# Quantitative genetic analysis of auxin-driven growth in *Arabidopsis thaliana*

Jennifer Wright

Master of Science (by Research)

University of York

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# Abstract

Growth and development in plants displays genetic complexity differently controlled by discrete environmental stimuli. The *Arabidopsis thaliana* hypocotyl is a useful model system for studying growth due to its simplicity. The hypocotyl elongates in response to a wide range of stimuli, including the phytohormone auxin. Auxin is a major regulator of growth and development with a role in every stage of a plant's life cycle. Previous work has shown that exogenous auxin can increase hypocotyl length in some cases, but decrease it in others. In this dissertation, I wanted to investigate the growth response to auxin in two ways. I added exogenous auxin, and increased auxin levels naturally by growing plants at warm temperatures. I also investigated the effect of the mutation *hsp90.2-3* on hypocotyl growth. This mutation increases hypocotyl length and reveals cryptic genetic variation within a plant. I studied the effect of auxin and the mutation on growth using a quantitative genetic approach.

I investigated growth using QTL analysis. This technique uses naturally occurring variation in a population to detect regions of the genome which influence a certain trait. It is useful for studying a trait like growth, which is controlled by many genes of small effect. I detected many QTL using this technique, including one which has a role in controlling growth and variation under several tested conditions. I also detected QTL that work through epistatic interactions. Some QTL control a change in hypocotyl length in response to a specific stimulus. Overall, I have studied the genetic basis of growth in several conditions and examined the change in hypocotyl length and variation in hypocotyl length due to changes in temperature and exogenous auxin. I envisage that the genetic architecture I reported will aid future studies into the way warmth and auxin affect growth and variation in plants.

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## Author's declaration:

I declare that this thesis is a presentation of original work and I am the sole author. This work has not previously been presented for an award at this, or any other, University. All sources are acknowledged as references.

# Chapter 1: Introduction

## 1.1 Overview

In this dissertation, I aim to investigate the genetics controlling the growth of *Arabidopsis thaliana* with special regard to the role of warmth and the phytohormone auxin. I will first use QTL mapping to determine which regions of the genome are important in this process. I will then compare my results from different environmental conditions and populations to find similarities and differences. I hope to better understand how differences in genotype and environment can result in similar phenotypes.

## 1.2 Growth of *Arabidopsis thaliana*

Research into why plants grow the way they do has been going on for hundreds of years (Darwin and Darwin 1880, Leonelli et al 2012). The process is extremely complex, involving many different genes and environmental factors (Scheel and Wasternack 2002, Davies 2004). This research has increased in scope and detail during the 20$^{th}$ century due to new genetic and molecular approaches (Leonelli et al 2012). In recent years, an expanding world population and a changing climate has made this research more important (Grieson et al 2011). The hope is that by understanding plant growth in greater detail we can improve crop yields and protect the forces that lead to biodiversity. I used quantitative genetic analysis to build an understanding of the genetic regions involved in growth that is driven by the phytohormone auxin.

*Arabidopsis thaliana* is the model species for plant research because it is quick and easy to grow, is available all over the world and has a relatively simple genetic structure. The growth of one specific organ, the hypocotyl, is established as a useful model system for plant growth. The hypocotyl has a very simple structure (only about 20 cells) and these

cells grow by expanding rather than dividing (Gendreau et al. 1997). Hypocotyl growth is affected by all known phytohormones (Davies 2004), all environmental conditions that affect growth of an adult plant including light (Arsovski et al. 2012) and temperature (Wigge 2013, Quint et al. 2016) and many genes not directly related to growth such as the circadian clock (Nusinow et al. 2011, Lu et al. 2012). Combined with the ease and speed of hypocotyl growth and the many mutants that are readily available, the *Arabidopsis thaliana* hypocotyl is an ideal model for investigating plant growth.

## 1.3 Auxin

In this dissertation, I focus on the effect of auxin on growth. Auxin, specifically indole-3-acetic acid (IAA), was the first phytohormone to be discovered. In the 1800s Darwin hypothesised that bending in grass coleoptiles was caused by a substance synthesised in the tip of the plant that moved to other regions to cause a change in growth (Darwin and Darwin 1880). The chemical was isolated much later (Went 1927) and was eventually identified as IAA (Wildman 1997). IAA is not the only auxin – there are others that occur naturally in plants and several synthetic auxins have commercial or research application. Auxin has a role in every stage of a plant's life cycle: patterning in the embryo (Chasan 1993), root and shoot growth (Boerjan et al. 1995), tropic responses to light and gravity (Friml 2003), flowering (Salisbury 1955) and senescence (Ellis et al. 2005). However, its main role is in cell growth and division, so it is most often found in growing regions of the plant or at wounds. Auxin can act on its own, but often works with in feedback loops with other hormones, including cytokinin (Coernen and Lomax 1997), ethylene (Chadwick and Burg 1970) and gibberellins (Fu and Harberd 2003, Frigerio et al. 2006). I focused on the effect of auxin on growth, specifically cell elongation in the hypocotyl.

Responses to environmental, hormonal or developmental stimuli often involve a change in auxin biosynthesis or auxin transport. Auxin is synthesised mainly in growing parts of a plant, such as young leaves and the tip of shoots and roots (Ljung et al. 2001), but is

transported to nearly all areas of a plant (Goldsmith 1977). There are several different auxin biosynthesis pathways (Zhao 2010). Some synthesise auxin from the amino acid tryptophan but tryptophan-independent pathways are also known. These signalling pathways are extremely complex due to auxins place at an intersection between many signalling pathways. There is also a high degree of redundancy, which has made characterising these pathways difficult. Once synthesised, auxin can be transported through the phloem (Hoad 1995) or between individual cells through transmembrane proteins. There are three main families of auxin carrier proteins: the influx carriers of the AUX1/LAX carriers (Bennett et al. 1996, Swarup et al. 2008), the efflux carriers of the PIN family (Vieten et al. 2007, Zazimalova et al. 2007) and another family of efflux carriers called ABCB proteins (Geisler et al. 2005, Santelia et al. 2005, Terasaka et al. 2005). These proteins use the polarity of IAA and other naturally occurring auxins to ensure that auxin only flows one way – towards the sink tissues where it is needed. This prevents auxin from causing unregulated growth in other areas. For this reason, it was important that I used a non-polar synthetic auxin, picloram, in my experiments. This auxin can be taken up from growth media and will flood all areas of the plant, making it easier for me to see the effect of auxin on hypocotyl growth. Similar experiments have been done with naturally occurring auxins, but these must be applied directly to the tip of the hypocotyl (Yang et al. 1996) or applied to isolated sections (Evans 1985). Using quantitative genetic analysis, I hoped to understand the genetics involved in the auxin response in the hypocotyl.

The effect of auxin on the *Arabidopsis* hypocotyl has been studied in great depth but is still not fully understood due to the complexity of auxin signalling. Generally, auxin stimulates growth. Applying a naturally-occurring polar auxin to isolated section of plants (Evans 1985), plants grown on nutrient deficient media (Smalle et al. 1997) or auxin-deficient mutants (Collett et al. 2000) will result in a rapid increase in growth. This growth is almost all cell elongation, not cell division (Evans 1985). However, applying auxin to a normal, healthy plant often has no effect and in some cases can inhibit growth (Collett et al. 2000). This has led to the hypothesis that auxin levels in the hypocotyl are naturally at their optimum level and adding more auxin inhibits growth. The effect of auxin seems to depend heavily on the dose, type of auxin and method of

application used. A very high dose of auxin will upset the balance between the many signalling pathways it is a part of which will inhibit growth, but a low concentration of auxin can stimulate growth, even in a healthy plant. The polarity of the auxin used and the method of application will determine which tissues the auxin acts in. I chose to introduce a non-polar auxin in to the growth media to flood the whole plant with a low concentration of auxin. This resulted in an increase of hypocotyl growth that was useful for my research.

## 1.4 Warmth

Auxin-driven changes in growth can be triggered naturally by any environmental condition that increases auxin levels. One of these is warmth. *Arabidopsis thaliana* grows optimally at temperatures between 12 and 27$^{o}$C (Griffing and Langridge 1963, Samach and Wigge 2005, Salinas and Sanchez-Serrano 2006). Temperatures lower than this range trigger cold-tolerance mechanisms while at higher temperatures plants start to go into heat shock (Levitt 1980). Warm temperatures at the upper end of this normal range produce changes in morphology, but do not harm the plant. Although there is some uncertainty about the precise signalling pathway, it is thought that changes in temperature are perceived by cell membranes through a change in fluidity (Kamada et al. 1995, Orvar et al. 2000, Penfield 2008). The signalling pathway up-regulates auxin production and transport to drive a change in growth (Gray et al. 1998, Stavang et al. 2009, Wigge 2013, Quint et al. 2016). These changes include elongation of the hypocotyl and petioles, changes in plant architecture with longer internodes, upward bending of the cotyledons and leaves known as hyponasty and early flowering (Wigge 2013, McClung et al. 2016, Quint et al. 2016). There is evidence that these changes in architecture aid in cooling the plant and preventing heat damage (Crawford et al. 2012). I was interested in the similarities and differences in genetic activity that cause similar changes in growth when auxin is increased naturally by warmth and artificially.

## 1.5 HSP90.2-3

Growth and hypocotyl length is under the control of many factors and can be increased by more than just an increase in auxin. One of these factors is a family of chaperone proteins called heat shock proteins. Chaperone proteins fold proteins into their proper shape so that they function properly (Thirumalai and Lorimer 2001, Young et al. 2004). Heat shock proteins (also called stress proteins) are chaperones induced by various types of stress including increases in temperature (Lindquist and Craig 1988, Vierling 1991, Hendrick and Hartl 1993, Parsell and Lindquist 1993, Kregel 2002). The study of heat shock proteins and their roles was helped greatly by the discovery of a mutation in one particular allele of one heat shock protein, HSP90.2-3 (Hubert et al. 2003). When this protein is mutated, it prevents the action of all proteins in an important family of heat shock proteins called the HSP90 family. Plants carrying this mutation have many of the same changes in growth caused by an increase in auxin, including increased growth, early flowering and increased variation within a population (Sangster and Quietsch 2005, Sangster et al. 2008).

The mutation is useful in QTL mapping studies as it can reveal cryptic genetic variation (Sangster et al. 2007). In wild-type plants, some variation is hidden because unusual proteins are forced into their proper shapes by heat shock proteins. The *hsp90.2-3* mutation allows this previously hidden variation to have an effect, often resulting in more QTL. I wanted to use this mutation to make my QTL mapping more effective, but I also wanted to investigate the changes in growth caused by the mutation.

## 1.6 QTL mapping

Quantitative genetic analysis, in particular QTL mapping, is a useful technique for studying genetics. Many methods used to study genetics are not suitable for studying very complex traits such as growth and variation. These traits are under the control of many different genes. Individual genes may only have a small effect which is difficult to detect using other methods. I aimed to study two of these complex traits. As discussed previously, the genetic control of growth is extremely complex. A greater understanding

of plant growth and the processes underpinning it is essential for issues such as food security, energy security and conservation. I also wanted to study variation, a trait that affects a population level as a whole. This was possible because of the plant population I used in QTL mapping (section 2.1). A difference in the variation of growth between two populations could reflect a difference in the consistency, reliability or rigidity of the genetic processes underlying growth. This in turn can provide information about how an entire population may behave in a natural environment, as opposed to an “average” individual in a controlled experiment. It is just as important to build up an understanding of variation as an aspect of growth as it is to know what drives or constrains growth in an individual plant.

QTL mapping is also useful for finding epistatic interactions. Some genes only have an effect when certain other genes are also present. These interactions can be additive, where two genes have an effect by simply coexisting, or synergistic, where the effect of two genes together is greater than the sum of each gene alone. The term “epistasis” can refer to either the concept of two genes needing to both be present to show their effect or specifically a synergistic interaction (Phillips 2008). QTL mapping, specifically two-dimensional, two-QTL scans, can detect both additive and synergistic interactions. This gives QTL mapping several advantages over other methods when studying a trait as complex as growth (Borevitz et al. 2002).

QTL mapping uses statistical analysis of the genotypes and phenotypes in a mapping population. There are several types of population which can be used, such as backcross or intercross. My mapping population is derived as a recombinant inbred line (RIL) collection. My population consists of 96 unique genetic lines descended from a cross between Col-0 and WS-2 parents for several generations (Young 1994, Collard et al. 2005). Each line has been genotyped at a number of markers. At each marker, each plant can be either homozygous for the Col-0 allele, homozygous for the WS-2 allele, or heterozygous. There is also a marker at the site of the *hsp90.2-3* mutation, which is present is half of the lines. I then gathered phenotypic information about each line. Specifically, I measured hypocotyl length in a range of conditions. QTL mapping compares the genotypic data to the phenotypic data to determine whether individuals with one genotype at a certain marker are significantly different from individuals with

another genotype at the same marker (Broman and Sen 2009). By repeating this for all markers each point on the genome is given a LOD score which represents how much influence that position has over the trait in question (Tanksley 1993, Young 1996). Single-QTL mapping considers each point on the genome independently. A 2-QTL scan considers each possible pair of points on the genome to find epistatic interactions in what is known as a two-dimensional genome scan. This can be done using either an additive model, where QTL simply coexist without changing each other's effect, or a full model, which considers synergistic interactions between QTL. Both models use differences in phenotypic and genotypic data to assess the likelihood that each possible pair of loci are linked QTL (Broman and Sen, 2009). However, detecting interactions between QTL is difficult. High statistical power is needed to avoid type-1 errors, and if the pattern of interaction controlling a trait is very complex, as with growth, then false identification becomes more likely (Laurie et al. 2014). Despite this, both single- and 2-QTL scans are powerful methods, and each can reveal important information.

There are various statistical methods that can be used in QTL mapping, but I chose to use composite interval mapping (CIM) and the multiple imputation method (Sen and Churchill 2001). I chose these methods because of their greater precision, especially when performing 2-QTL scans for epistatic relationships between markers (Jansen 1993, Zeng 1993, Jansen and Stam 1994, Zeng 1994). Using a permutation test (Churchill and Doerge 1994) I can determine how high a LOD score must be to consider that point on the genome as significant to the trait. This method can be used for both single- and 2-QTL scans. By using this technique, I hoped to understand which regions of the genome are important in hypocotyl growth under a range of conditions.

## 1.7 Aims and hypotheses

I have three main aims for this project:

1. Investigate hypocotyl growth in a range of conditions. This includes two genotypes, wild type and *hsp90.2-3*, and three environmental conditions, standard, warm and with added auxin. I expect all changes in condition to result

in an increase in hypocotyl length compared to wild-type plants in standard conditions, but I am interested to see whether a natural rise in auxin concentration as a result of increased temperature or artificially increasing auxin concentrations by adding it to growth media will result in the greatest increase in hypocotyl length.

2. Find QTL for hypocotyl growth and variation in all conditions tested. I expect to find at least one QTL in every condition since hypocotyl length is controlled by many genes.
3. Compare the results of QTL mapping between genotypes and environmental conditions to determine which QTL are for general growth and which are specific to growth in a certain condition. I expect that growth in warmth and with auxin will have some QTL in common since both use the auxin signalling pathway. I also expect most conditions to have some QTL in common with the wild-type genotypes in standard conditions, since some genes are needed for growth in any condition.

# Chapter 2: Materials and methods

## 2.1 Plant material

The Recombinant Inbred Line (RIL) I used for QTL mapping was previously derived from Col-0 and WS-2 genetic backgrounds. The full map can be seen in appendix 1. The Col-0 parent contained the *hsp90.2-3* mutation (Hubert et al. 2003) on chromosome 5. The WS-2 parent contained a luciferase transgene in a *CCR2:LUC* construct. The first generation progeny of these parents (F1) was crossed with WS-2 *CCR2:LUC* to generate the first generation backcross population (BC1-F1). From BC1-F1, sixteen seeds heterozygous for *hsp90.2-3* and hemizygous for *CCR2:LUC* were chosen and self-fertilized. Three seeds from each genetic line were chosen, all heterozygous for *hsp90.2-3* and hemizygous for *CCR2:LUC*. This population of 48 unique genetic lines was BC1-F2. The population was bred by single-seed descent, maintaining heterozygosity for *hsp90.2-3*, until generation BC1-F7. From population BC1-F7, one individual homozygous for *hsp90.2-3* and one wild-type individual from each unique line were selected to create the final RIL, containing 96 unique genotypes.

I used a RIL because it carries several advantages in QTL mapping over other types of mapping populations. Each genotype is almost completely homozygous. This means that genetically identical plants can be used in different experiments. This was important as I wanted to test the effect of different environmental stimuli on the same plants. Furthermore, since I could grow groups of genetically identical plants I could map the standard deviation of a population of a population of plants. This was essential for studying variation (section 1.6). However, the RIL I used has an unusual structure because of the presence of *hsp90.2-3* in only half the genotypes. The population is effectively made up of 48 genotypes, each of which has a wild-type and mutant form, to make a total of 96 genotypes. This allowed me to map traits in the presence and absence of the mutation. Without this characteristic in my mapping population, I could have assessed the effect of the mutation by assigning it as a co-factor. However, in some types of population co-factor analysis has been shown to increase the likelihood

of type-1 errors, especially in low-power experiments (Sahana et al. 2006). As my mapping population was relatively small, and therefore had low statistical power, the benefits of co-factor analysis were outweighed by the risk of false positives. I decided to instead to investigate the effect of *hsp90.2-3* using this unusual RIL which allowed me to map wild-type and mutant populations separately.

## 2.2 Chemicals

Growth media for collecting seed:

33% COIR compost – Melcourt Profressional Growing Media

33% F2 + S compost with sand – Levington Advance

33% Vermiculite compost additive – Sinclair Pro

0.07% osmocote NPK fertiliser – Keith Singleton Horticultural Products

Chemicals for sterilising seed:

Ethanol absolute – VWR Chemicals

33% Bleach in sterile $H_2O$

0.01g/100ml Select agar water in sterile $H_2O$ - Invitrogen

Growth media for hypocotyl length assays (MS3):

4.4 g/L Murashige & Skoog (MS) medium basal salt mixture without vitamins – Duchefa Biochemie

30 g/L sucrose – Fisher Scientific UK

0.5 g/L MES free acid buffer – Melford Biolaboratories Ltd

15 g/L phyto agar – Duchefa Biochemie

pH adjusted to 5.7 with KOH

To create MS3 + hygromycin media: 0.1% 30mg/ml hygromycin B – Duchefa Biochemie

To create MS3 + 3µM picloram media: 0.3 ml/L picloram stock solution

Solvent: sterile $H_2O$

Picloram stock solution (10mM):

2.4 mg/ml picloram – Duchefa Biochemie
Solvent: Dimethyl sulfoxide (DMSO) – Fisher Scientific UK

## 2.3 Software

R in RStudio, including packages R/qtl (Broman et al. 2003) and pylr, used to analyse data and perform qtl mapping.

