674	0.8607	0.3153	-0.4190	0.4597	-0.5980	0.1878	-0.9085	0.1335	0.0001
890.8926	-0.5157	0.0878	-0.1614	0.6856	-0.5234	0.0735	-0.4283	0.2080	-0.1611	0.5718	0.3595	0.9880	-0.6722	0.0203	1.9689	1.4956	0.0067
892.2291	0.2314	0.7490	2.0486	0.1648	-0.5375	0.6905	-0.7625	0.4637	-0.1334	0.2783	1.0145	1.2287	-1.0597	0.6114	-0.6353	0.5571	0.0005
900.1860	1.5996	1.2123	-0.2068	0.5217	-0.4937	0.3437	-0.4864	0.5077	-0.8791	0.0539	0.6844	0.8317	-0.7427	0.1188	0.3280	1.0256	0.0067
900.7910	-0.5619	0.0888	2.3284	0.9444	-0.3401	0.4778	-0.5454	0.1655	-0.6281	0.3312	-0.1756	0.9570	-0.5878	0.2144	0.9916	1.2925	0.0007
903.7539	0.0480	0.4456	-0.1127	0.3398	-0.2296	0.0973	-0.3617	0.0318	1.3536	0.5511	-0.4580	0.0760	-0.3935	0.0341	-0.4030	0.0637	0.0000
905.6084	-0.1812	0.0816	-0.2165	0.0409	-0.1445	0.1048	-0.2879	0.0318	-0.4841	0.1726	-0.3925	0.0905	-0.3127	0.0381	2.0731	2.0365	0.0098
910.0385	-0.1806	0.0816	2.6217	2.4479	-0.1438	0.1048	-0.2873	0.0318	-0.4836	0.1728	-0.3920	0.0906	-0.3120	0.0381	-0.3301	0.0702	0.0076
911.5159	-0.7545	0.0937	-0.6467	0.3884	-0.5979	0.3944	1.7806	0.7331	0.1989	1.2414	-0.0012	0.9627	-0.7481	0.3053	0.6104	1.1002	0.0096
911.8218	-0.4283	0.1531	0.2962	0.8912	-0.4109	0.4027	-0.7361	0.0442	-0.3698	0.3847	1.7349	0.7041	-0.4008	0.4935	-0.1018	0.1703	0.0004
912.5696	-0.1794	0.0816	2.7274	2.5823	-0.1424	0.1049	-0.2862	0.0318	-0.4826	0.1731	-0.3909	0.0909	-0.3108	0.0382	-0.3289	0.0704	0.0089
922.5841	-0.3873	0.0851	-0.4420	0.0443	2.8905	1.7905	-0.3130	0.2829	-0.6490	0.1230	-0.5719	0.0509	0.8527	1.2332	-0.5290	0.0529	0.0005
925.4217	-0.4376	0.6628	-0.9675	0.4065	-0.3320	0.6009	1.1622	0.7679	0.2293	1.1305	-0.2261	0.9482	1.2360	0.5080	-0.9003	0.5299	0.0099
926.0136	-0.3014	0.0835	0.4045	1.3180	-0.2806	0.0930	-0.4059	0.0323	-0.5802	0.1437	-0.4972	0.0674	-0.4419	0.0316	2.4231	1.5367	0.0016
927.3377	-0.6599	0.1530	0.0333	0.7825	0.7518	0.8826	-0.9894	0.0591	0.8388	0.4184	-0.9334	0.1879	0.8971	0.7105	-0.0761	0.9966	0.0046
930.0736	-0.4846	0.0871	-0.0135	0.9361	-0.4882	0.0761	-0.5857	0.0373	-0.7268	0.0996	2.1303	1.7251	0.1637	0.4004	0.2045	0.7029	0.0061
931.4566	-0.9923	0.5764	-0.6500	0.6676	0.2025	0.5210	1.5904	1.2045	-0.4754	0.1292	-0.4766	0.4532	1.7296	0.7529	-0.6143	0.3783	0.0004
933.4460	-0.7823	0.2958	-0.6441	0.3726	1.5344	0.0872	-1.0047	0.0601	-0.8355	0.4060	0.3924	0.7034	1.8852	0.4838	-0.3240	0.1588	0.0000
937.4430	-0.0597	0.3675	-0.1461	0.4129	-0.3655	0.3664	-0.6265	0.0389	-0.1948	1.0332	-0.6928	0.0242	2.7437	1.4254	-0.0701	0.4988	0.0004
937.4800	-0.5557	0.6592	-1.1109	0.1369	0.5628	0.5151	2.1041	1.5704	0.1366	0.6585	-0.5185	0.3861	0.1420	0.4421	-0.6291	0.5016	0.0020
944.4604	-0.3643	0.4010	-0.7162	0.2798	-0.4974	0.2627	0.1678	0.9446	0.6866	0.7337	-0.4372	0.7112	1.3534	0.7384	-0.5928	0.5839	0.0092
946.8817	-0.1246	0.4398	-0.2216	0.7942	-0.6512	0.0645	-0.4852	0.3781	-0.0556	0.4049	-0.3770	0.3637	-0.2601	0.4765	2.1584	1.2751	0.0013
959.8997	-0.2421	0.4268	-0.4244	0.3574	-0.5503	0.0715	-0.6396	0.0395	0.1752	0.7843	1.5884	0.5125	-0.5317	0.2978	-0.0512	0.2782	0.0001
961.4392	-0.1525	0.2943	-0.8260	0.5465	0.3150	0.2861	-0.8929	0.2735	0.2190	0.7642	-0.7512	0.2429	2.3851	0.0869	-0.0463	1.1764	0.0001