ImageJ (Schneider et al. 2012) used to measure seedling hypocotyls after imaging.

MS Excel used to analyse data.

Corel Paintshop Pro used to process seedling images.

## 2.4 Collecting seed

I grew 10 plants of each of the RIL genotypes and harvested their seed to use in hypocotyl assays. I also harvested seed from Col-0 and WS-2 parental plants, both with and without the *hsp90.2*-3 mutation. Before growing I sterilised seeds by washing once with 70% ethanol, once with 33% bleach with triton and twice with sterile water. I washed seeds by suspending them in 400µL of solution. I then suspended the seeds in sterile agar water and plated them onto MS3 media. I stratified the plates by storing them at $4^{o}$C for two days. After stratification, I germinated the seeds at room temperature in a 12-hour light/12-hour dark cycle. After 10 days the seedlings were large enough to be handled with tweezers. I transferred them to pots containing damp compost. The plants were grown in greenhouses with regular watering until mature siliques formed. After this, watering was stopped and the siliques were dried. Seeds were harvested from the dried siliques, cleaned of debris and stored at room temperature until use.

## 2.5 Hypocotyl length assay

I sterilised seeds by washing them in a series of solutions. I suspended seeds in 400μL of each solution. I washed the seeds once with ethanol, once with bleach and twice with sterile water. I then suspended the seeds in sterile agar water and plated individual seeds onto the correct growth media for each treatment. All genotypes in the RIL were grown on media containing hygromycin B to reduce the risk of contamination. The Col-0 and WS-2 genotypes are not resistant to this antibiotic, so they were grown on basic MS3. I spaced all seeds evenly with approximately 1cm of clear space around each seed. I grew 20 seeds from each genetic line. I stratified the seed by storing them at $4^{o}$C for two days. After stratification, I moved the plates to growth cabinets with the following conditions for each treatment:

| Treatment | Temperature ($^{o}$C) | Media |
|---|---|---|
| Control | 22 | MS3 |
| Warmth | 27 | MS3 |
| Auxin | 22 | MS3 + picloram |

All conditions took place under a 12-hour photoperiod. I sealed the agar plates with parafilm to maintain humidity.

I grew the plants for 10 days. I then pushed the seedlings flat and scanned the agar plate using a flatbed scanner. I obtained an image of the seedlings with a ruler next to them. Using ImageJ, I compared the length of a line traced over each hypocotyl to the known length of the ruler to find the length of each hypocotyl.

## 2.6 Analysis

I used Microsoft Excel to perform statistical analysis. This included finding the mean hypocotyl length, standard deviation, standard error and change in growth due to a certain condition of each genotype. I calculated the change in growth using the formula:

$$\frac{\text{hypocotyl length with treatment-hypocotyl length without treatment}}{\text{hypocotyl length with treatment}}$$

Using this metric allowed me to map the change in hypocotyl length due to the treatment applied (Reymond et al. 2006). Once I had collected this phenotypic data I used Microsoft Excel to create a file containing phenotypic data about a single trait, marker locations and the genotype of each RIL at each marker location.

I imported the file containing phenotype and genotype data into R for analysis. I used the package R/qtl to perform interval mapping (IM) using the multiple imputation method (Sen and Churchill 2001). The multiple imputation method has an advantage over alternative IM methods since it can consider the spaces between markers as well as the markers themselves. During IM each position on the genome is considered as the possible location of a QTL. H1 is the likelihood of a QTL being at that location. H0 is the likelihood of there being no QTL at that location. Using both H1 and H0 a likelihood of odds (LOD) score is calculated for each position on the genome. The higher a positions LOD score is, the more likely it is that there is a QTL at that position. The output of single-QTL analysis is a set of LOD scores for each position on the genome that can be described graphically.

As well as the single-QTL scans described above, I analysed my data using two-QTL scans. This type of analysis has the advantage of detecting epistatic interactions – loci that are only QTL if another QTL is in the same genome. The method for two-QTL scans is similar to single-QTL scans. I used IM and the multiple imputation method, but instead of considering the possibility of a QTL at each position, the analysis considered to possibility of two QTL at each possible pair of positions. My analysis used two models: the additive model, which considers each pair of positions as QTL that coexist but do not interact, and the full model, which considers each pair of positions as QTL that have an epistatic interaction. The output of this analysis is two sets of LOD scores

(one set from each model) for each pair of positions in the genome reflecting the likelihood that there is a pair of QTL there.

To interpret my data, I calculated the genome-wide significance thresholds using permutation tests. These tests randomly shuffle the phenotypic data while keeping the genotype data intact and perform QTL mapping on each permutation using the method described above. After 10,000 permutations for a single-QTL scan or 1000 permutations for a two-QTL scan, the genome-wide maximum LOD scores for each permutation were collected and compared. This data reflects the LOD scores that can be expected by random chance if there is no QTL. Using this data, I calculated thresholds for 20% significance levels to help me interpret my data.

## 2.7 Validity of tests

For every QTL analysis I performed, I first investigated the variation and distribution of my trait data to ensure that QTL analysis was appropriate. The calculations used are explained here and the results are displayed in the Validity of Test sections in chapters 4, 5 and 6.

Firstly, I investigated the variation in my data using ANOVA as a wide range of phenotypes in the trait being mapped improves QTL analysis. ANOVA is a simple yet powerful way to estimate how much variation exists in a certain trait. Some QTL analysis use heritability to estimate genetic control of a trait, and therefore how likely it is that QTL will be detected. However, this can be inconsistent (Huang et al. 2007). Furthermore, since growth and variation are both subject to a complex combination of genetic and environmental control, heritability would be insufficient. I decided that ANOVA was a more appropriate statistical test. If there was a significant amount of variation in the trait being mapped and that variation was affected by genotype, I was confident that QTL mapping would yield some useful results.

Secondly, I investigated the distribution of my trait data because one of the assumptions of QTL mapping is that the trait data are normally distributed. If the data were normally distributed, they were appropriate for QTL mapping. Once I confirmed that my data met the assumptions of the test, I proceeded with my analysis.

# Chapter 3: Growth of *Arabidopsis thaliana*

## 3.1 Overview

I wanted to understand the effect of auxin and the *hsp90.2-3* mutation on hypocotyl growth. My experimental system employed the parental genotypes of my RIL, Col-0 and WS-2. Using these genotypes meant that, as well as understanding the behaviour of the hypocotyl generally, I could learn how effective QTL analysis would be. If the hypocotyl length of Col-0 and WS-2 were different from each other I would expect more variation in a mapping population generated from these parents. This is because growth is a complex trait controlled by many genes. If the parents were different from each other they provided a greater number of possible combinations of alleles in my mapping population. This led to greater variation in the mapping population which increased the power of QTL analysis. QTL mapping works by comparing differences in phenotype to differences in genotype, so more variation makes the analysis more powerful. I examined the effects of warmth, auxin and the mutation *hsp90.2-3*. To examine the effect of warmth I grew seedlings at 27$^{o}$C. I examined a range of concentrations of auxin between 0.3µM and 50µM. I studied the concentration 3µM in more detail. Auxin application increased hypocotyl length in both parental genotypes. In contrast warmth and *hsp90.2-3* increased hypocotyl length in one genotype, but not the other. These results are displayed in figure 1 and illustrated further in figure 2. The full data can be seen in appendix 2. The results presented in this chapter show that warmth, auxin and *hsp90.2-3* all share a similar phenotype. They also provided a good base for QTL mapping, as the differences between Col-0 and WS-2 meant that the population generated from these parents would have a wide range of alleles. This created more variation in the mapping population which made QTL analysis more powerful and lead to the identification of QTL.

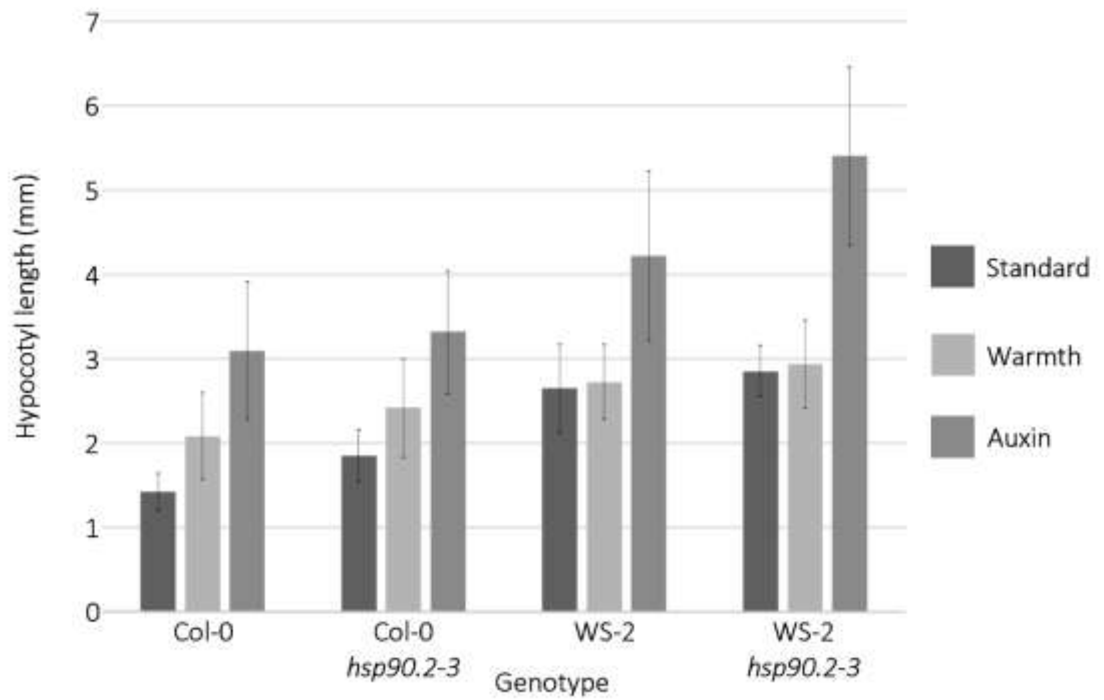



Figure 1: Mean hypocotyl length of parental genotypes, both wild type and with *hsp90.2-3* mutation, in standard conditions, at 27°C and with 3µM picloram media.

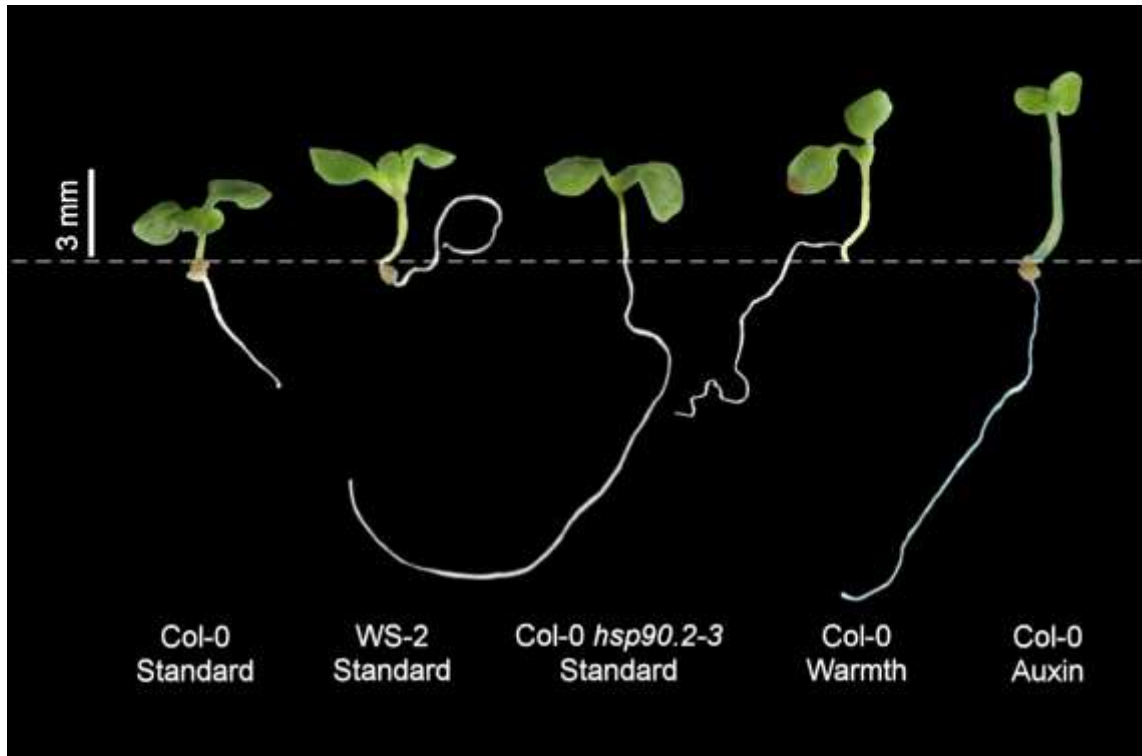



Figure 2: The effect of genotype, the *hsp90.2-3* mutation, warmth and exogenous auxin on *Arabidopsis thaliana* seedlings. All seedlings were grown using the method in section 2.5.

## 3.2 General and auxin-driven growth of WS-2 and Col-0

I investigated the differences in growth between the parents of my mapping population to examine whether the genotypes were different from each other. This would provide a wide range of alleles in my mapping population, which would make QTL mapping more powerful. The mapping population I used in my QTL mapping experiments was generated from these genetic backgrounds (section 2.1). I wanted to know if QTL analysis of hypocotyl length in my population would be effective.

### Standard growth

The hypocotyl length of WS-2 was greater than Col-0 in standard conditions. The mean hypocotyl length of 20 seedlings after 10 days was 1.4 mm for Col-0 and 2.7 mm for WS-2. The hypocotyl length of WS-2 seedlings was also more variable than Col-0 seedlings. WS-2 had a standard deviation of 0.520, compared to a standard deviation of 0.224 for Col-0. WS-2 was significantly taller than Col-0 (ANOVA: $F = 93.58$, $d.f = 1, 38$, $p = 8.51e-12$). Because the parents displayed phenotypic differences, I could expect to find genetic differences underlying these phenotypes. This would provide a range of alleles in my mapping population that would be helpful for finding QTL.

### Warmth and auxin

The hypocotyl of WS-2 was longer than the hypocotyl of Col-0 both at warm temperatures and in the presence of exogenous auxin. After 10 days at 27$^{o}$C, the mean hypocotyl length of 20 WS-2 seedlings was 2.7 mm, while the mean hypocotyl length of Col-0 was 2.1 mm. The genotypes seemed to have roughly equal variation in height. The standard deviation of WS-2 was 0.445 and the standard deviation of Col-0 was 0.508. I found this to be a significant difference in height (ANOVA: $F = 17.55$, $d.f = 1, 37$, $p = 0.000167$). At 22$^{o}$C with picloram added to the growth medium, the mean hypocotyl length of WS-2 seedlings was 4.2 mm. The mean hypocotyl length of Col-0 was 3.1 mm. With added auxin, there was more variation in the hypocotyl length of WS-2 than Col-0. The standard deviation of hypocotyl length for WS-2 was 1.101, while it was 0.812 for Col-0. This was also a significant difference in height (ANOVA: $F = 15.03$, $d.f = 1, 38$, $p =$

0.000406). Since I found phenotypic differences between the parent genotypes of my mapping population, I could expect to find QTL at warm temperatures and with exogenous auxin.

## 3.3 The effect of the mutation *hsp90.2-3*

My mapping population consisted of pairs of genotypes with and without *hsp90.2-3*. This mutation is known to increase hypocotyl length and variation (section 1.5). I next investigated the effect of this mutation in warmth and with added auxin and its relationship to other genes using QTL mapping. Before proceeding with QTL mapping, I wanted to examine the effect of this mutation on hypocotyl growth. I used Col-0 and WS-2 seedlings containing the mutation to understand its effect in both parental genetic backgrounds.

### Standard growth

The effect of *hsp90.2-3* on growth seemed to depend on what genetic background it was in. The mean hypocotyl length of Col-0 was 1.4 mm in standard conditions. This increased to 1.9 mm in Col-0 *hsp90.2-3*. The mutation also appeared to increase variation, as standard deviation increased from 0.224 in Col-0 to 0.306 in Col-0 *hsp90.2-3*. This was a significant increase in hypocotyl length (ANOVA: $F = 25.12$, $d.f = 1, 37$, $p = 1.36e-05$). The mutation appeared to have less effect in a WS-2 background. The mean hypocotyl length of 20 WS-2 seedlings in standard conditions was 2.7 mm, increasing to 2.9 mm in WS-2 *hsp90.2-3*. The standard deviation in WS-2 was 0.520, while the standard deviation in WS-2 *hsp90.2-3* was only 0.298. I found that this was not a statistically significant increase in height (ANOVA: $F = 2.287$, $d.f = 1, 38$, $p = 0.139$). The decrease in standard deviation was similar to the way standard deviation in WS-2 was reduced in higher temperatures. The fact that the mutation seems to behave differently in different genetic backgrounds suggests that QTL mapping will be a powerful tool for investigating its effect. The effect of the mutation would depend on which alleles from the parent lines it combined with, which would lead to variation in the mapping

population. I reasoned that QTL mapping would be a powerful tool for investigating the role of the mutation, especially two-dimensional scans to search for QTL that HSP90.2-3 interacts with.