961.4812	**-0.5837**	0.2771	**-0.8649**	0.0708	**-0.6662**	0.1359	**1.5638**	0.7478	**-0.2294**	0.4772	**0.3832**	1.1764	**0.3653**	0.8116	**-0.4513**	0.1674	0.0031
964.1459	**-0.6757**	0.4878	**-0.0910**	0.7947	**-0.8385**	0.3137	**-0.3473**	0.6269	**0.0939**	0.5426	**1.5695**	0.6860	**-1.0253**	0.2054	**0.8956**	0.3584	0.0002
967.1177	**-0.5581**	0.2374	**0.2833**	0.3486	**-0.8763**	0.0913	**-0.5633**	0.3017	**0.6454**	0.9231	**0.8563**	0.5785	**-0.4571**	0.5030	**-0.0056**	0.5835	0.0053
967.6324	**-0.5517**	0.9471	**0.3162**	1.4199	**1.1813**	0.3960	**1.9836**	0.7264	**-0.5117**	1.1358	**-0.5347**	0.1640	**-0.6368**	0.4611	**-0.6897**	0.1979	0.0048
970.6230	**2.2244**	0.9131	**0.1447**	0.8076	**-0.3520**	0.8135	**0.2246**	0.2909	**-0.1336**	0.8082	**-0.7493**	0.2290	**-0.7262**	0.3455	**-0.6687**	0.2430	0.0005
970.8519	**-0.2761**	0.1467	**-0.4199**	0.2524	**-0.3167**	0.2772	**-0.2339**	0.6250	**1.4805**	0.5967	**-0.4018**	0.2651	**-0.0164**	0.6771	**-0.3343**	0.1082	0.0008
974.8276	**-0.3103**	0.0836	**-0.3577**	0.0428	**-0.2907**	0.0921	**-0.4146**	0.0324	**2.0978**	1.5519	**-0.5049**	0.0657	**-0.4514**	0.0312	**-0.0110**	0.4115	0.0006
977.4422	**-0.5977**	0.1671	**-0.8365**	0.0530	**-0.7863**	0.0566	**0.5984**	1.6777	**-0.5726**	0.6513	**-0.1504**	0.6731	**1.0399**	0.6505	**1.6555**	0.5234	0.0045
980.6964	**-0.4112**	0.0855	**-0.0580**	0.6677	**-0.4051**	0.0827	**-0.3948**	0.2388	**0.5111**	1.0616	**-0.5928**	0.0463	**-0.4528**	0.1978	**1.2855**	0.6713	0.0050
986.3792	**-0.4352**	0.0860	**0.0853**	0.4932	**-0.3471**	0.1149	**-0.5372**	0.0355	**1.8023**	1.1078	**-0.2116**	0.3288	**-0.5130**	0.1014	**-0.3381**	0.4376	0.0003
987.9033	**-0.3970**	0.0928	**-0.3708**	0.3761	**-0.2887**	0.3538	**-0.6019**	0.0379	**1.6214**	0.5101	**-0.1771**	0.5060	**0.1545**	0.6936	**-0.4516**	0.3517	0.0002
989.5447	**-0.8128**	0.0671	**-0.5543**	0.4008	**-0.6859**	0.3825	**1.1866**	0.6492	**0.5446**	0.6520	**0.1176**	0.5805	**0.3186**	0.4544	**-0.7894**	0.4020	0.0006
992.4180	**-0.7204**	0.0928	**-0.6562**	0.2669	**-0.1543**	0.5939	**0.1073**	0.4135	**0.3006**	0.0661	**-0.6669**	0.3511	**2.5485**	0.9418	**-0.6775**	0.3279	0.0000
999.1421	**-0.5110**	0.2936	**0.9076**	0.8027	**-0.9155**	0.0513	**-0.4656**	0.5452	**1.1905**	0.1488	**0.2861**	0.6665	**-0.3455**	0.6397	**-0.7027**	0.4742	0.0008
1003.8723	**-0.4685**	0.0867	**-0.5309**	0.0460	**-0.2549**	0.3503	**0.1004**	1.1593	**1.3835**	0.4976	**-0.6427**	0.0352	**-0.1000**	0.5967	**-0.0104**	0.4917	0.0079
1007.2114	**-0.2112**	0.7447	**2.2556**	0.2583	**-0.1833**	0.9769	**0.0590**	0.5158	**-0.4009**	0.3664	**0.6620**	1.5959	**-0.7994**	0.4743	**-1.0018**	0.2342	0.0028
1016.7776	**-0.5813**	0.4300	**0.0202**	0.9285	**-0.0847**	1.0197	**-0.9787**	0.0585	**0.7910**	0.8296	**0.3094**	0.6923	**-1.0691**	0.0061	**1.1324**	0.5179	0.0075
1017.4628	**-0.2741**	0.4166	**-0.5833**	0.6182	**-0.2559**	0.2410	**0.1845**	0.7940	**0.5972**	0.8777	**-0.4027**	0.4988	**1.2073**	0.0670	**-0.8815**	0.3385	0.0051
1018.3235	**0.4542**	0.7650	**-0.0878**	0.8725	**-0.3269**	0.0479	**-0.4143**	0.5160	**1.3084**	0.3412	**-0.8804**	0.2313	**0.0022**	0.6297	**-0.4686**	0.5055	0.0063
1023.1869	**-0.1152**	0.1486	**-0.4103**	0.3487	**-0.4548**	0.0423	**-0.1515**	0.0770	**1.0010**	1.0049	**-0.6225**	0.1517	**0.4312**	0.2511	**-0.2156**	0.2320	0.0029
1023.6785	**0.2042**	0.9456	**-0.4458**	0.0443	**-0.3819**	0.0845	**-0.4936**	0.0341	**0.5735**	1.0807	**-0.5749**	0.0502	**1.3223**	0.6684	**-0.5323**	0.0527	0.0061
1026.4577	**-0.4569**	0.5039	**-0.3881**	0.1257	**-0.7973**	0.1396	**0.6594**	0.9165	**0.9107**	0.5796	**-0.4948**	0.6201	**0.5779**	0.8119	**-0.6049**	0.3773	0.0094
1026.5231	**-0.4998**	0.5519	**-0.5919**	0.6724	**0.6325**	0.9120	**-0.5053**	0.6963	**-0.6526**	0.3537	**0.3653**	0.9644	**1.9295**	0.8692	**-0.4730**	0.6522	0.0056
1026.9612	**-0.4332**	0.0860	**-0.4923**	0.0452	**-0.0127**	0.6982	**-0.3301**	0.3884	**1.1636**	0.6349	**0.1094**	0.6742	**-0.5836**	0.0246	**0.0519**	0.6116	0.0099
1029.6801	**-0.3137**	0.0837	**-0.3615**	0.0429	**-0.2946**	0.0918	**-0.4180**	0.0325	**-0.5901**	0.1407	**-0.5079**	0.0650	**0.3816**	0.8829	**2.2711**	1.3548	0.0003
1029.9818	**-0.4684**	0.0867	**1.8061**	1.0193	**-0.4698**	0.0776	**-0.5698**	0.0366	**0.2967**	0.9703	**0.0308**	1.1834	**-0.1464**	0.4531	**-0.6071**	0.0468	0.0076
1035.5774	**-0.6630**	0.2431	**-0.6226**	0.1855	**-0.1220**	0.6483	**0.0704**	0.4557	**1.2341**	0.5642	**-0.4624**	0.4998	**-0.0684**	0.2777	**0.0621**	0.6847	0.0046
1040.0544	**-0.7381**	0.0932	**-0.8259**	0.0528	**0.5105**	0.3243	**0.2408**	1.0985	**-0.9297**	0.0387	**1.1002**	0.1920	**-0.6075**	0.5277	**1.1076**	1.0905	0.0011
1040.9484	**-0.4136**	0.0856	**-0.4708**	0.0448	**0.8196**	1.1187	**-0.0423**	0.7941	**-0.6700**	0.1167	**1.8974**	0.9723	**-0.5067**	0.1133	**-0.5543**	0.0509	0.0008