## Warmth

The mutation *hsp90.2-3* had a reduced effect at 27ºC in both Col-0 and WS-2 backgrounds. The mean hypocotyl length of Col-0 seedlings at 27ºC was 2.1 mm. In Col-0 *hsp90.2-3* seedlings the mean hypocotyl length was 2.4 mm. There was a slight increase in standard deviation, from 0.508 in Col-0 to 0.584 in Col-0 *hsp90.2-3*. This was not a statistically significant difference in hypocotyl length (ANOVA: $F = 3.437$, $d.f = 1, 36$, $p = 0.072$). The results in the WS-2 seedlings were similar. Mean hypocotyl length was 2.7 mm in WS-2 seedlings and 2.9 mm in WS-2 *hsp90.2-3* seedlings. Standard deviation increased from 0.445 in WS-2 to 0.523 in WS-2 *hsp90.2-3*. This was also not a significant increase in height (ANOVA: $F = 1.835$, $d.f = 1, 38$, $p = 0.183$). These results suggested that the *hsp90.2-3* mutation has less of an effect at warm temperatures. This could have been due to hypocotyls approaching their maximum possible lengths under the conditions and further growth being inhibited by something else such as the plant's capacity for auxin biosynthesis. Since the parents had similar phenotypic responses, it could be expected that fewer QTL would be detected in QTL mapping.

## Auxin

The mutation *hps90.2-3* increased growth in the presence of exogenous auxin in the WS-2 genetic background but not in the Col-0 genetic background. The mean hypocotyl length was 4.2 mm in WS-2 seedlings and 5.4 mm in WS-2 *hsp90.2-3* seedlings. There was a slight increase in standard deviation, from 1.010 in WS-2 to 1.063 in WS-2 *hsp90.2-3*. This was a significant increase in height (ANOVA: $F = 12.93$, $d.f = 1, 38$, $p = 0.00092$). In contrast, there was no significant increase in height in the Col-0 background. The mean hypocotyl length was 3.1 mm in Col-0 and 3.3 mm in Col-0 *hsp90.2-3*. There was a slight decrease in standard deviation, from 0.812 in Col-0 to 0.726 in Col-0 *hsp90.2-3*. This was not a significant increase in height (ANOVA: $F = 0.804$, $d.f = 1, 38$, $p = 0.376$). The mutation appeared to have a greater effect on growth

and variation in a WS-2 genetic background when auxin is added. This could have been due to Col-0 approaching its maximum possible height, or the WS-2 background could be more sensitive to auxin in general, enhancing the effect of the mutation.

## 3.4 The effect of warmth on growth

Temperatures of about 27$^{o}$C, at the upper limit of the range *Arabidopsis thaliana* can grow at without obvious damage, increase hypocotyl length and variation (Gray et al. 1998). Interestingly, my results showed that warmth has a greater effect on Col-0 than on WS-2. The mean hypocotyl length of Col-0 seedlings was 1.4 mm in control conditions and 2.1 mm at 27$^{o}$C. Warmth also increased standard deviation in Col-0, from 0.224 in control conditions to 0.508 in warmth. This was a significant increase in hypocotyl length (ANOVA: $F$ = 28.21, *d.f* = 1, 37, $p$ = 5.38e-06). In contrast, there was no significant increase in the growth of WS-2 seedlings. Mean hypocotyl length was 2.7 mm in control conditions and 2.7 mm at 27$^{o}$C. There was a slight decrease in standard deviation, from 0.520 in control conditions to 0.445 in warmth. This was not a significant increase in growth (ANOVA: $F$ = 0.251, *d.f* = 1, 38, $p$ = 0.619). This could have been due to a natural reduction in temperature sensitivity in WS-2, or it could have been that WS-2 was close to its maximum height after 10 days and something else was limiting growth, such as the plant's natural capacity for auxin biosynthesis. These results suggested that effect of warmth on growth depends on genotype, increasing growth in Col-0 but having little effect in WS-2, but this deserves further research as my experimental design may have been flawed.

## 3.5 The effect of auxin on growth

Auxin is an important phytohormone known to be capable of increasing growth (Jouve et al. 1999, Collett et al. 2000). In experiments, adding exogenous auxin has been shown to increase or decrease hypocotyl length, depending on the type of auxin and the method of application. Some people have hypothesised that natural levels of auxin

in the hypocotyl are at their optimum and adding more inhibits growth by destabilising the balance between different signalling pathways (Evans 1985, Smalle et al. 1997, Collett et al. 2000). I added a range of concentrations of the non-polar synthetic auxin picloram to growth media and carried out my normal hypocotyl-length assay. I found that low concentrations of picloram resulted in a dramatic increase in growth, but very high concentrations inhibited growth (figure 3). Because of this, I decided to use 3µM picloram when I carried out further studies on the effect of auxin on growth. This concentration produced an increase in growth, but not the maximum increase possible. I reasoned that at this concentration, a small change in auxin sensitivity could result in a large difference in hypocotyl length. This wider range of phenotypic response could increase the power of QTL analysis to reveal more QTL.

Adding the synthetic non-polar auxin picloram to growth media increased hypocotyl length in all genotypes tested. The mean hypocotyl length of Col-0 seedlings increased from 1.4 mm in control conditions to 3.1 mm with exogenous auxin. Standard deviation increased from 0.224 in control conditions to 0.812 with exogenous auxin. This was a significant increase in growth due to auxin (ANOVA: $F = 78.94$, $d.f = 1, 38$, $p = 8.2e\text{-}11$). The mean hypocotyl length of WS-2 seedlings increased from 2.7 mm in control conditions to 4.2 mm with added auxin. Standard deviation increased from 0.520 in control conditions to 1.010 with exogenous auxin. This was a significant increase in hypocotyl length (ANOVA: $F = 38.28$, $d.f = 1, 38$, $p = 3.16e\text{-}07$). In all genotypes tested, I found the greatest hypocotyl length when I added exogenous auxin to the growth media. This could mean that in other conditions where plants appeared to reach their maximum possible height, such as warmth, the factor limiting their growth could have been the amount of auxin available to them through their own biosynthesis pathways.

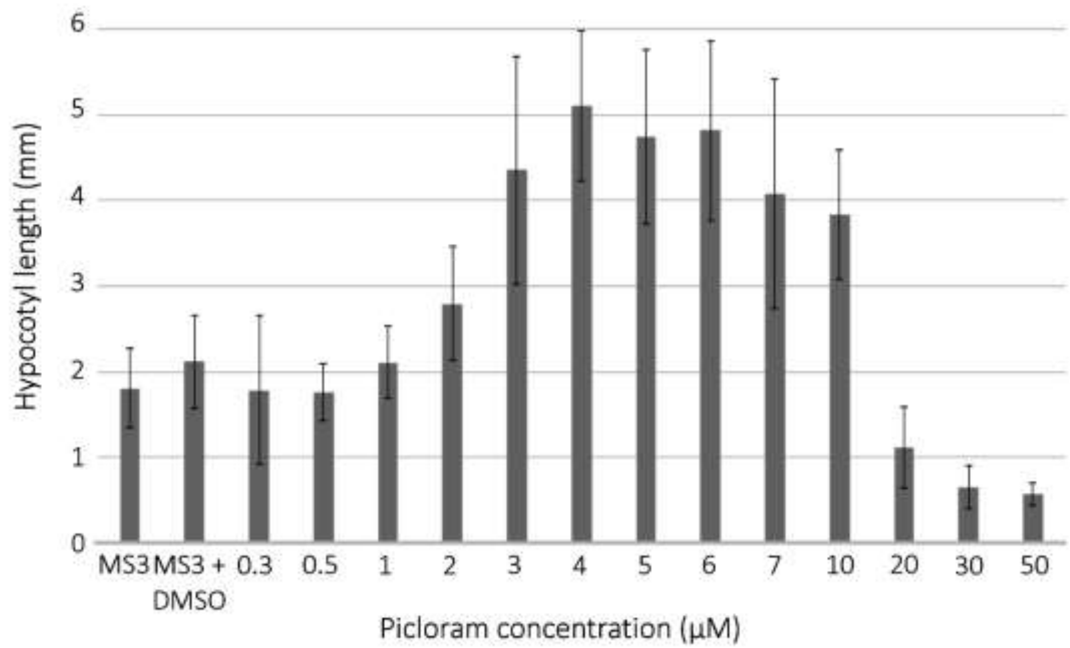



Figure 3: Mean hypocotyl length of *Arabidopsis thaliana* seedlings after 10 days grown on a range of picloram concentrations. Error bars show 1 standard error.

## 3.6 Summary of results

The results presented in this chapter confirm some previously existing hypotheses and add detail to others. Firstly, warm temperatures, exogenous auxin and the mutation *hsp90.2-3* often had the effect on growth that I expected. Each increased hypocotyl length and standard deviation. However, warmth and *hsp90.2-3* had reduced effects on certain genotypes. Warmth only produced a significant increase in hypocotyl length in Col-0 plants. The mutation increased growth only in Col-0 plants in standard conditions but increased growth only in WS-2 plants with exogenous auxin. The mutation did not increase the hypocotyl length of either genotype at warm temperatures. In many cases, a non-significant increase in growth was accompanied by a decrease, or a marginal increase, in standard deviation. This consistency in height suggested to me that these plants are reaching their maximum possible length under the conditions. These results highlighted a flaw in my experimental design. Measuring the plants at more time points, especially earlier than 10 days, may have revealed differences in growth that may have evened out after 10 days.

Secondly, I investigated the effect of a range of auxin concentrations on hypocotyl growth. The effect of auxin on the hypocotyl was complex. Adding auxin increased growth in some cases but decreased it in others. This was consistent with previous hypotheses (Evans 1985, Collett et al. 2000). Some people have hypothesised that auxin levels are naturally at their optimum and adding more inhibits growth. I found that adding low concentrations of auxin to the growth media resulted in a dramatic increase in growth, but high concentrations inhibited growth. I used the non-polar synthetic auxin picloram. Non-polar auxins are able to “flood” every area of the plant, in contrast to naturally occurring polar auxins which are transported to sink tissues (section 1.3). My results suggest that the effect of adding exogenous auxin to a plant relies heavily on the concentration used. It is also possible that the type of auxin used and the method of application could have affected these results, which could be tested in further research.

These results provided a good base to begin QTL mapping. I found differences in growth between Col-0 and WS-2, the parent genotypes of my mapping population. This led me to expect a wide range of hypocotyl lengths in the mapping population, which makes

QTL detection more likely. I also found differences in hypocotyl length due to warmth and auxin. This is important as I am interested in QTL for changes in growth due to these conditions. The changes in growth described in this chapter meant that QTL mapping was an appropriate analysis and was likely to yield useful results.

# Chapter 4: QTL analysis of general growth and variation

## 4.1 Overview

This chapter presents the results of my analysis of growth and variation in standard conditions. I looked for QTL for two traits, hypocotyl length and the standard deviation of hypocotyl length, in three mapping populations: the full mapping population, the wild-type half of the mapping population and the half of the mapping population containing the *hsp90.2-3* mutation. I performed single-QTL and two-QTL scans to search for QTL that act on their own and those that act through epistatic interactions (section 2.6). I found several QTL using these methods, including one on chromosome 5 that controls both growth and variation through interactions with several different genes. The results presented in this chapter provide an idea of what regions of the genome are important in regulating growth in normal conditions.

## 4.2 QTL mapping of hypocotyl length

I investigated QTL for growth. I used hypocotyl length, collected using the method in section 2.5, as a convenient measure of growth in each genotype in my mapping population. These data are presented in appendix 3. By using hypocotyl length as a trait in QTL mapping, I found QTL that are important in increasing or decreasing the growth of a seedling.

### Results of analysis

QTL analysis of the general growth of the mapping population revealed one QTL. I mapped the average hypocotyl length of 20 individuals from 89 genotypes in standard conditions using the method in section 2.6. Figure 4 displays the QTL map obtained from this analysis. I found one peak located on chromosome 5 at position 13.5 with a LOD score of 3.17. This peak is a possible QTL for growth under control conditions.

My analysis also yielded some inconclusive results. I found one other peak on chromosome 5 at position 95.28. This peak had a LOD score of 2.5, which put it very close to the significance threshold (2.85) but did not quite cross it. It is difficult to tell whether this is due to a lack of statistical power or a genuinely negative result. This position should be noted as it is important in several other QTL maps I made.

## Validity of test

Variation:

Figure 5 displays the mean hypocotyl length of each genotype in my mapping population in standard conditions.

ANOVA: $F = 8.868$, $d.f = 86, 1611$, $p = <2^{e-16}$

Distribution:

Figure 6 displays the distribution of mean hypocotyl length under control conditions.

Shapiro-Wilks: $W = 0.979$, $p = 0.172$

See section 2.7 for a full explanation of these statistics.

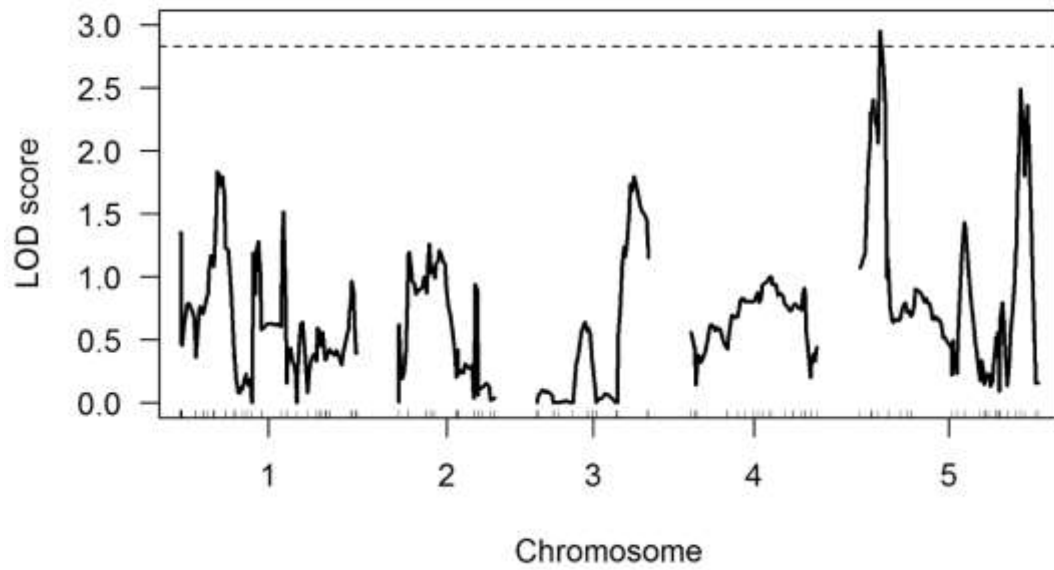



Figure 4: QTL map of mean hypocotyl length in standard conditions using the multiple-imputation method. The dotted line indicates the 20% significance threshold.

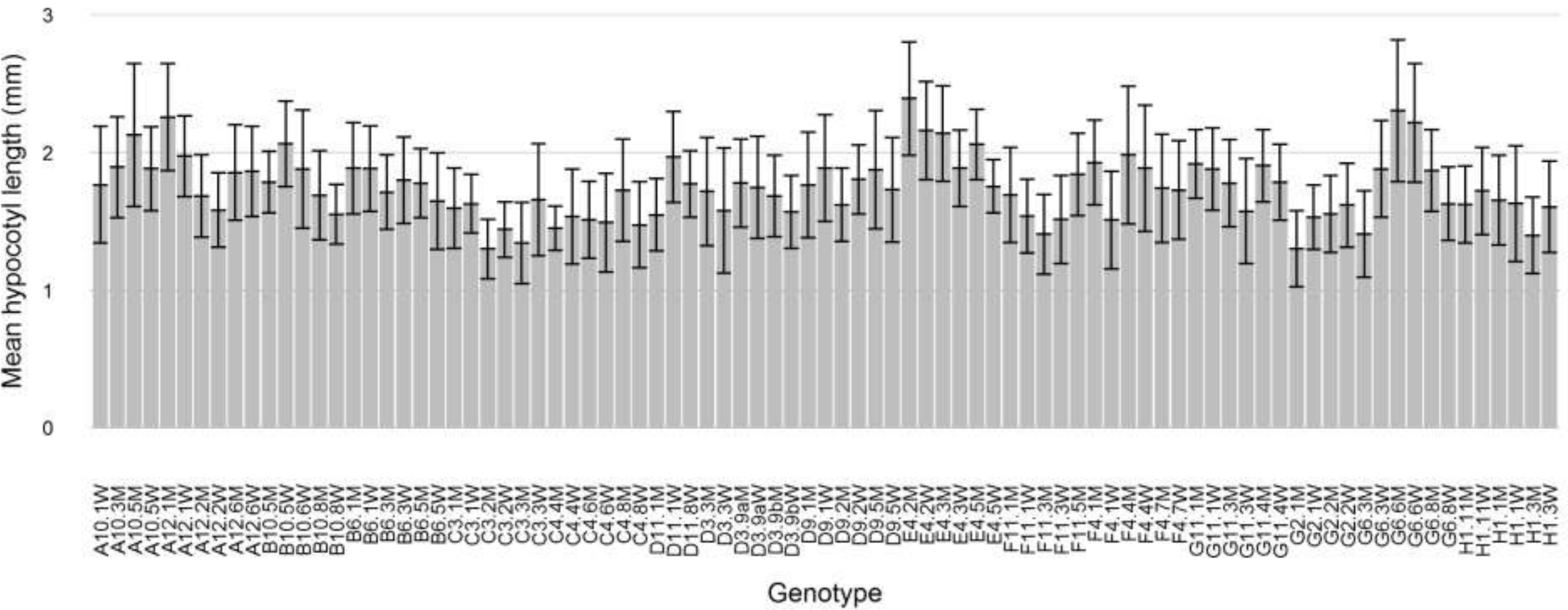



Figure 5: Mean hypocotyl length of each genotype in the mapping population in standard conditions. Error bars show 1 standard deviation.

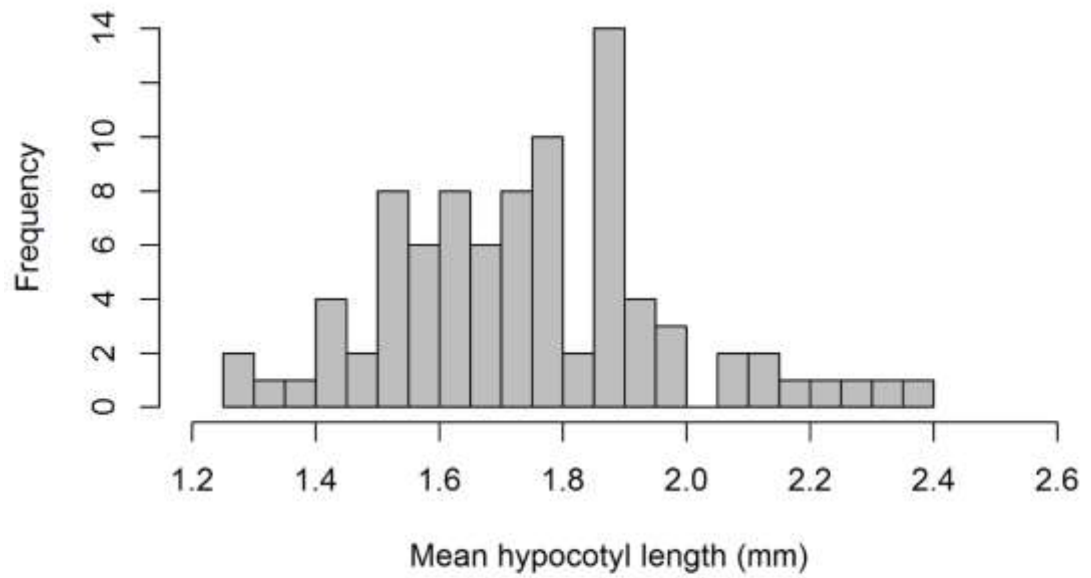



Figure 6: Histogram of mean hypocotyl length in the mapping population in standard conditions.

## Discussion

There are no known strong candidate genes for a QTL near position 13.5 on chromosome 5. It is likely to be a gene with a role in general growth and development, which would create variation in hypocotyl length if altered. However, I am hesitant to make guesses about what the gene may be.

There are some possibilities as to what may be under the peak at position 95.28. This peak is close to the mutation *hsp90.2-3*, which was used as a genetic marker in the map. The mutation makes seedlings grow taller (section 1.5) so it is reasonable to find it as a QTL when hypocotyl length is mapped. However, the mutation often produces a QTL even when the mutation does not affect the trait in question due to the unusual structure of the mapping population. The population is made of pairs of similar genotypes in which one genotype contains the mutation and the other is wild type. Because the marker is present in half the population, it is likely that there will be differences between genotypes with the mutation and wild-type genotypes. This leads to QTL analysis registering the mutation as a QTL. In combination with the physiological effect of *hsp90.2-3*, it is likely that the possible QTL on chromosome 5 has something to do with the mutation.

It should be noted that although the peak is close to the mutation, it is not at the marker *hsp90.2-3* (position 83.09). Under normal circumstances the two positions are close enough to expect them to be tightly linked. However, because the population was constructed to retain heterozygosity for the mutation, the areas around it are not linked to it. At the position of the possible QTL, 34 out of 89 lines have genetic material from the Col-0 parent and 51 have genetic material from the WS-2 parent. Four lines are heterozygous. There does not appear to be a disproportional influence from one parent at this position. Because of this I think it is very unlikely that the peak is due to the mutation. I think that this possible QTL is due to a gene that controls growth that happens to be close to the *hsp90.2-3* mutation. I found this QTL in several other QTL maps, and will refer to it as QTL-A.

## 4.3 Two-QTL scan of hypocotyl length

My single-QTL analysis did not detect every QTL that is relevant to growth in my mapping population. Single QTL mapping considers all possible QTL in isolation. Although this is a powerful and useful analysis, there is always the possibility that some important areas of the genome were not given a high LOD score. There are various reasons for this. Some QTL exist in pairs that only show their effect when both are present in the same organism. Other pairs only seem relevant if they are allowed to interact with each other. A two-dimensional, two-QTL scan considers all possible pairs of QTL (section 2.6). I analysed my data this way to reveal QTL with epistatic interactions that were invisible to a single-QTL scan.

### Results of analysis

I performed a two-dimensional, two-QTL scan on the same mapping data used in my previous analysis using the method in section 2.6. Figure 7 displays the results of this analysis. The high $LOD_{fv1}$ scores in the lower right triangle show evidence for pairs of QTL if interaction is allowed, particularly on chromosome 5. The high $LOD_i$ scores in the upper left triangle show clear evidence for interactions between QTL rather than a purely additive relationship.

I found three pairs of QTL with LOD scores high enough to be considered statistically significant. One pair is on chromosome 1 position 44.4 and chromosome 5 position 99.5. These positions can only be considered QTL in a model that allows interaction. The second pair is on chromosome 3 position 58.4 and chromosome 5 position 99.5. These positions can only be considered QTL in a model that allows interaction. The third pair is on chromosome 5 position 14.5 and chromosome 5 position 99.5. Although there is some evidence for this pair when an additive model is used, LOD scores are maximized by allowing interaction. All of these positions are likely to be pairs of QTL which only show their effect when they are allowed to interact with each other.

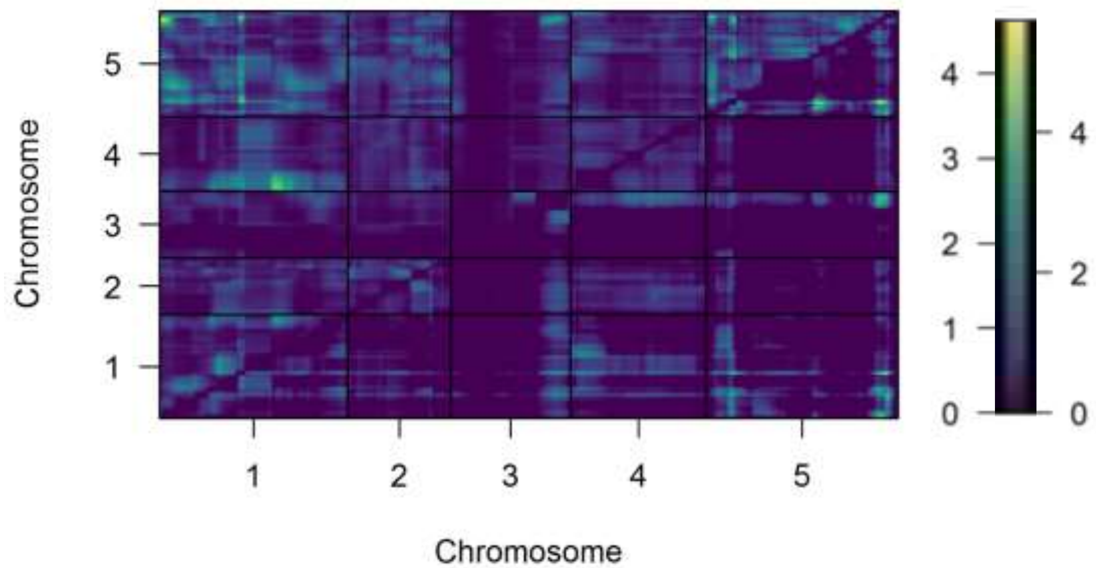



Figure 7: LOD scores from a two-QTL scan using the multiple-imputation method of mean hypocotyl length in standard conditions. The lower right triangle contains $LOD_{fv1}$ scores showing the probability of one half of a pair of epistatic QTL existing at that position. The right side of the key indicates the magnitude of $LOD_{fv1}$ scores. The upper left triangle contains $LOD_i$ scores showing the probability of synergistic interactions as opposed to an additive relationship. The left side of the key indicates the magnitude of $LOD_i$ scores.

### Discussion

The results from my two-QTL scan give a clearer picture of QTL-A. In my single-QTL scan, chromosome 5 position 95.28 had a high LOD score but was not statistically significant, so I was unsure whether I should consider it as a QTL. In a two-dimensional model where interaction is allowed it becomes clear that there is a QTL at this position, but its effect depends upon epistatic interactions. All three pairs of QTL with significant LOD scores include QTL-A. I am confident in concluding that all three pairs include the same QTL, even though positions in two-QTL scans are only estimated. Although the positions in the single-QTL and two-QTL models are not exactly the same, their close proximity and the fact that positions in two-QTL scans are only estimated makes me confident in assuming that the underlying QTL is the same in each case. It is possible that QTL-A is the *hsp90.2-3* mutation since the estimated position is very close, but as discussed previously (section 4.2), it is unlikely that this is the case. It is now clear that there is a QTL on chromosome 5 that affects growth through epistatic interactions.

## 4.4 Growth QTL present in mutant population only

### Results of analysis

To examine the effect of the *hsp90.2-3* mutation on growth, I mapped wild-type and mutant populations separately. The mapping population I used is made up of pairs of genotypes that were almost identical to each other. One genotype in the pair is wild type and the other contains the *hsp90.2-3* mutation. I used this to divide the full mapping population in half, producing two smaller populations – mutant and wild type. I then performed QTL analysis on each separately. Each population contained 44 genotypes. I mapped the average hypocotyl length of 20 individuals from each genotype in standard conditions using the method in section 2.6. Figure 8 displays the QTL maps obtained from this analysis layered over one another for easy comparison. I found no significant peaks in the wild-type population. I found one significant peak in

the mutant population on chromosome 5 at position 95.28 with a LOD score of 3.39. These peaks are possible QTL for general growth under control conditions.

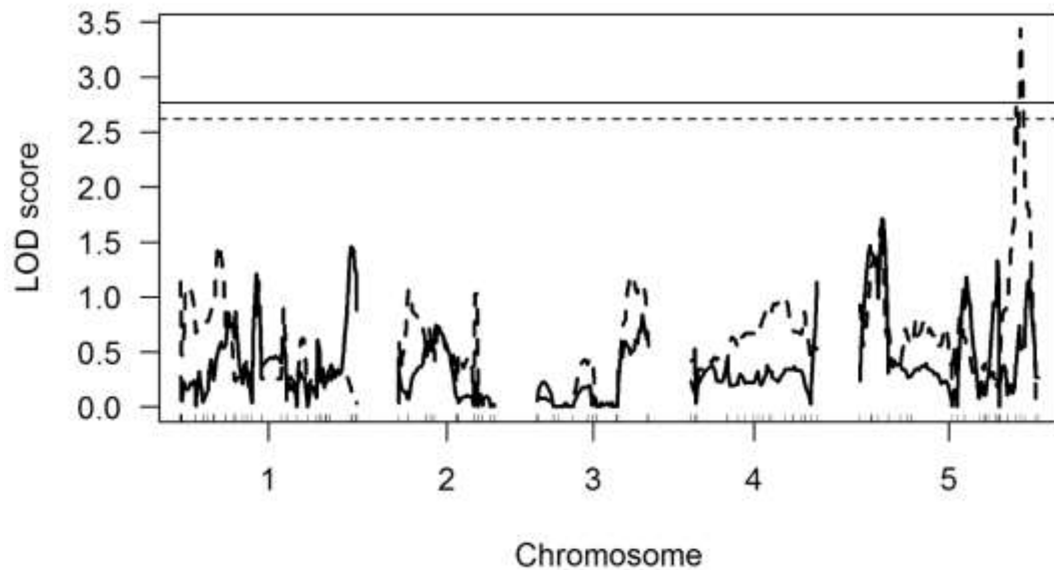



Figure 8: QTL map of mean hypocotyl length of wild-type and mutant populations in standard conditions using the multiple-imputation method. The solid line shows LOD scores of the wild-type population. The horizontal solid line shows the 20% significance threshold for the wild-type population. The dotted line shows LOD scores of the *hsp90.2-3* mutant population. The horizontal dotted line shows the 20% significance threshold for the mutant population.

## Validity of test

Wild-type population

Variation:

Figure 9A displays the mean hypocotyl length of each genotype in the wild-type population in standard conditions.

ANOVA: $F$ = 6.19, $d.f$ = 43, 818, $p$ = $<2^{e-16}$

Distribution:

Figure 9B displays the distribution of mean hypocotyl length in standard conditions in the wild-type population.

Shapiro-Wilks: $W$ = 0.950, $p$ = 0.056

This data is approximately normally distributed. I used a p-value of 0.05 as my threshold for significance, but this is arbitrary. It could easily be argued that the distribution of the trait data for the wild-type population is not appropriate for QTL mapping. I decided to proceed using this data, but its distribution could explain why I found negative results.

Mutant population

Variation:

Figure 10A displays the mean hypocotyl length of each genotype in the mutant population under control conditions.

ANOVA: $F$ = 11.76, $d.f$ = 42, 793, $p$ = $<2^{e-16}$

Distribution:

Figure 10B displays the distribution of mean hypocotyl length in standard conditions in the mutant population.

Shapiro-Wilks: $W = 0.975$, $p = 0.475$

See section 2.7 for a full explanation of these statistics.

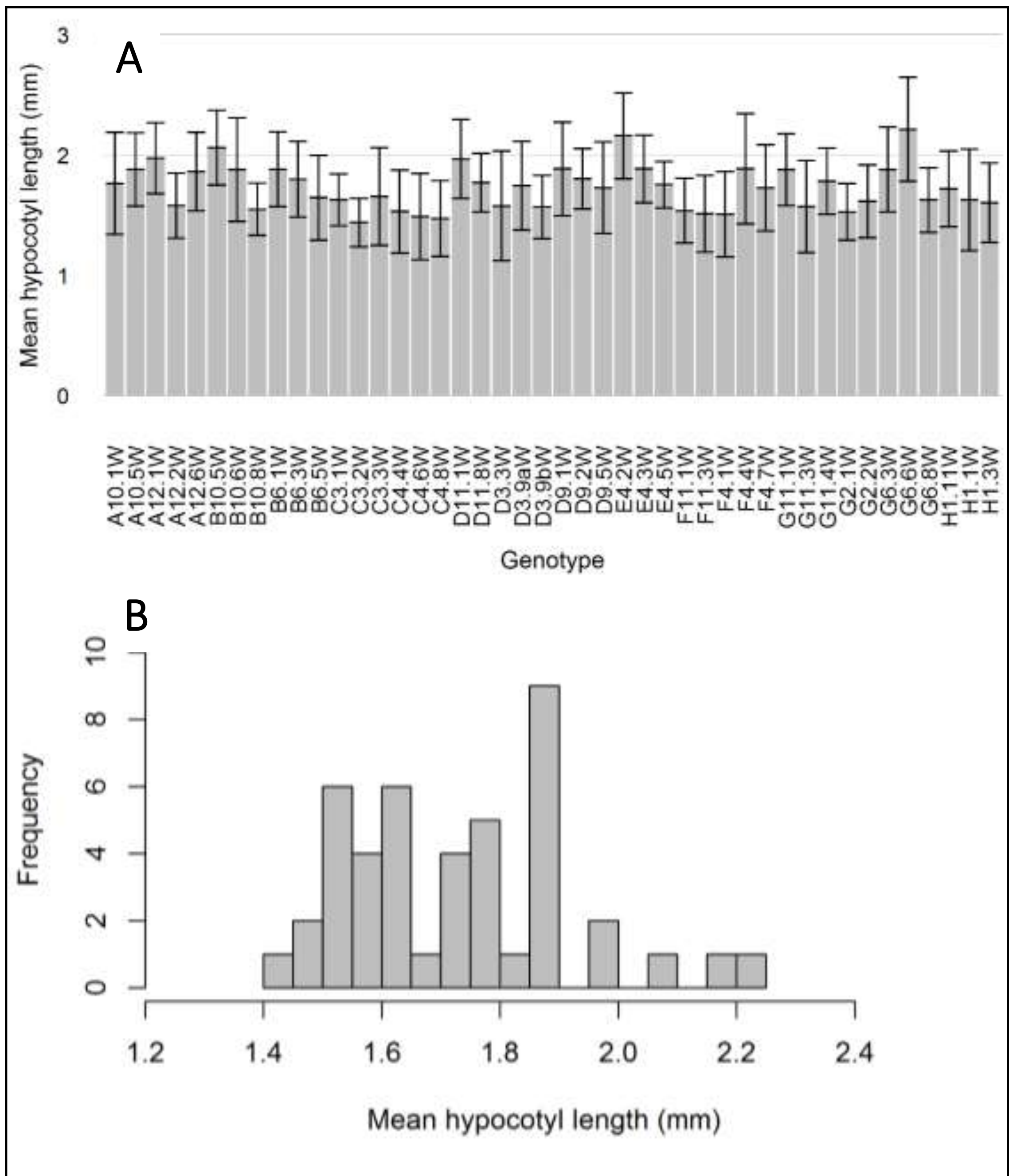



Figure 9: Validity of wild-type population hypocotyl length data in standard conditions for QTL mapping.

A: Mean hypocotyl length of each genotype in the population. Error bars show 1 standard deviation.

B: Histogram of mean hypocotyl length of each genotype in the population.

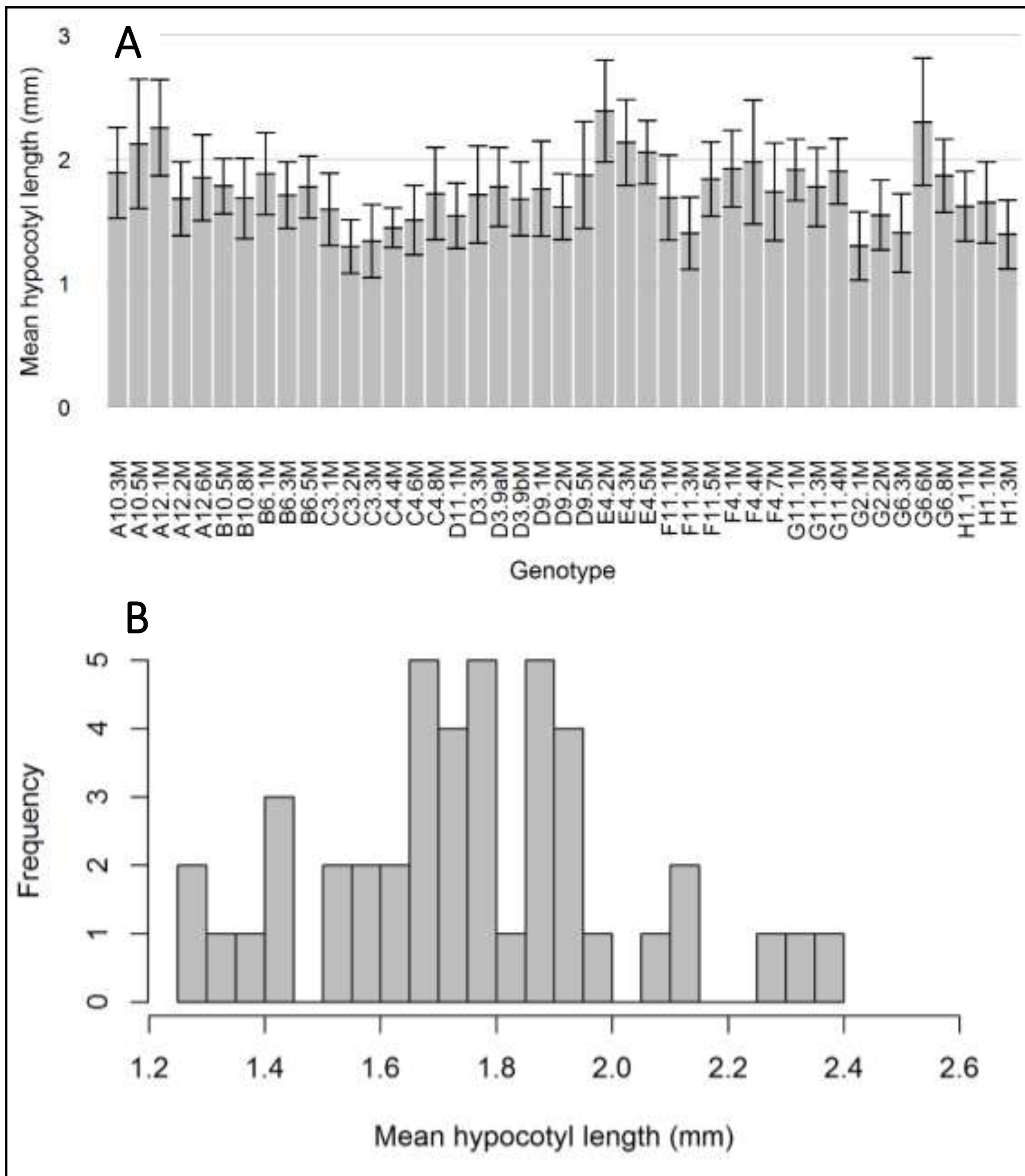



Figure 10: Validity of *hsp90.2-3* mutant population hypocotyl length data in standard conditions for QTL mapping.