1041.8123	**-0.5953**	0.0896	**-0.5587**	0.2336	**0.3125**	0.8813	**-0.2659**	0.2100	**1.0919**	0.6610	**0.0986**	0.4282	**-0.2671**	0.4327	**-0.4432**	0.4584	0.0096
1043.1429	**-0.8474**	0.0963	**0.3672**	0.7124	**-0.3795**	0.5652	**-0.5915**	0.3294	**0.4201**	0.1456	**1.2484**	0.7669	**-0.5168**	0.8837	**-0.1903**	0.5684	0.0073
1054.3799	**-0.1800**	0.0816	**2.7316**	2.5713	**-0.1431**	0.1049	**-0.2867**	0.0318	**-0.4831**	0.1729	**-0.3915**	0.0907	**-0.3114**	0.0382	**-0.3295**	0.0703	0.0085
1055.7027	**-0.4585**	0.0865	**-0.5199**	0.0457	**-0.4586**	0.0784	**2.4335**	0.7927	**-0.2946**	0.7624	**-0.1692**	0.8233	**-0.1751**	0.7314	**-0.1969**	0.7205	0.0003
1055.7695	**-0.4788**	0.2543	**0.0331**	0.8126	**-0.2501**	0.4606	**-0.3064**	0.3768	**1.1896**	0.8740	**0.5031**	0.6666	**-0.7171**	0.2333	**-0.5801**	0.2510	0.0095
1055.9851	**-0.5174**	0.0878	**0.1075**	1.1546	**2.4015**	0.9254	**0.2237**	1.4242	**-0.3536**	0.6008	**-0.4972**	0.3000	**-0.4705**	0.3325	**-0.6543**	0.0433	0.0034
1062.5117	**-0.8123**	0.5789	**-0.5279**	0.6607	**-0.2032**	0.1548	**0.8410**	0.8925	**0.6244**	0.5463	**-0.2634**	0.2309	**1.3188**	1.4716	**-1.1279**	0.3995	0.0089
1062.8397	**-0.3184**	0.0838	**-0.3666**	0.0430	**-0.2998**	0.0913	**-0.4225**	0.0325	**-0.5938**	0.1396	**-0.1458**	0.5700	**-0.4601**	0.0307	**2.5722**	0.5140	0.0000
1065.1154	**2.2898**	0.7872	**0.1725**	0.8678	**-0.7187**	0.0603	**-0.1963**	0.4873	**-0.8896**	0.0507	**-0.2119**	1.0763	**0.5401**	1.3031	**-0.4877**	0.5752	0.0033
1068.5185	**-0.9616**	0.0997	**1.1932**	1.5092	**0.3941**	0.3600	**-1.0540**	0.0633	**-0.8846**	0.3869	**0.5296**	0.3879	**1.6422**	0.5471	**-0.3349**	0.6796	0.0005
1068.5915	**-0.4308**	0.5466	**-0.5423**	0.2542	**-0.8097**	0.1017	**0.8580**	0.5234	**1.2713**	0.2865	**-0.3653**	1.1217	**0.1031**	0.4828	**-0.7161**	0.2655	0.0013
1070.4872	**-0.7603**	0.3439	**-0.6434**	0.3769	**-0.8042**	0.4480	**1.1793**	0.9729	**0.9540**	0.7500	**0.0907**	1.1376	**0.3064**	0.2854	**-0.7480**	0.4333	0.0064
1074.5351	**-0.2353**	0.1650	**-1.0255**	0.1003	**-0.0068**	0.6661	**0.1607**	0.2318	**1.2511**	0.7739	**-0.5624**	0.2448	**0.7642**	0.2788	**-0.7632**	0.5295	0.0001
1077.8730	**-0.4866**	0.0871	**-0.5507**	0.0464	**-0.4905**	0.0760	**-0.3545**	0.4045	**0.5885**	0.9190	**-0.0457**	0.4846	**-0.5235**	0.1818	**1.2451**	0.6002	0.0012
1086.2541	**-0.5037**	0.4520	**-0.3173**	0.3596	**-0.7747**	0.0572	**-0.4784**	0.2963	**0.1996**	0.2561	**2.0029**	0.9932	**-0.2363**	0.9145	**-0.2972**	0.1472	0.0003
1095.0362	**-0.4194**	0.0857	**0.2791**	1.3209	**-0.4143**	0.0819	**-0.5217**	0.0350	**-0.6747**	0.1153	**-0.5999**	0.0447	**0.4521**	0.8710	**2.2197**	1.1558	0.0016
1095.8095	**-0.3430**	0.3090	**-0.1964**	0.3543	**-0.4942**	0.0757	**-0.3155**	0.2435	**1.0741**	0.7905	**0.4007**	0.4442	**-0.4390**	0.2263	**-0.4455**	0.1392	0.0012
1099.8122	**-0.2326**	0.5045	**0.0124**	0.3412	**-0.2905**	0.4910	**-0.0336**	0.5350	**1.4333**	0.3283	**-0.5136**	0.3700	**-0.7363**	0.0172	**-0.2759**	0.2694	0.0002
1105.9608	**1.2012**	1.3390	**-0.0428**	0.1342	**-0.7419**	0.0590	**-0.8056**	0.0480	**-0.7487**	0.2995	**-0.1163**	0.6536	**-0.5189**	0.6144	**1.9069**	1.0833	0.0012
1107.2115	**-0.7478**	0.0935	**-0.8365**	0.0530	**0.3378**	0.9274	**-0.4831**	0.6606	**-0.1908**	1.3114	**0.0818**	0.1630	**-0.1575**	0.7776	**2.1279**	0.8612	0.0044
1108.1670	**-0.4709**	0.0868	**-0.5335**	0.0460	**-0.3513**	0.1744	**0.3588**	1.5821	**-0.7159**	0.1029	**1.6774**	0.3517	**-0.6241**	0.0226	**0.4747**	0.3409	0.0018
1113.6372	**-0.2428**	0.0825	**-0.2839**	0.0418	**-0.2143**	0.0987	**2.3110**	1.7132	**-0.5334**	0.1578	**-0.1107**	0.6250	**-0.3789**	0.0348	**-0.3900**	0.0649	0.0012
1123.4284	**0.0119**	0.7869	**-0.7498**	0.1585	**-0.5877**	0.4515	**-0.3742**	0.4938	**1.2950**	0.6227	**0.3473**	0.5676	**-0.4512**	0.2589	**-0.1091**	0.6873	0.0066
1124.8759	**-0.2882**	0.3670	**-0.4728**	0.2140	**-0.1647**	0.6556	**-0.4934**	0.2380	**1.7235**	1.3922	**-0.5132**	0.3576	**-0.4614**	0.4130	**0.3386**	0.6068	0.0073
1125.1921	**-0.5371**	0.0882	**0.3077**	0.6011	**-0.5477**	0.0717	**-0.6373**	0.0394	**0.8516**	1.3657	**-0.4278**	0.4535	**1.0243**	0.2978	**-0.4818**	0.3596	0.0084
1125.6033	**-0.5874**	0.0894	**-0.1814**	0.7863	**-0.4055**	0.3169	**-0.0038**	0.5864	**-0.5359**	0.5098	**0.6092**	1.2396	**1.8752**	0.7403	**-0.7216**	0.0389	0.0029
1127.5316	**-0.4626**	0.0866	**-0.5244**	0.0458	**-0.4633**	0.0781	**2.3974**	0.5257	**-0.1946**	0.9396	**-0.4268**	0.3287	**-0.6152**	0.0231	**0.3263**	1.0196	0.0001
1137.5147	**-0.8821**	0.3722	**-0.7221**	0.4286	**-0.3347**	0.7725	**1.4762**	0.4919	**0.1738**	1.2603	**0.8759**	0.3158	**-0.5910**	0.2046	**-0.3783**	0.8546	0.0055