A: Mean hypocotyl length of each genotype in the population. Error bars show 1 standard deviation.

B: Histogram of mean hypocotyl length of each genotype in the population.

## Discussion

The lack of any significant QTL in the wild-type population for hypocotyl length could have been due to the statistical test. One possibility is that the smaller population (half the size of the full mapping population) created a lack of statistical power. This would mean that some of the non-significant peaks are actually QTL, but more data was needed to increase their LOD scores. However, I do not think the size of the population was a problem as the similarly sized mutant population provided positive results. An alternative explanation is that the wild-type population could have contained many QTL of small effect. Difficulty detecting QTL of small effect is a common problem with QTL analysis but would have been made worse by a smaller mapping population. QTL of small effect often show their effect more clearly when a certain combination of QTL is present in the same organism. For this reason, a two-QTL scan could reveal QTL that did not appear to be significant in a single-QTL scan. A third explanation is that that QTL in the wild-type population are being hidden by the action of heat shock proteins, and the wild-type population contains QTL similar to the mutant population. Another explanation is that the test did not work as it should due to the distribution of the wild-type population. This would also mean that QTL that exist were not detected. This possibility could be investigated further by transforming the trait data and creating a new QTL map. It is possible that there were simply no QTL for this trait in the wild-type population. I find this unlikely due to the significant amount of variation in the trait, and the fact that this variation is due in part to genotype, but it is worth considering. I think it is likely that the QTL analysis did not detect any QTL due to unsuitable data and there are significant QTL in the population.

The QTL in the mutant population is QTL-A, which was detected when the hypocotyl length of the full population was mapped. QTL-A was especially important in a model that allowed epistasis. It is interesting that QTL-A was detected in a single-QTL scan of growth in the mutant population but not the wild-type population. One explanation is that QTL-A is either the mutation itself or a gene linked to it, but I explained why I think this is unlikely in section 4.2. I think that QTL-A is a gene close to the mutation. Its

presence in the mutant population but not the wild-type population could be due to the effect that *hsp90.2-3* has on QTL mapping (section 1.5).

## 4.5 Two-QTL scan for epistatic interactions in mutant population

My single-QTL analysis did not detect every QTL relevant to growth in my mapping population. As discussed previously (section 4.3) single-QTL mapping rarely reveals every significant QTL, making further analysis using two-QTL scans necessary. I analysed my data this way to reveal QTL with epistatic interactions that were invisible to a single-QTL scan.

### Results of analysis

I performed a two-dimensional, two-QTL scan on the same mapping data used in my previous analysis using the method in section 2.6. I found no significant QTL in the wild-type population. Figure 11 displays the results of my analysis for the mutant population. The high $LOD_{fv1}$ scores in the lower right triangle show evidence for pairs of QTL if interaction is allowed, especially on chromosome 5. The high $LOD_{i}$ scores in the upper left triangle show clear evidence for epistatic interactions rather than an additive relationship.

I found two pairs of QTL in the mutant population with LOD scores high enough to be considered statistically significant. The first pair is on chromosome 3 position 56.4 and chromosome 5 position 95.5. These positions can only be considered as QTL in a model that allows interaction.  The second pair is on chromosome 5 position 15.5 and chromosome 5 position 95.5. These positions can only be considered as QTL in a model that allows interaction. All of these positions are likely to be pairs of QTL which only show their effect when they are allowed to interact with each other.

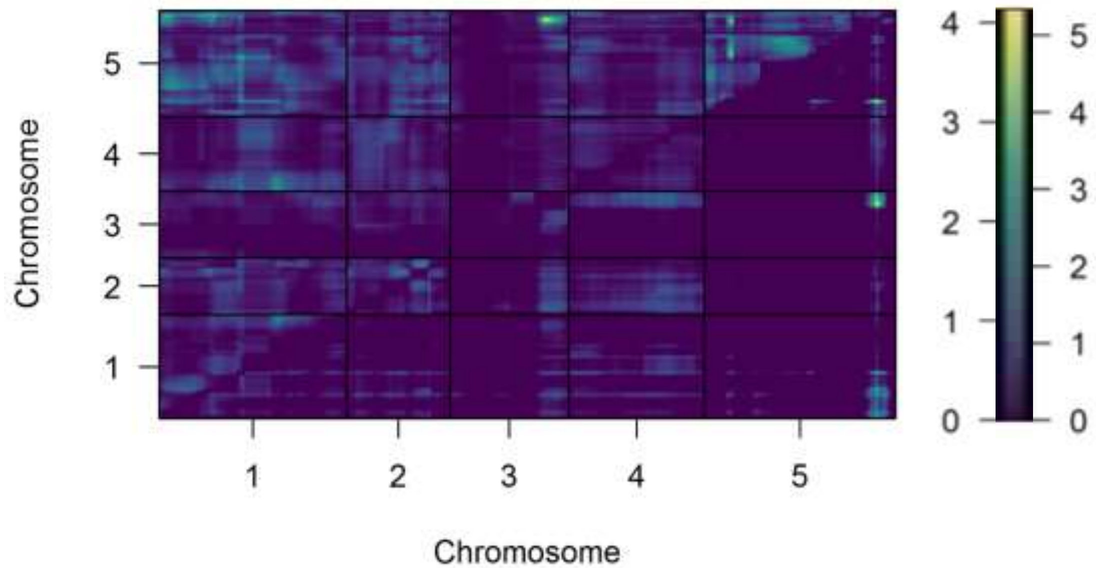



Figure 11: LOD scores from a two-QTL scan using the multiple-imputation method of mean hypocotyl length of a population containing the *hsp90.2-3* mutation in standard conditions. The lower right triangle contains $LOD_{fv1}$ scores showing the probability of one half of a pair of epistatic QTL existing at that position. The right side of the key indicates the magnitude of $LOD_{fv1}$ scores. The upper left triangle contains $LOD_i$ scores showing the probability of synergistic interactions as opposed to an additive relationship. The left side of the key indicates the magnitude of $LOD_i$ scores.

### Discussion

The pairs of QTL found in the mutant population make it clear that QTL-A has epistatic interactions with several other QTL. This supports my results from section 4.3. It is interesting that there are fewer epistatic pairs in the mutant population than there were in the full population. This could be a natural result of using a smaller population, but it could also mean that one or more of the genes that QTL-A interacts with is part of the HSP90 family, which is inactivated by the mutation.

## 4.6 QTL mapping of standard deviation

I wanted to investigate QTL that affected variation. I used the standard deviation of the mean hypocotyl length of each genotype as a measure of how consistent the growth of seedlings was. By using standard deviation as a trait in QTL mapping I found QTL that were associated with regulating growth.

### Results of analysis

QTL analysis of the variation of the mapping population in standard conditions revealed one possible QTL. I mapped the standard deviation of the mean hypocotyl length of 20 individuals from each of 89 genotypes in standard conditions using the method in section 2.6. Figure 12 displays the QTL map obtained from this analysis. I found one peak located on chromosome 5 at position 96.52 with a LOD score of 2.90. This puts it close to the significance threshold (2.99) but not crossing it. This peak is a possible QTL for growth under control conditions.

### Validity of test

Variation:

Figure 13 displays the standard deviation of the mean hypocotyl length of each genotype in my mapping population in standard conditions.

Distribution:

Figure 14 displays the distribution of the standard deviation of the mean hypocotyl length in standard conditions.

Shapiro-Wilks: $W = 0.978$, $p = 0.144$

See section 2.7 for a full explanation of these statistics.

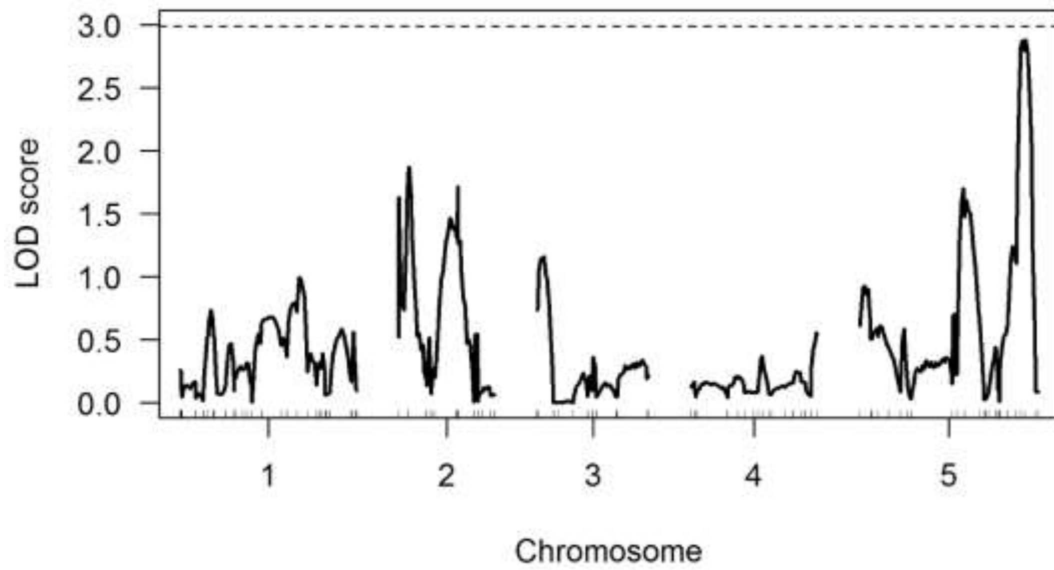



Figure 12: QTL map of the standard deviation of mean hypocotyl length in standard conditions using the multiple-imputation method. The dotted line indicates the 20% significance threshold.

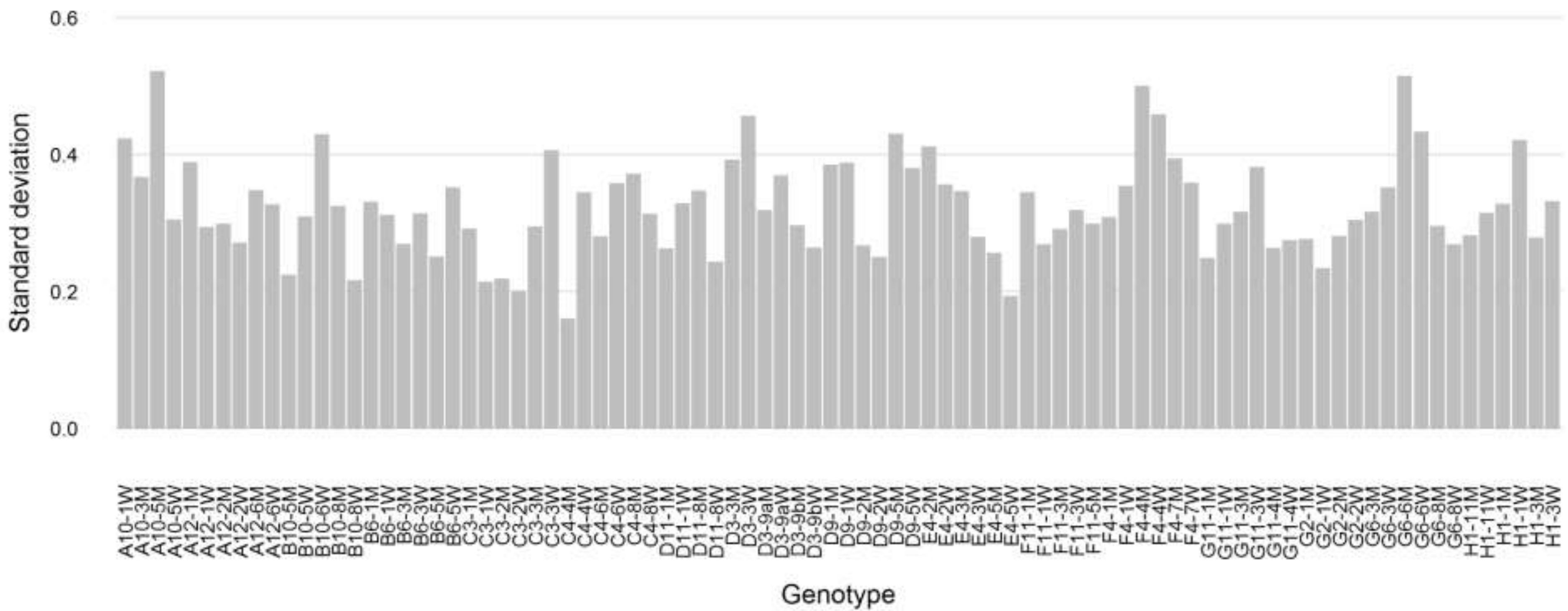



Figure 13: Standard deviation of hypocotyl length for each genotype in the full mapping population in standard conditions.

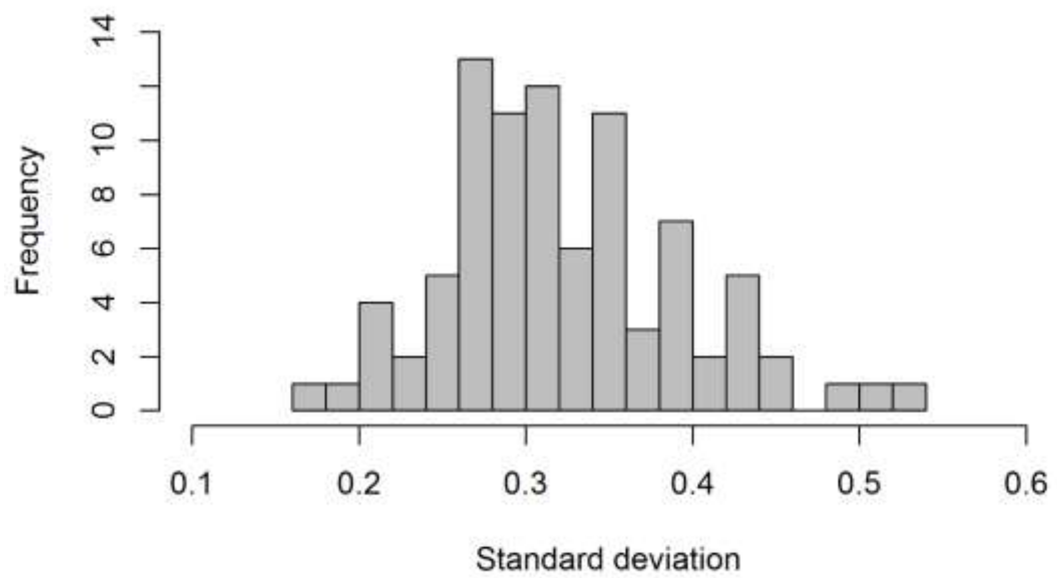



Figure 14: Histogram of the standard deviation of hypocotyl length of the full mapping population in standard conditions.

### Discussion

The possible QTL on chromosome 5 is QTL-A, which I found in several other QTL maps (sections 4.2 and 4.3). A peak at this position appeared when growth of the whole population and mutant population was mapped under control conditions and with exogenous auxin. Two-QTL scans of the same populations revealed that this QTL exerts its effect through epistatic interactions with several other QTL. The positions are close enough for me to assume that these peaks all represent QTL-A. I concluded that QTL-A is a gene close to the *hps90.2-3* mutation and may interact with other heat shock proteins (section 4.2). The QTL in this new analysis of standard deviation is close enough to QTL-A for me to assume that they represent the same gene or genes. This new evidence suggests two things to me.  Firstly, the QTL affects variation as well as growth. Secondly, its effect on variation may be similarly reliant on epistatic interactions, as the peak does not quite reach statistical significance in a way similar to when I mapped growth for the whole population. It is clear that QTL-A plays a significant role in growth and development.

## 4.7 QTL for standard deviation in wild-type population only

### Results of analysis

As explained previously (section 4.4), I examined the effect of the *hsp90.2-3* mutation on standard deviation by performing QTL analysis on the wild-type and mutant populations separately. Each population contained 44 genotypes. I mapped the standard deviation of the mean hypocotyl length of 20 individuals from each genotype in the wild-type population in standard conditions using the method in section 2.6. I also mapped the log-transformed standard deviation of mean hypocotyl length in the mutant population. Figure 15 displays the QTL maps obtained from this analysis layered over one another for easy comparison. I found no significant peaks in the mutant population. I found one peak with a high LOD score in the wild-type population on

chromosome 2 at position 35.51 with a LOD score of 2.296. This peak is a possible QTL for variation in standard conditions in the wild-type population.

## Validity of test

Wild-type population

Variation:

Figure 16B displays the standard deviation of the mean hypocotyl length of each genotype in the wild-type population in standard conditions.

Distribution:

Figure 16A displays the distribution of standard deviation of the mean hypocotyl length in standard conditions in the wild-type population.