1144.9082	**-0.2763**	0.0831	**-0.3205**	0.0423	**-0.2522**	0.0954	**-0.3813**	0.0320	**-0.5602**	0.1498	**-0.4753**	0.0722	**2.9976**	1.3261	**-0.2095**	0.3072	0.0000
1147.0861	**-0.4459**	0.0862	**0.0890**	1.0648	**-0.4443**	0.0795	**-0.5477**	0.0358	**1.2870**	0.2781	**-0.6230**	0.0396	**-0.1503**	0.4145	**0.3061**	0.8994	0.0068
1160.2816	**-0.4454**	0.0862	**-0.5056**	0.0455	**-0.4437**	0.0796	**1.3607**	1.7770	**-0.4950**	0.4044	**-0.3529**	0.4273	**-0.5966**	0.0240	**1.4302**	0.7881	0.0070
1161.8915	**-0.0792**	0.6178	**-0.4684**	0.0447	**-0.4052**	0.0827	**-0.5139**	0.0347	**-0.6683**	0.1172	**1.8022**	0.4769	**0.7853**	1.6168	**-0.5522**	0.0510	0.0022
1167.5126	**-0.0791**	0.4616	**-1.1071**	0.0605	**1.5113**	0.8417	**-0.2468**	0.7255	**0.4959**	1.0391	**0.2623**	0.3023	**-0.0063**	1.2316	**-1.0598**	0.0709	0.0088
1170.9997	**-0.5899**	0.0895	**-0.6637**	0.0488	**0.4534**	1.0103	**-0.2462**	0.7287	**1.7592**	0.8760	**-0.2985**	0.7834	**-0.1866**	0.5633	**-0.3640**	0.6348	0.0090
1171.2235	**-0.4483**	0.4072	**-0.1306**	1.0017	**-0.0643**	1.0340	**-0.7530**	0.0451	**-0.6116**	0.4648	**1.9328**	0.4613	**-0.1625**	0.8368	**0.2174**	0.8764	0.0090
1186.1558	**2.2084**	0.8041	**-0.7753**	0.0515	**-0.4643**	0.4139	**-0.7892**	0.0471	**0.8810**	1.3368	**-0.5907**	0.2200	**-0.5070**	0.3548	**0.0033**	0.2786	0.0001
1190.0333	**2.4727**	1.6430	**-0.3619**	0.0429	**0.7383**	0.9758	**-0.4184**	0.0325	**-0.5904**	0.1406	**-0.5082**	0.0649	**-0.4556**	0.0310	**-0.4586**	0.0589	0.0005