Shapiro-Wilks: $W$ = 0.981, $p$ = 0.686

Mutant population

Distribution:

Figure 17 displays the distribution of the standard deviation of the mean hypocotyl length in standard conditions in the mutant population.

Shapiro-Wilks: $W$ = 0.934, $p$ = 0.014

These data were not normally distributed so were not suitable for QTL mapping. I transformed the mutant population data using log(x +1).

Figure 18A displays the distribution of the transformed data.

Shapiro-Wilks: $W$ = 0.948, $p$ = 0.048

Variation:

Figure 18B displays the log-transformed standard deviation of the mean hypocotyl length of each genotype in the mutant population in standard conditions.

See section 2.7 for a full explanation of these statistics.

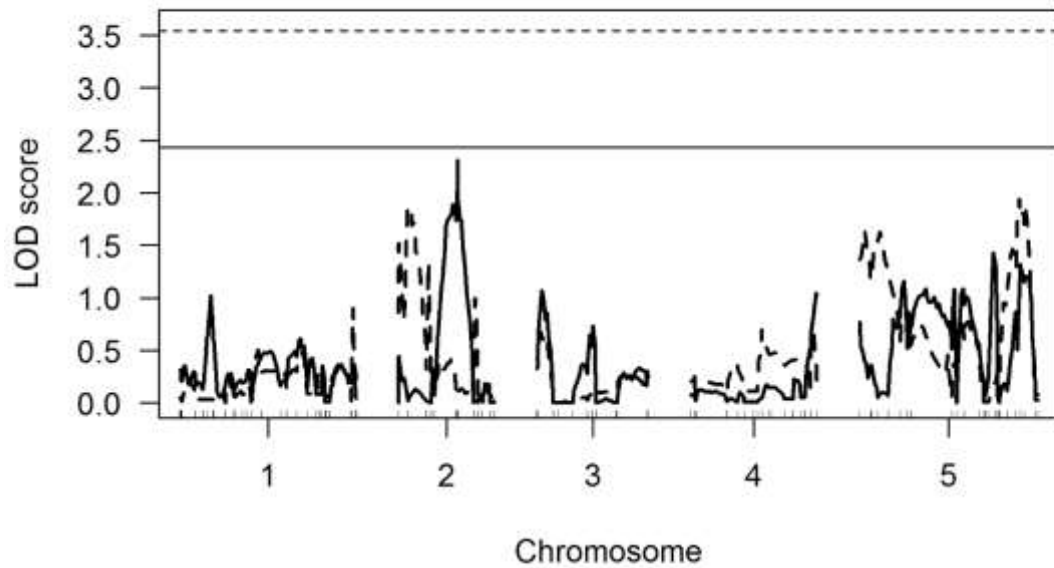



Figure 15: QTL map of the standard deviation of mean hypocotyl length in standard conditions using the multiple-imputation method. The solid line shows LOD scores of the wild-type population. The horizontal solid line shows the 20% significance threshold for the wild-type population. The dotted line shows LOD scores of the *hsp90.2-3* mutant population. The horizontal dotted line shows the 20% significance threshold for the mutant population.

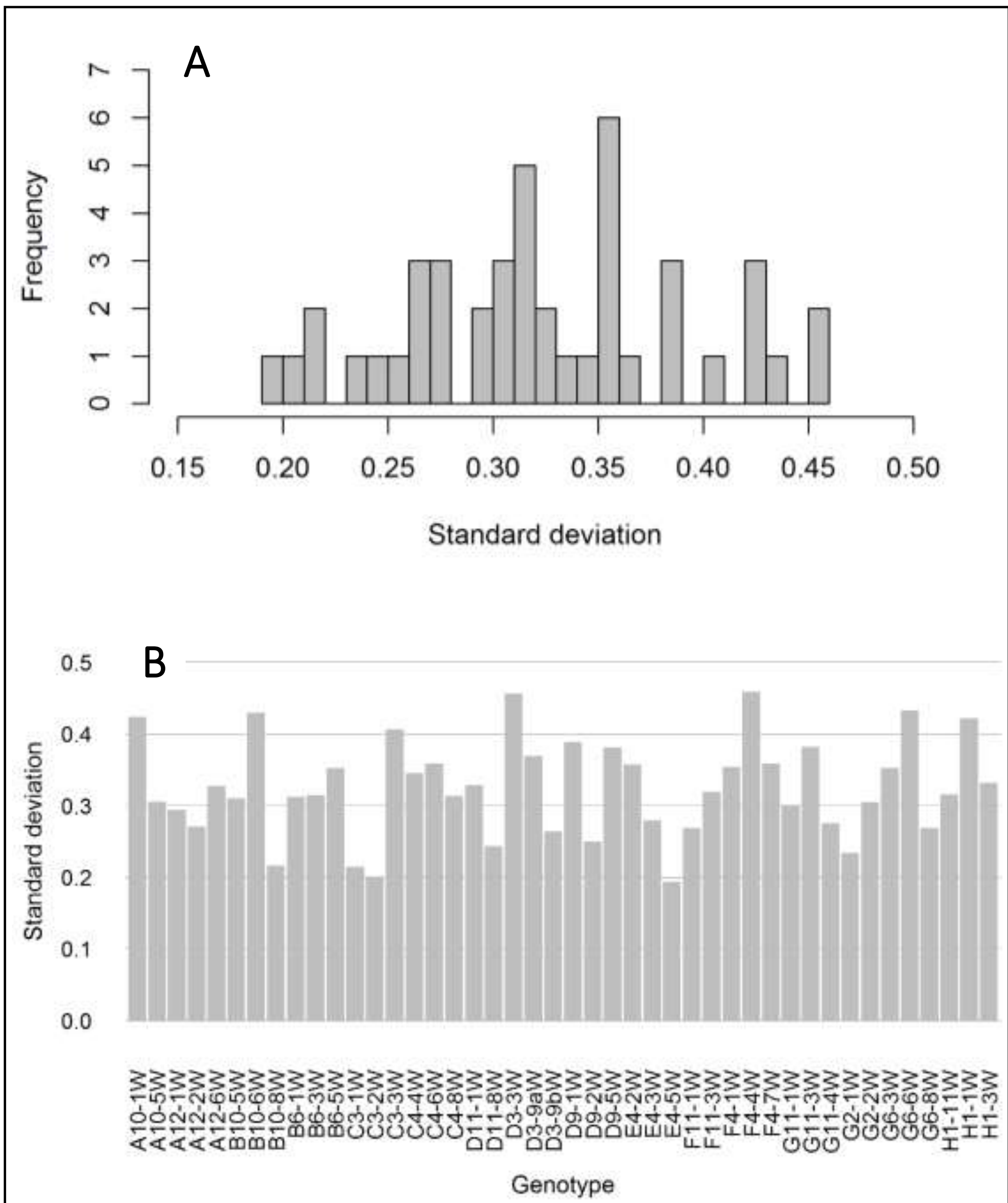



Figure 16: Validity of wild-type population standard deviation of hypocotyl length data for QTL mapping.

A: Histogram of standard deviation of hypocotyl length of each genotype in the population.

B: Standard deviation of hypocotyl length of each genotype in the population.

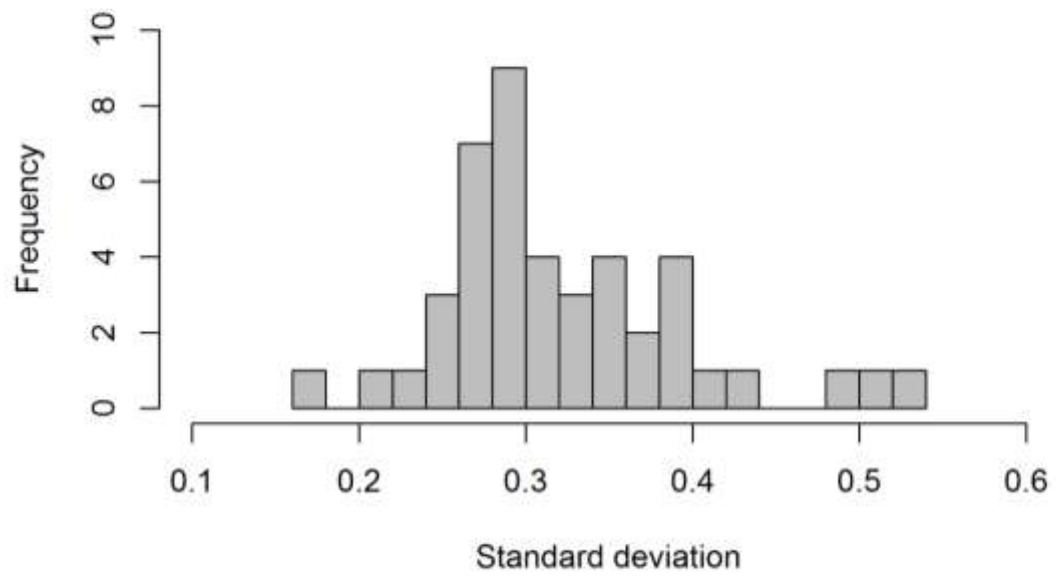



Figure 17: Histogram of the standard deviation of hypocotyl length in the *hsp90.2-3* mutant population in standard conditions.

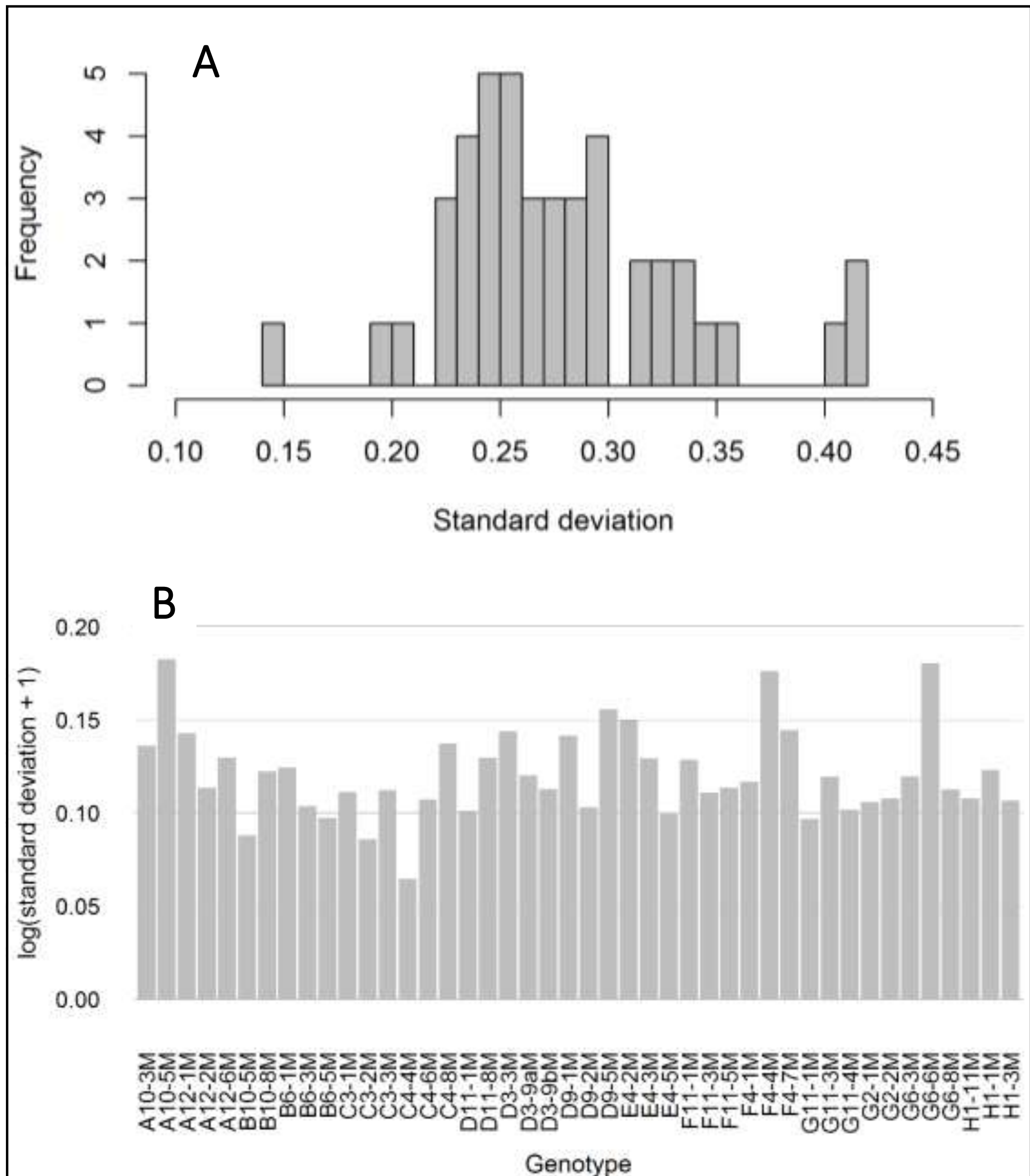



Figure 18: Validity of *hps90.2-3* mutant population log-transformed standard deviation of hypocotyl length data for QTL mapping.

A: Histogram of log-transformed standard deviation of hypocotyl length of each genotype in the population.

B: Standard deviation of log-transformed hypocotyl length of each genotype in the population.

### Discussion

There are several possible explanations for why the peak on chromosome 2 in the wild-type population falls short of statistical significance. These explanations are discussed in detail in section 4.4. In short, there are three possibilities for this population. They are a lack of statistical power caused by a relatively small population size, many QTL of small effect which could be detected by a two-QTL scan, or a false positive result. All of these explanations also apply to the mutant population. If the possible QTL in the wild-type population is a genuine result, it appears to be a QTL unique to this trait, condition and population, as it is not seen in any other QTL map. Although it is impossible to be sure, this position on chromosome 2 may be a QTL for variation in the wild-type population.

## 4.8 Summary of results

The most interesting result presented in this chapter is the QTL on chromosome 5 near position 95, which I have called QTL-A. It is clear through mapping hypocotyl length and standard deviation in the full mapping population that QTL-A is important for both growth and variation. Further discussion of QTL-A can be found in chapter 7.

Two-QTL scans revealed that QTL-A interacts with several others. These other QTL were only detected in a full model that allowed QTL to truly interact, as opposed to an additive model where QTL simply co-exist. QTL-A appears to play a major role in growth and a more minor role in regulating variation, as seen by its reduced LOD score when standard deviation was mapped. Any effect QTL-A has on variation appears to be through its own action alone, as two-QTL scans returned negative results. It is unknown what may be under QTL-A, but its role in growth is clear.

# Chapter 5: QTL analysis of growth and variation with exogenous auxin

## 5.1 Overview

This chapter presents the results of my analysis of growth and variation in the presence of exogenous auxin, specifically the non-polar synthetic auxin picloram. I looked for QTL for two traits, hypocotyl length and the standard deviation of hypocotyl length, in three mapping populations: the full mapping population, the wild-type half of the mapping population and the half of the mapping population containing the *hsp90.2-3* mutation. I performed single-QTL and two-QTL scans to search for QTL that act on their own and those that act through epistatic interactions. I also analysed a third trait, the change in hypocotyl length found by comparing hypocotyl length with exogenous auxin to the same genotype in standard conditions. I found several QTL using these methods. Some were not found in any other QTL map and may be unique to growth with exogenous auxin. I found some QTL that have a role in regulating variation in the presence of exogenous auxin. The role of QTL-A, found as a growth QTL in standard conditions, is supported and made more detailed by the results in this chapter. The results presented in this chapter build on the results presented previously and identify QTL important to growth with exogenous auxin.

## 5.2 QTL mapping of hypocotyl length with exogenous auxin

I wanted to investigate QTL for auxin-induced growth. Exogenous auxin increases hypocotyl length at certain concentrations (Evans 1985, Collett et al. 2000). I measured the hypocotyl length of each genotype in my mapping population with 3µM picloram added to the growth medium using the method in section 2.5. The full data are presented in appendix 4. By using hypocotyl length as a trait in QTL mapping I found QTL that control seedling growth when exogenous auxin is present.

## Results of analysis

QTL mapping of growth with exogenous auxin revealed one QTL. I mapped the mean hypocotyl length of 20 individuals from 85 genotypes grown on media containing 3µM picloram using the method in section 2.6. Figure 19 displays the QTL map obtained from this analysis. I found one significant peak on chromosome 5 at position 95.3 with a LOD score of 2.85. This peak is a possible QTL for growth with exogenous auxin.

## Validity of test

Variation:

Figure 20 displays the mean hypocotyl length of each genotype in my mapping population when grown with exogenous auxin.

ANOVA: $F$ = 22.82, $d.f$ = 8, 1644, $p$ = $<2^{e-16}$

Distribution:

Figure 21 displays the distribution of mean hypocotyl length when grown with exogenous auxin.

Shapiro-Wilks: $W$ = 0.988, $p$ = 0.587

See section 2.7 for a full explanation of these statistics.

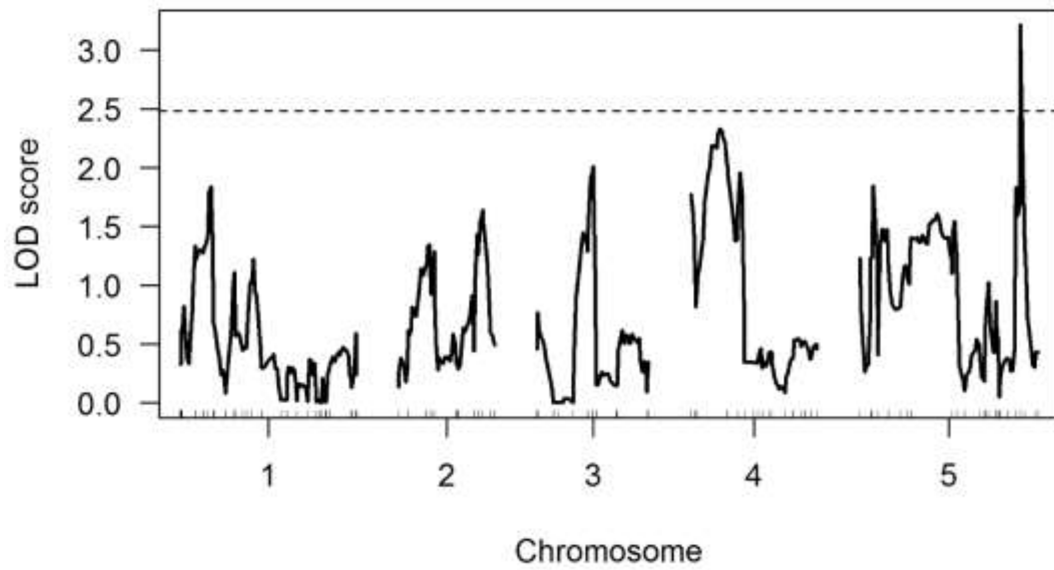



Figure 19: QTL map of mean hypocotyl length with exogenous auxin using the multiple-imputation method. The dotted line indicates the 20% significance threshold.