Appendix 5

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PCR Primers

Primer name	Sequence	Official symbol	Full name	Aliases / Notes
8474	5'-ATAATAACGCTGCGGACATCTACATTTT-3'		for GABI-Kat lines	
At1g13220 qPCR Fwd	5’-TCCTCCGCCTCCGATTG-3'	CRWN2, LINC2	Crowded nuclei2, Little nuclei2	nuclear matrix constituent protein, housekeeping gene for qPCR analysis
At1g13220 qPCR Rev	5’-TATCATCAGTGTGACCTCGAGAAA-3'	CRWN2, LINC2	Crowded nuclei2, Little nuclei2	nuclear matrix constituent protein, housekeeping gene for qPCR analysis
Bar Fwd	5’-GTCTGCACCATCGTCAACC-3’	BlpR	Basta resistance	bialophos/ phosphinothricin resistance, bar
Bar Rev	5’-GAAGTCCAGCTGCAAGAAAC-3’	BlpR	Basta resistance	bialophos/ phosphinothricin resistance, bar
FAH2 TOPO Fwd	5'-CACCATGGTTGCAGAACGATACACAG-3'	FAH2, At4g20870	Fatty acid hydroxylase 2	
FAH2 TOPO Rev	5'-GCTCTTCTTCGCAGCGGCTTTAATAC-3'	FAH2, At4g20870	Fatty acid hydroxylase 2	
GAPDH qPCR Fwd	5’-GCCATCCCTCAATGGAAAATT-3'	GAPC-2, At1g13440	Glyceraldehyd-3-phosphate dehydrogenase	housekeeping gene for qPCR analysis
GAPDH qPCR Rev	5’-GAGACATCAACGGTTGGAACA-3'	GAPC-2, At1g13440	Glyceraldehyd-3-phosphate dehydrogenase	housekeeping gene for qPCR analysis
GEN006	5'-CTACCCCGACCACATGAAGC-3'	GFP, YFP, CFP	Greenish fluorescent protein primer forward	
GEN007	5'-CTTCTCGTTGGGGTCTTTGC-3'	GFP, YFP, CFP	Greenish fluorescent protein primer reverse	

gentamycinR Fwd	5'-TTAGGTGGCGGTACTTGGGT-3'	aacC1	gentamycin acetyltransferase-3-I	gentamycin resistance gene, AAC(3)-I
gentamycinR Rev	5'-ACCTACTCCCAACATCAGCC-3'	aacC1	gentamycin acetyltransferase-3-I	gentamycin resistance gene, AAC(3)-I
HM1 qPCR Fwd	5'-TGGTGGGATCAGAGGTTTTGGAG-3'		At4g26870	
HM1 qPCR Rev	5'-CGTTCCTGTTTACGGAGCAACTTC-3'		At4g26870	
HM1-25C Fw:	5'- GAAGGGGTTCAAATGCTTAAGGAAG-3’			
HM1-25N Rev:	5'-GATTCGCGACTCAGCTGCTTG-3’			
HM1-50N-Rev:	5'-CAGCAGAACCTCCTTCACTAC-3’			
HM1-75C Fw:	5'- CAAGCAGCTGAGTCGTGAATC-3’			
HM1-75N-Rev:	5'-CTTCCTTAAGCATTTGAACCCCTTC-3’			
HM-50C-Fw:	5'-GTAGTGAAGGAGGTTCTGCTG-3’			
IBI1 Fwd	5'-GGATCCGAAAGCCGGGAAGTG-3'	IBI1, At4g31180	impaired in BABA-induced immunity 1	IBI1 WT-specific Fwd primer for genotyping
IBI1 qPCR Fwd	5'-GAGCGAGTGGTCATGCTTTTC-3'	IBI1, At4g31180	impaired in BABA-induced immunity 1	At4g31180
IBI1 qPCR Rev	5'-CGAGGGAAGAGGGATGTTTTC-3'	IBI1, At4g31180	impaired in BABA-induced immunity 1	At4g31180
IBI1, ibi1-1 common Rv	5'-AACCTGCTGCGTAGTTCCCGTCAG-3'			IBI1/ ibi1-1 common Rev primer for genotyping
IBI1/HM1 qPCR Rev	5'-ACAGCAGAACCTCCTTCACTAC-3'			IBI1/HM1 common Rev primer for qPCR (total Asp-RS expression)
IBI1/HM1 qPCR Fwd	5'-AGAACACCGGCTAATCAAGCCATC-3'			IBI1/HM1 common Rev primer for qPCR (total Asp-RS expression)
ibi1-1 Fwd	5'-GGATCCGAAAGCCGGGAAGTA-3'			*Ibi1-1*-specific Fwd primer for genotyping