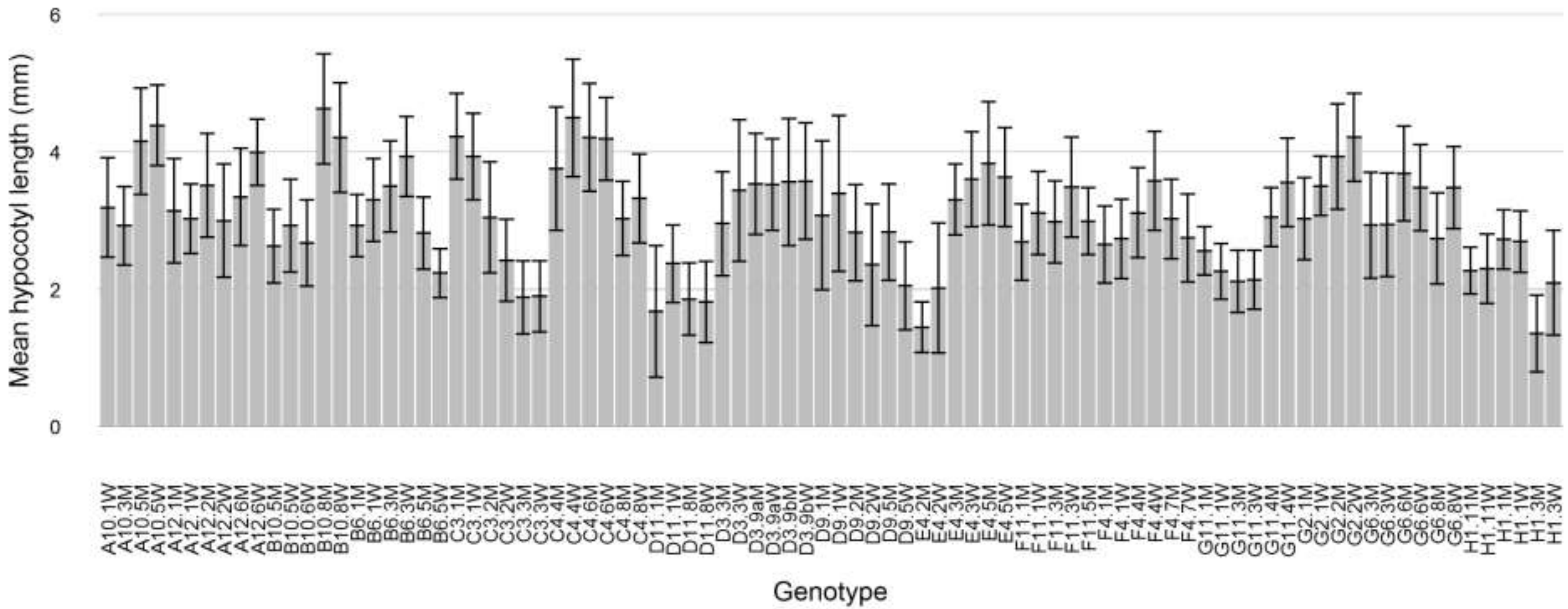



Figure 20: Mean hypocotyl length of each genotype in the mapping population with 3µM picloram media. Error bars show 1 standard deviation.

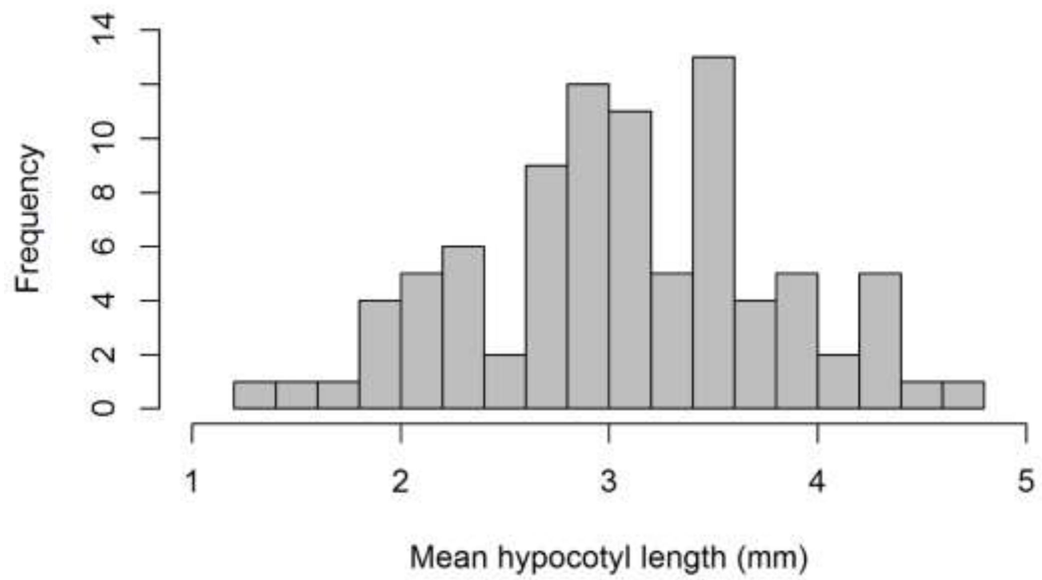



Figure 21: Histogram of mean hypocotyl length in the full mapping population with 3µM picloram media.

### Discussion

The significant peak on chromosome 5 at position 95.3 is shared between several of my QTL maps. I previously called it QTL-A. As well as this map, QTL-A is present in the QTL maps for growth in standard conditions (although it does not quite pass the significance threshold) and growth in standard conditions in the mutant half of the population. Two-QTL scans of growth in standard conditions revealed that QTL-A interacts with several other QTL. QTL-A appeared to have a small effect on variation as well as growth (section 4.). I previously hypothesised that QTL-A was a general growth QTL which exerted its effect through epistatic interactions. This new evidence suggests that the effect of QTL-A can be enhanced using exogenous auxin. QTL-A may be part of an auxin signalling pathway. It is also possible that one of the QTL that QTL-A can interact with is part of an auxin signalling pathway. This could be further investigated by a two-QTL scan. If QTL-A is part of an auxin signalling pathway, I would expect to see it not only in the results of this chapter, but also in the QTL maps for growth at warm temperatures, as warmth naturally increases auxin levels within the plant (Stavang et al. 2009, Wigge 2013). The role of QTL-A is complex and involves many different genes, but it appears to be linked to an auxin signalling pathway in some way.

## 5.3 Two-QTL scan of hypocotyl length with exogenous auxin

As discussed previously (section 4.8) single-QTL mapping rarely reveals every significant QTL, making further analysis using two-QTL scans necessary. This is especially important in the case of QTL-A, which has already been shown to interact with several other QTL in standard conditions. I analysed my data using a two-dimensional, two-QTL scan to investigate interactions between QTL-A and other loci in the presence of exogenous auxin.

### Results of analysis

I performed a two-dimensional, two-QTL scan on the same mapping data using the method in section 2.6. Figure 22 displays the results of this analysis. The high $LOD_{fv1}$

scores in the lower right triangle show evidence for pairs of QTL if interaction is allowed, particularly on chromosome 2. The high $LOD_i$ scores in the upper left triangle show clear evidence for epistatic interactions rather than an additive relationship.

I found four pairs of QTL with LOD scores high enough to be considered statistically significant. One pair is on chromosome 1 position 1.38 and chromosome 1 position 9.38. These positions can only be considered QTL in an additive model. The second pair is on chromosome 1 position 44.4 and chromosome 2 position 36.5. These positions can only be considered QTL in a model that allows interaction. The third pair is on chromosome 1 position 43.4 and chromosome 3 position 35.4. These positions can only be considered QTL in a model that allows interaction. The final pair is on chromosome 2 position 35.5 and chromosome 3 position 36.4. These positions can only be considered QTL in a model that allows interaction. All of these positions are likely to be pairs of QTL which only show their effect when they are allowed to interact with each other.

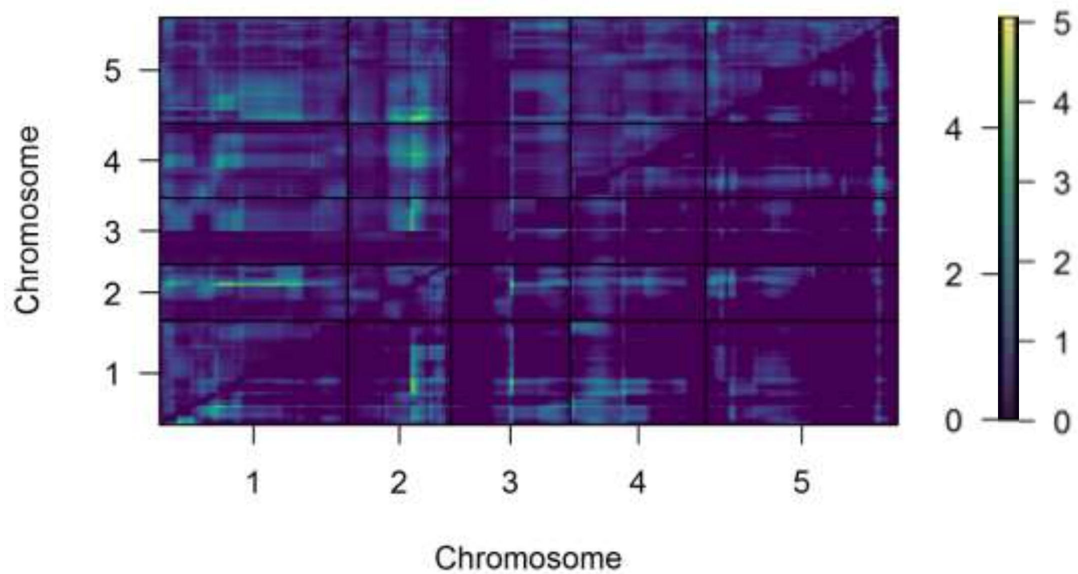



Figure 22: LOD scores from a two-QTL scan using the multiple-imputation method of mean hypocotyl length with exogenous auxin. The lower right triangle contains $LOD_{fv1}$ scores showing the probability of one half of a pair of epistatic QTL existing at that position. The right side of the key indicates the magnitude of $LOD_{fv1}$ scores. The upper left triangle contains $LOD_i$ scores showing the probability of synergistic interactions as opposed to an additive relationship. The left side of the key indicates the magnitude of $LOD_i$ scores.

### Discussion

Most of the QTL found in this analysis seem to be unique to growth with exogenous auxin. The only position seen in another analysis is chromosome 1 position 44.4, which was part of an epistatic pair for growth in standard conditions. The QTL found in this analysis are likely to be genes with a role in auxin production or signal transduction, as changes to these genes would result in variation in hypocotyl length with exogenous auxin.

One notable absence is QTL-A. Since it seemed to have an effect on growth with exogenous auxin, and in standard conditions it exerted its effect through epistatic interactions, I expected QTL-A to be part of at least one epistatic pair in this analysis. This unexpected result could be due to three things. Firstly, QTL-A could have a strong effect on growth through its own action alone. Secondly, the effect of the genes QTL-A interacts with could be much smaller with exogenous auxin, while the action of other genes is enhanced. This would lead to the pairs including QTL-A not being detected by the analysis while others were. Finally, the genes QTL-A was previously interacting with could now be interacting with other QTL, as seems to be the case with the QTL on chromosome 1 position 44.4. In reality, all three elements in combination probably produced this result. QTL-A must still have a significant effect on its own or it would not have been detected in the single-QTL scan, while the change in conditions could have altered the action of other genes.

## 5.4 QTL for growth with exogenous auxin in mutant population only

### Results of analysis

As explained previously (section 4.4), I examined the effect of the *hsp90.2-3* mutation on growth in the presence of exogenous auxin by mapping the wild-type and mutant populations separately. Each population contained 44 genotypes. I mapped the mean

hypocotyl length of 20 individuals from each genotype grown on media containing 3µM picloram using the method in section 2.6. Figure 23 displays the QTL maps obtained from this analysis layered over one another for easy comparison. I found no significant QTL in the wild-type population. I found two regions of high LOD score in the mutant population that did not quite cross the significance threshold. The peaks were both on chromosome 5. One is at position 0.52 and has a LOD score of 2.26. The other is at position 95.28 with a LOD score of 2.24. The significance threshold in this population is a LOD score of 2.39. It is possible that the two peaks on chromosome 5 are QTL with a role in growth in the presence of exogenous auxin.

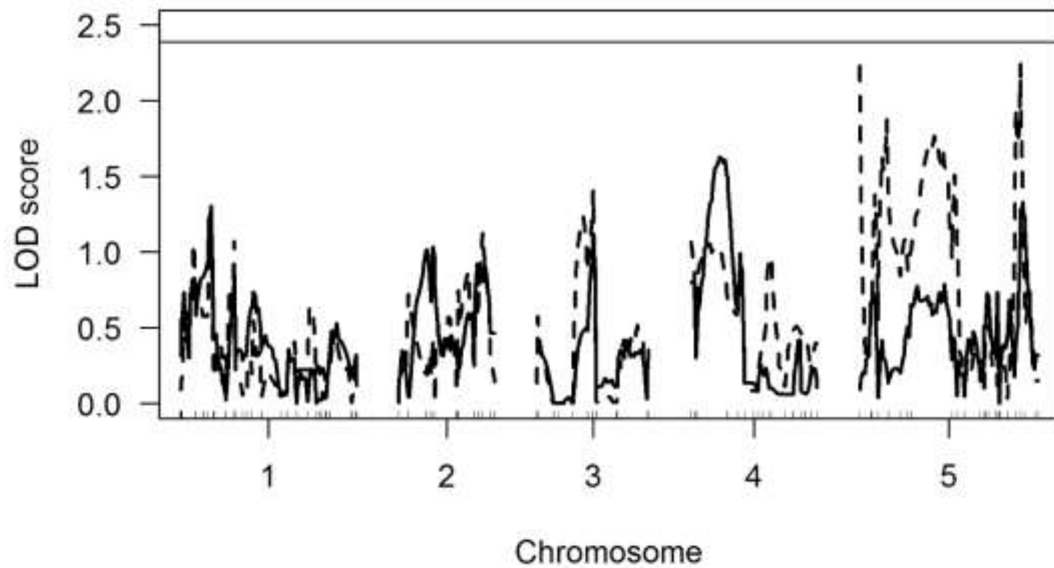



Figure 23: QTL map of mean hypocotyl length in wild-type and mutant populations with exogenous auxin using the multiple-imputation method. The solid line shows LOD scores of the wild-type population. The horizontal solid line shows the 20% significance threshold for the wild-type population. The dotted line shows LOD scores of the *hsp90.2-3* mutant population. The horizontal dotted line shows the 20% significance threshold for the mutant population.

## Validity of test

Wild-type population

Variation:

Figure 24A displays the mean hypocotyl length of each genotype in the wild-type population with exogenous auxin.

ANOVA: $F$ = 23.05, $d.f$ = 43, 818, $p$ = $<2^{e-16}$

Distribution:

Figure 24B displays the distribution of mean hypocotyl length in the wild-type population with exogenous auxin.

Shapiro-Wilks: $W$ = 0.961, $p$ = 0.140

Mutant population

Variation:

Figure 25A displays the mean hypocotyl length of each genotype in the mutant population with exogenous auxin.

ANOVA: $F$ = 22.83, $d.f$ = 43, 826, $p$ = $<2^{e-16}$

Distribution:

Figure 25B displays the distribution of mean hypocotyl length in the mutant population with exogenous auxin.

Shapiro-Wilks: $W$ = 0.968, $p$ = 0.265

See section 2.7 for a full explanation of these statistics.

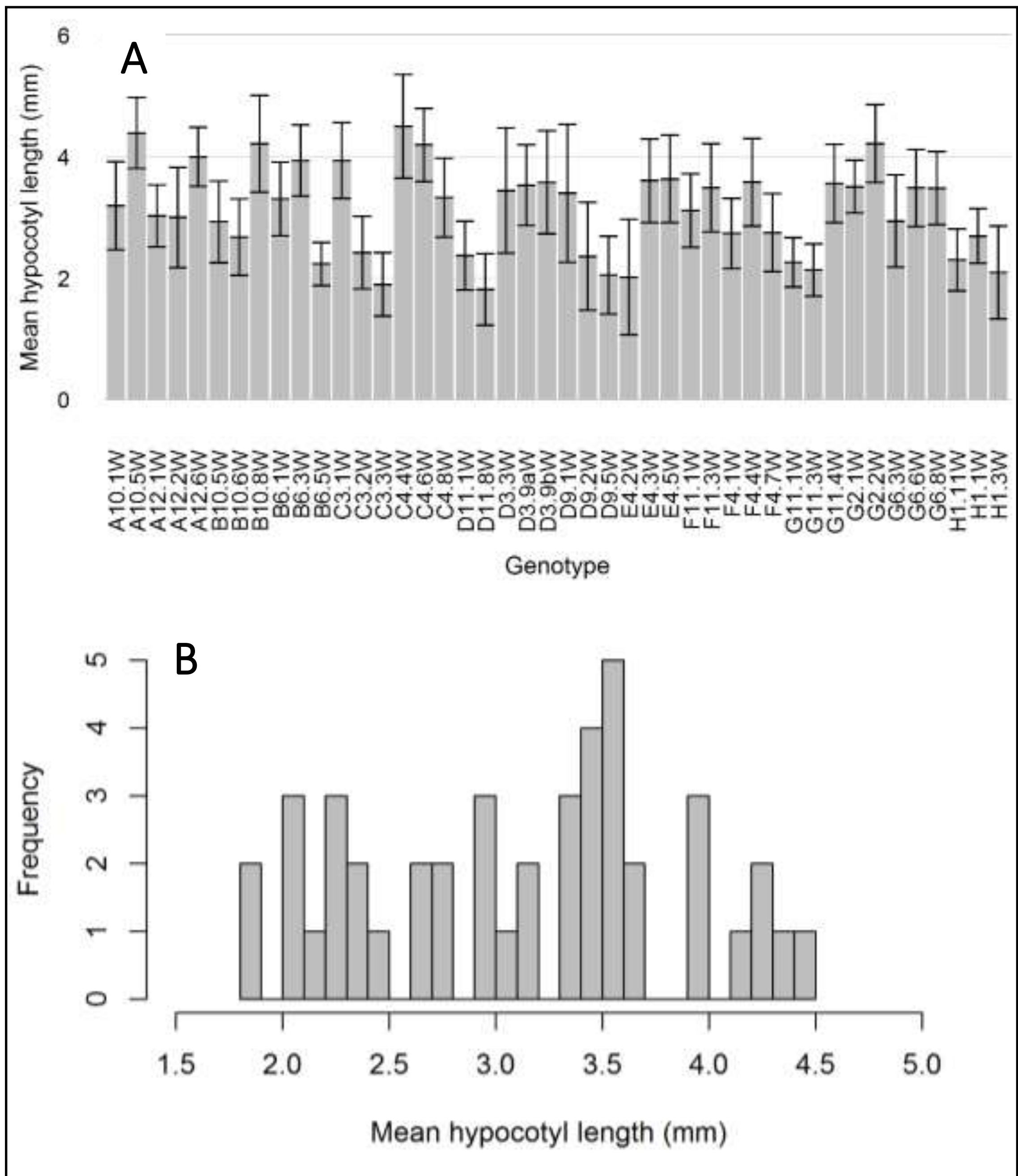



Figure 24: Validity of wild-type population hypocotyl length data with 3µM picloram media for QTL mapping.