IBI1-25C Fw:	5'- GAAGGGGTTCAAATGCTTAAGGAAG-3’			
IBI1-50C-Fw:	5'-GTAGTGAAGGAGGTTCTGCTG-3’			
IBI1-50N Rev:	5'-CAGCAGAACCTCCTTCACTAC-3’			
IBI1-75C Fw:	5'-CAAGCAGCTGAGTCGCGAATC-3’			
IBI1-75N Rev:	5'- CTTCCTTAAGCATTTGAACCCCTTC -3’			
IBI-25N Rev:	5'-GATTCACGACTCAGCTGCTTG-3’			
LBb1.3	5’-ATTTTGCCGATTTCGGAAC-3’			T-DNA-specific primer for genotyping of SALK T-DNA insertion lines
M13 FW:	5'-GTTGTAAAACGACGGCCAGTC-3’			
M13 Rev:	5'-CCAGGAAACAGCTATGACCATG-3			
MDH PCR Fwd	5'-TGGTGGACATGCTGGAATCAC-3'	MDH, pNAD-MDH	Plastidic malate dehydrogenase	
MDH TOPO Fwd new	5'-CACCATGGCAACAGCAACATCAGCTTCTC-3'	MDH, pNAD-MDH	Plastidic malate dehydrogenase	
MDH TOPO Rev new	5'-GTTAGCTGCTGCAGCAGCTGG-3'	MDH, pNAD-MDH	Plastidic malate dehydrogenase	
MIOX4 qPCR Fwd	5'-TCAATGGGCTGTTGTTGGTGAC-3'	MIOX4, At4g26260.2	myoinositol oxygenase 4	
MIOX4 qPCR Rev	5'-TCCACACCCTTCAGAGTAAATCCC-3'	MIOX4, At4g26260.2	myoinositol oxygenase 4	
p745	5’-AACGTCCGCAATGTGTTATTAAGTTGTC-3’		LB primer for WiscDsLox lines	binds T-DNA insertion in WiscDsLox
pEG201-YN Rev	5’-CGTCGCCGTCCAGCTCGACCAG-3’			Reverse primer binding to (E)GFP, YFP
PR1 qPCR Fwd	5’-GTCTCCGCCGTGAACATG-3'	PR1, At2g14610.1	Pathogenesis-related gene 1	

PR1 qPCR Rev	5’-CGTCTTCGCAGCGTAGTTGT-3'	PR1, At2g14610.1	Pathogenesis-related gene 1	
SALK_018395 (MIOX4) LP	5'-TTGACTTACCAACAACAGCCC-3'	miox4-1	MIOX4 T-DNA insertion line from SALK	SALK_018395.54.50.x, NASC N518395
SALK_018395 (MIOX4) RP	5'-CTGTTGAGAAGCCGATTTTTG-3'	miox4-1	MIOX4 T-DNA insertion line from SALK	SALK_018395.54.50.x, NASC N518395
SALK_027238 (MIOX4) LP	5'-TTTCGAGGACAAAACTCATGC-3'	SALK_027238.49.75.x	homozygous MIOX4 T-DNA insertion line	miox4, NASC N670466
SALK_027238 (MIOX4) RP	5'-AATCCTCCGTCCAATTTCAAC-3'	SALK_027238.49.75.x	homozygous MIOX4 T-DNA insertion line	miox4, NASC N670466
SAND family protein qPCR Fwd	5'-AT2G28390AACTCTATGCAGCATTTGATCCACT-3'	At2g28390		from Czechowski et al., Plant Phys, 2005
SAND family protein qPCR Rev	5'-TGATTGCATATCTTTATCGCCATC-3'	At2g28390		from Czechowski et al., Plant Phys, 2005
spectinomycinR Fwd	5'-CGCTATGTTCTCTTGCTTTTG-3'	aadA	spectinomycin resistance gene, adenylyltransferase	ANT
spectinomycinR Rev	5'-TGATTTGCTGGTTACGGTGAC-3'	aadA	spectinomycin resistance gene, adenylyltransferase	ANT
UBC qPCR Fwd	5'-CTGCGACTCAGGGAATCTTCTAA-3'	At5g25760		from Czechowski et al., Plant Phys, 2005
UBC qPCR Rev	5'-TTGTGCCATTGAATTGAACCC-3'	At5g25760		from Czechowski et al., Plant Phys, 2005
voz1-2 LP	5'-CTCACTCTCCAGAACCGTGAG -3'			voz1-2 = WISCDSLOX489-492O10
voz1-2 RP	5'-AGAACCGTGTTGATGATTTGC -3'			voz1-2 = WISCDSLOX489-492O10
VOZ2 PCR Fwd	5'-CCCAGTGTGTGAAGGAGCTG-3'		Vascular plant one zinc finger protein 2	
VOZ2 TOPO Fwd	5'-CACCATGTCAAACCACCCG-3'	VOZ2, At2g42400	Vascular plant one zinc finger protein 2	
VOZ2 TOPO Rev	5'-CTCCTTACGACCTTTGGTTGG-3'	VOZ2, At2g42400	Vascular plant one zinc finger protein 2	
voz2-2 LP	5'-CTCTTGTCGTCTGCTGTCTCC -3'			voz2-2 = SALK_115813

voz2-2 RP	5'-AAGTGTGCACTATGGGATTGC -3'			voz2-2 = SALK_115813

Appendix 6

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Transcription factor binding motif enrichment analysis

The promoter regions (500bp upstream from the transcription start site; [500 +0]) of the 139 genes whose expression values on the microarray differed significantly between Col-0 and *ibi1-1* under conditions of BABA-primed defence against *H. arabidopsidis* (Chapter 7, Fig. 7.3; Appendix 2) were analysed using the Pscan web interface (http://159.149.160.88/pscan/) to check for transcription factor binding motif enrichment.

Settings

Organism:	Arabidopsis thaliana
Region:	500 +0
Descriptors:	Jaspar 2016

Top 10 hits:

TF name	P-value
ABI5	7.02E-19
bZIP68	2.55E-18
ABF2	5.11E-18
PIF4	1.68E-17
ABF3	2.42E-17
HY5	2.43E-17
PIF3	1.49E-15
SPT	2.86E-14
BEE2	7.45E-14
MYC3	1.14E-14

Red = directly interacts with phytochrome B.

Blue = indirectly regulated by phyB or plays a role in phyB signalling.

TF_NAME	MATRIX_ID	Z_SCORE	P_VALUE	SAMPLE_ AVERAGE	BACKGROUND_ AVERAGE	SAMPLE_ DEVSTD
ABI5	MA0931.1	8.76597	7.02E-19	0.864705	0.813368	0.0896129
OJ1058_F05.8	MA1033.1	8.6367	2.24E-18	0.844965	0.786974	0.100645
bZIP68	MA0968.1	8.6261	2.55E-18	0.915562	0.88204	0.0536701
ABF2	MA0941.1	8.54572	5.11E-18	0.861229	0.819685	0.068666
PIF4	MA0561.1	8.40771	1.68E-17	0.872276	0.823336	0.0821057
ABF3	MA0930.1	8.36669	2.42E-17	0.804965	0.746925	0.0949257
HY5	MA0551.1	8.35442	2.43E-17	0.766523	0.722727	0.0877289
PIF3	MA0560.1	7.8664	1.49E-15	0.849767	0.806622	0.0799786
SPT	MA1061.1	7.49555	2.86E-14	0.872043	0.835065	0.0633826
BEE2	MA0956.1	7.36986	7.45E-14	0.899895	0.870601	0.0507088
MYC3	MA0568.1	7.312	1.14E-13	0.891992	0.857522	0.0620884
PHYPADRAFT_143875	MA0988.1	7.28183	1.42E-13	0.868989	0.834487	0.0630143
ABF1	MA0570.1	7.21025	2.28E-13	0.748425	0.718352	0.066124
BIM3	MA0966.1	7.18467	2.97E-13	0.864008	0.83239	0.0552365
BIM2	MA0965.1	7.16767	3.36E-13	0.876317	0.843872	0.0574373
PHYPADRAFT_72483	MA1011.1	7.09119	5.81E-13	0.892445	0.864261	0.0522288
BZR2	MA0549.1	6.97374	1.32E-12	0.842134	0.805828	0.0750716
BZR1	MA0550.1	6.96241	1.41E-12	0.853588	0.823094	0.0659508
BHLH13	MA0958.1	6.93794	1.74E-12	0.832201	0.794202	0.0732112
PIF1	MA0552.1	6.87773	2.65E-12	0.822558	0.785628	0.0737641
BHLH3	MA0957.1	6.87359	2.73E-12	0.8206	0.782535	0.075359
MYC4	MA0569.1	6.79915	4.65E-12	0.850022	0.812621	0.0722845
MYC2	MA0566.1	6.68352	1.05E-11	0.859142	0.828173	0.0583117
BIM1	MA0964.1	6.65307	1.29E-11	0.895211	0.867809	0.0513407
UNE10	MA1074.1	6.59973	1.84E-11	0.834303	0.788055	0.091334
PIF5	MA0562.1	6.30096	1.33E-10	0.870354	0.836549	0.0704058
EmBP-1	MA0128.1	6.21891	2.24E-10	0.868517	0.835487	0.0725447
PHYPADRAFT_48267	MA1021.1	6.12785	4.03E-10	0.832154	0.792658	0.0845661
HBI1	MA1025.1	5.50829	1.68E-08	0.799153	0.768854	0.0697159
BHLH104	MA0960.1	5.50083	1.74E-08	0.715314	0.685181	0.0732267
BHLH34	MA0962.1	5.33994	4.33E-08	0.857686	0.827225	0.0720891
CMTA2	MA0969.1	4.85571	5.69E-07	0.874593	0.850187	0.0602081
CMTA3	MA0970.1	4.67288	1.41E-06	0.890575	0.867395	0.0592435
TGA5	MA1047.1	4.37942	5.62E-06	0.874006	0.853363	0.0646779
NAC025	MA0935.1	4.23418	1.09E-05	0.876709	0.858308	0.0567372
NAC055	MA0937.1	3.98348	3.24E-05	0.866186	0.84988	0.0550146
AIB	MA0959.1	3.94653	3.82E-05	0.791889	0.772788	0.0588788
bZIP911	MA0097.1	3.86353	5.37E-05	0.744202	0.726388	0.0588911
bZIP910	MA0096.1	3.60653	0.000149441	0.861299	0.840452	0.0771713
HAT5	MA0008.1	3.57241	0.000172142	0.934253	0.919566	0.0469677
TGA2	MA1068.1	3.31132	0.000448711	0.845021	0.829257	0.0656236
TGA6	MA1069.1	3.04722	0.00111898	0.802428	0.787027	0.0723747

DOF2.5	MA0977.1	2.92517	0.00168627	0.937585	0.927173	0.0427728
TGA1	MA0588.1	2.8872	0.00190487	0.858503	0.846787	0.0486995
PHYPADRAFT_140773	MA0987.1	2.88692	0.00191898	0.977661	0.973728	0.0134219
SPL3	MA0577.1	2.87955	0.00194544	0.854239	0.844409	0.0442449
CDF3	MA0974.1	2.87798	0.00196273	0.934356	0.926503	0.0322878
ATHB-5	MA0110.1	2.86161	0.00206196	0.887105	0.874593	0.0552048
TGA7	MA1070.1	2.85844	0.00207681	0.859122	0.84959	0.0447937
SPL11	MA1056.1	2.7766	0.002687	0.777702	0.76361	0.065986
DOF5.3	MA1071.1	2.76551	0.00281706	0.99427	0.99168	0.00768388
BHLH78	MA0963.1	2.75932	0.00284525	0.699468	0.681251	0.0776018
DOF1.8	MA0981.1	2.75662	0.00288133	0.974198	0.969553	0.0172068
PHYPADRAFT_153324	MA0989.1	2.73606	0.0030624	0.973893	0.968806	0.0203332
CDF2	MA0973.1	2.69155	0.0034964	0.937565	0.928814	0.0382074
ATHB-9	MA0573.1	2.62482	0.00426001	0.750848	0.742187	0.0402377
SPL4	MA1058.1	2.62231	0.00429003	0.895534	0.88637	0.0430795
CCA1	MA0972.1	2.59016	0.0046979	0.89255	0.880675	0.0618942
ATHB-5	MA0110.2	2.55686	0.00519858	0.83065	0.821513	0.0424487
SPL5	MA1059.1	2.50202	0.00607043	0.861649	0.850853	0.0543001
PHYPADRAFT_38837	MA1022.1	2.48903	0.00632487	0.960998	0.956098	0.021378
KAN1	MA1027.1	2.47603	0.00655199	0.944591	0.936389	0.0378548
BZIP60	MA0967.1	2.4678	0.00665865	0.818999	0.80791	0.0630942
SPL12	MA1057.1	2.38819	0.00834438	0.876402	0.865775	0.0538483
ATHB-51	MA0952.1	2.36734	0.00884523	0.917288	0.906836	0.0504794
NAC080	MA0939.1	2.34338	0.00942457	0.856591	0.847086	0.0486889
37865	MA0563.1	2.27054	0.0114532	0.896494	0.889461	0.0352126
F3A4.140	MA0575.1	2.26821	0.0114583	0.804	0.795213	0.0537219
ERF018	MA1048.1	2.26331	0.0116636	0.82308	0.811564	0.0597291
SPL7	MA1060.1	2.25488	0.0118797	0.794331	0.783253	0.0657719
EDT1	MA0990.1	2.2544	0.0119425	0.902844	0.89583	0.0359685
NAC083	MA1043.1	2.23347	0.0125834	0.840196	0.830517	0.0545376
ARR11	MA0946.1	2.21528	0.0132219	0.87107	0.862381	0.0446609
ATHB-15	MA1026.1	2.19078	0.0140585	0.932959	0.923543	0.0520594
HAT5	MA0008.2	2.0824	0.0184433	0.839128	0.831065	0.0468567
PHYPADRAFT_64121	MA1010.1	2.0627	0.0193724	0.819049	0.810858	0.0459512
TCP16	MA0587.1	2.02312	0.0213002	0.75368	0.742617	0.0661514
DREB1E	MA0978.1	2.00946	0.0219625	0.807473	0.79794	0.0628621
PBF	MA0064.1	1.98807	0.0233304	0.99187	0.987986	0.012742
ARALYDRAFT_897773	MA1054.1	1.96752	0.0243096	0.790438	0.781753	0.0535788
TGA1A	MA0129.1	1.95602	0.0248978	0.91012	0.902896	0.0512147
WRKY45	MA1087.1	1.91649	0.027346	0.841868	0.832629	0.0621346
ANT	MA0571.1	1.88282	0.0295808	0.738624	0.733141	0.035217
RAV1	MA0582.1	1.8745	0.0301512	0.870978	0.863967	0.0447824
AHL25	MA0934.1	1.87164	0.0304228	0.946902	0.940512	0.0349162
KAN4	MA1028.1	1.86714	0.0306624	0.835332	0.826859	0.0539502

GATA8	MA1017.1	1.85904	0.0313161	0.922146	0.916224	0.0314468
WRKY38	MA1084.1	1.85434	0.0314926	0.835439	0.82658	0.063476
ARR18	MA0948.1	1.84949	0.031933	0.855857	0.849832	0.0370502
DOF5.6	MA0983.1	1.8354	0.0329857	0.978916	0.975789	0.0183171
POPTR_0002s00440g	MA0955.1	1.81786	0.034189	0.870881	0.862903	0.0570302
FHY3	MA0557.1	1.81236	0.034703	0.762056	0.753053	0.055324
WRKY12	MA1075.1	1.80932	0.0348135	0.837359	0.829258	0.0605238
ATHB-6	MA0953.1	1.8111	0.0348141	0.942377	0.936885	0.0328128
DOF2.4	MA0982.1	1.76699	0.0383551	0.97769	0.974335	0.0204653
TCP19	MA1063.1	1.764	0.0384657	0.801653	0.793833	0.0592658
ARALYDRAFT_496250	MA1096.1	1.75777	0.0390722	0.736073	0.725024	0.0749185
squamosa	MA0082.1	1.72987	0.0415048	0.898072	0.893362	0.0319444
DRE1C	MA0985.1	1.7285	0.041537	0.792381	0.783162	0.0708523
BHLH112	MA0961.1	1.71452	0.042813	0.834873	0.825316	0.0726661
GATA10	MA1013.1	1.68755	0.0454572	0.84217	0.834135	0.0518832
TCP5	MA1067.1	1.67461	0.046625	0.76397	0.754722	0.0683802
DREB1A	MA0971.1	1.6704	0.0470273	0.815311	0.806877	0.0633264

Appendix 7

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GO term enrichment analysis

Genes associated with significantly enriched GO terms in clusters identified in Chapter 7, Fig. 7.3:

response to water deprivation	response to ABA	response to desiccation	leaf senescence
CBF1	ABF3	LTI78	COR15A
DREB2A	COR15A	P5CS1	COR15B
DREB2B	COR15B	RD22	LTI78
GolS2	ERD10		NRT1.5
HAI1	LTI78		
NAC019	P5CS1		
NCED3	RD22		
ZF2			

transcription, DNA template	ABA-activated signalling	neg. regulation of ABA signalling	response to water deprivation
ABF2	ABI2	ABI2	ABF2
AT4G28140	PP2CA	HAI2	ABI2
HB-7	ABF2	PP2CA	EDL3
HFR1	LTI65		LTI65
HSFC1	EDL3		PP2CA
NAC032	AT4G28140		
NF-YB2	HFR1		
PIL1	HFR1		
PIL2	AFP1		