A: Mean hypocotyl length of each genotype in the population. Error bars show 1 standard deviation.

B: Histogram of mean hypocotyl length of each genotype in the population.

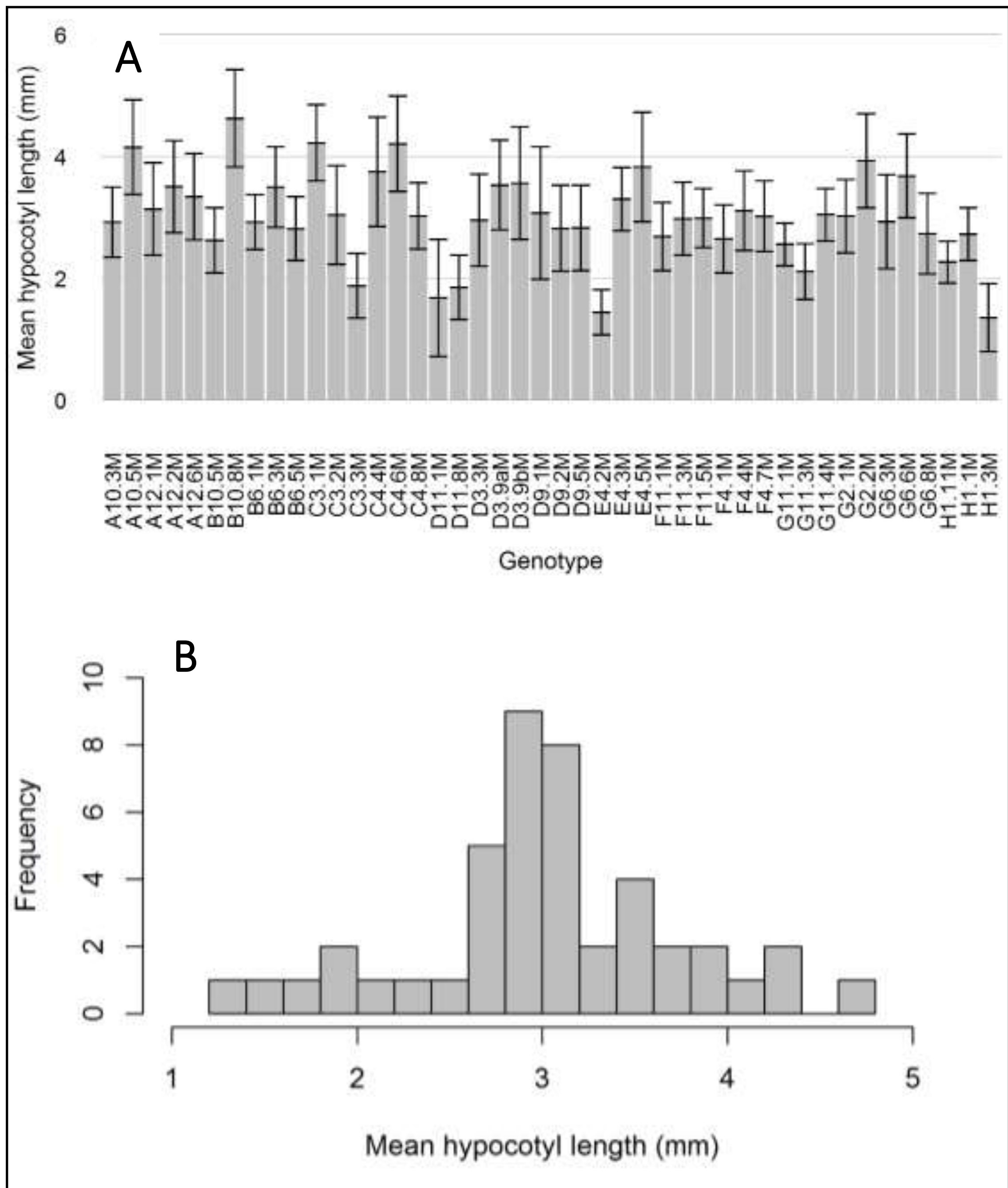



Figure 25: Validity of *hsp90.2-3* mutant population hypocotyl length data with 3µM picloram media for QTL mapping.

A: Mean hypocotyl length of each genotype in the population. Error bars show 1 standard deviation.

B: Histogram of mean hypocotyl length of each genotype in the population.

## Discussion

Both regions of high LOD score in the mutant population are similar to the QTL I found when I mapped the whole population under control conditions. The peak at position 95.28 is QTL-A, which has been seen in several other maps. This result is similar to QTL map of hypocotyl length in standard conditions. It was also the case there that QTL-A appeared in single-QTL scans of the full population and the mutant population, but not the wild-type population. QTL-A appears to have similar effects with exogenous auxin as it did in standard conditions.

The region of high LOD score at position 0.52 is close to the QTL I found at position 3.17 in the QTL map for growth under control conditions. I think that these two peaks are too far apart to consider that they may indicate the same candidate gene. I think it is more likely that the peak at position 0.52 is a new QTL not yet seen in the QTL maps of any other condition.

It is worth noting that there have been no QTL in the wild-type population under any conditions. I previously laid out several explanations for this (section 4.4). The data used in this particular analysis met all the assumptions of the test, removing the possibility of the test failing due to it being invalid. It is unlikely that there is a genuine lack of QTL as I tested for variation in growth linked to genotype and found a positive result, but it should still be considered. QTL of small effect or those that work through epistatic interactions may be detectable using a two-QTL scan. I performed a two-QTL scan using the same data and received negative results. I think it is most likely that cryptic variation in the wild-type population is being hidden by heat shock proteins. If cryptic QTL are being hidden by the action of heat shock proteins, it is unlikely that any QTL scan of the wild-type population could detect them. The absence of QTL in the wild-type population deserves further research, particularly into what effect heat shock proteins are having on the population.

## 5.5 QTL mapping of standard deviation with exogenous auxin

I wanted to investigate QTL that affected variation. I used the standard deviation of the mean hypocotyl length of each genotype as a measure of how consistent the growth of seedlings was. By using standard deviation as a trait in QTL mapping I found QTL that are involved in regulating growth with exogenous auxin.

### Results of analysis

QTL mapping of standard deviation revealed one QTL. I calculated the standard deviation of the mean hypocotyl length of 20 individuals from 85 genotypes grown on media containing 3µM picloram. I created a QTL map using standard deviation as a trait using the method in section 2.6. Figure 26 displays the QTL map obtained from this analysis. There was one significant peak on chromosome 5 at position 20.5 with a LOD score of 4.72. This peak could be a compound peak made up of lots of smaller peaks; the highest LOD score occurs at position 20.5, but the entire region from position 0.52 to position 22.52 has a LOD score well above the significance threshold. Given the width of the peak, it is likely that multiple QTL for variation in the presence of exogenous auxin are within this region.

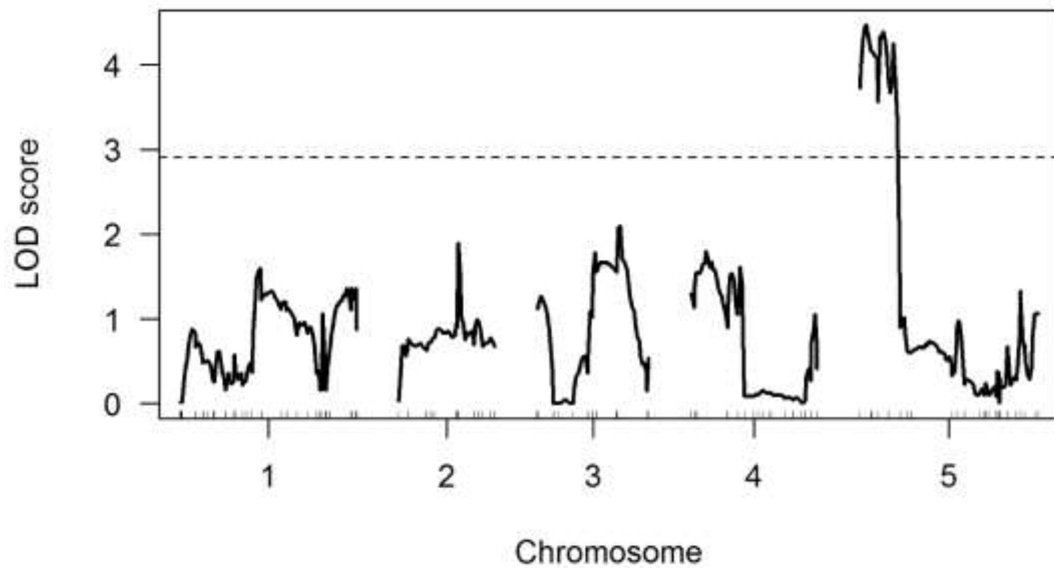



Figure 26: QTL map of the standard deviation of mean hypocotyl length with exogenous auxin using the multiple-imputation method. The dotted line indicates the 20% significance threshold.

## Validity of test

Variation:

Figure 27 displays the standard deviation of the mean hypocotyl length of each genotype in my mapping population when grown with exogenous auxin.

Distribution:

Figure 28 displays the distribution of the standard deviation of the mean hypocotyl length when grown with exogenous auxin.

Shapiro-Wilks: $W = 0.979$, $p = 0.198$

See section 2.7 for a full explanation of these statistics.

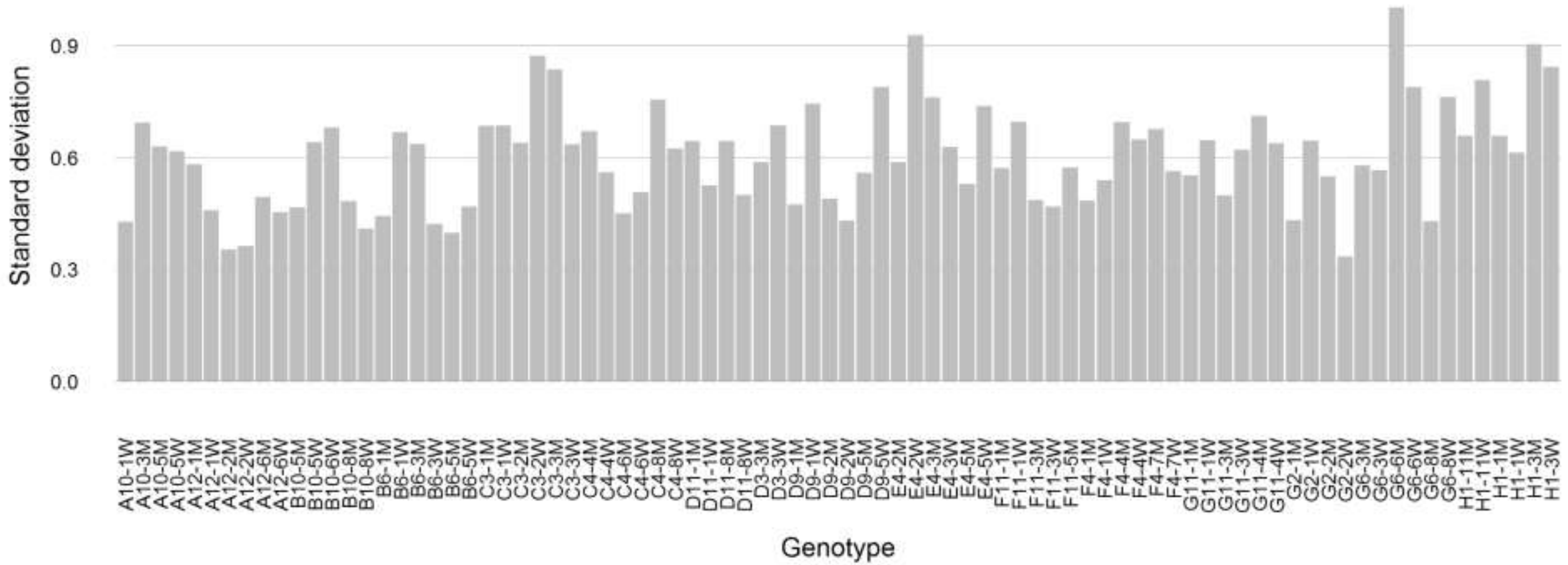



Figure 27: Standard deviation of hypocotyl length of each genotype in the full mapping population with 3µM picloram

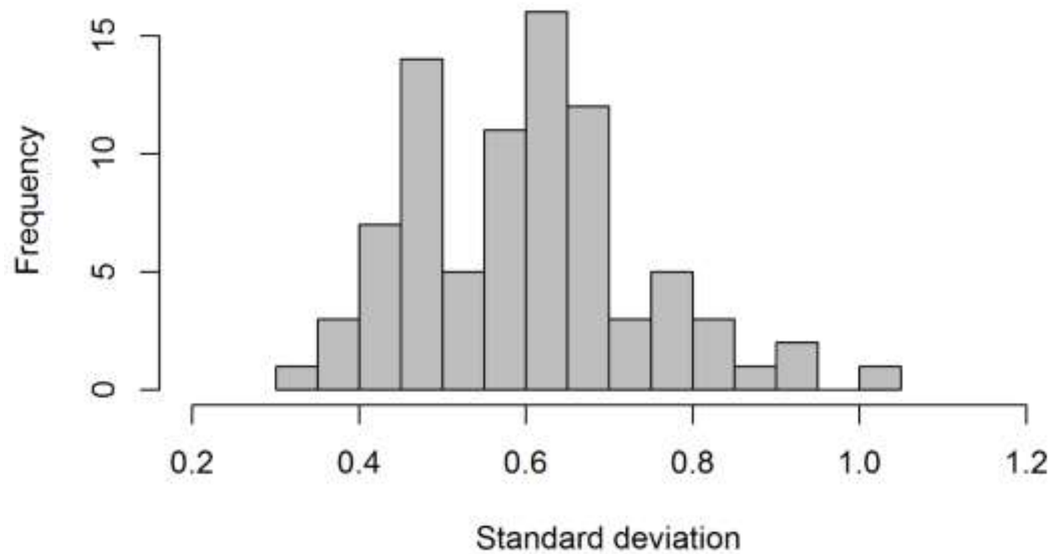



Figure 28: Histogram of the standard deviation of hypocotyl length in the full mapping population with 3µM media.

### Discussion

The QTL, or multiple QTL, I found in this analysis were similar to those found in other maps using different traits. I found possible QTL within this region of chromosome 5 when I mapped growth in standard conditions (position 3.17) and growth of the mutant population in the presence of exogenous auxin (position 0.52). There was also a QTL that is part of an epistatic pair for growth in standard conditions in this region (position 14.5). It is possible that this region of the genome contains multiple genes that affect growth and variation. I think it is very likely that this peak is a compound peak, representing several different QTL. It is difficult to know whether the QTL mentioned previously could have an effect on variation, or the peak could represent new QTL not seen in any other analysis. It is important to note that the point of highest LOD score occurs far away from the position of any QTL seen previously. In previous research that cloned and isolated QTL the genetic bases that affected the trait have always occurred very close to the point of maximum LOD score (Price 2006). I think it is most likely that this peak represents a combination of new QTL unique to this trait and QTL I found in other QTL maps.

## 5.6 Two-QTL scan of standard deviation with exogenous auxin

My single-QTL analysis did not reveal all significant QTL in my mapping population. As discussed previously (section 4.3) single-QTL mapping rarely reveals every significant QTL, making further analysis using two-QTL scans necessary. I analysed my data this way to reveal QTL with epistatic interactions that were invisible to a single-QTL scan.

### Results of analysis

I performed a two-dimensional, two-QTL scan on the same mapping data using the method in section 2.6. Figure 29 displays the results of this analysis. The high $LOD_{fv1}$ scores in the lower right triangle show evidence for pairs of QTL if interaction is allowed, particularly on chromosomes 1, 4 and 5. The high $LOD_i$ scores in the upper left triangle show clear evidence for epistatic interactions rather than an additive relationship.

I found four pairs of QTL with LOD scores high enough to be considered statistically significant. The first pair is on chromosome 1 position 45.4 and chromosome 1 position 95.4. These positions can only be considered QTL in a model that allows interaction. The second pair is on chromosome 3 position 63.4 and chromosome 5 position 21.5. There is evidence for these QTL in both an additive model and a full model, but LOD scores are maximized by allowing interaction. The third pair is on chromosome 4 position 42.5 and chromosome 5 position 21.5. These positions can only be considered QTL in a model that allows interaction. The final pair is on chromosome 5 position 20.5 and chromosome 5 position 60.5. There is evidence for these QTL in both an additive model and a full model, but LOD scores are maximized by allowing interaction. All of these positions are likely to be pairs of QTL which only show their effect when they are allowed to interact with each other.

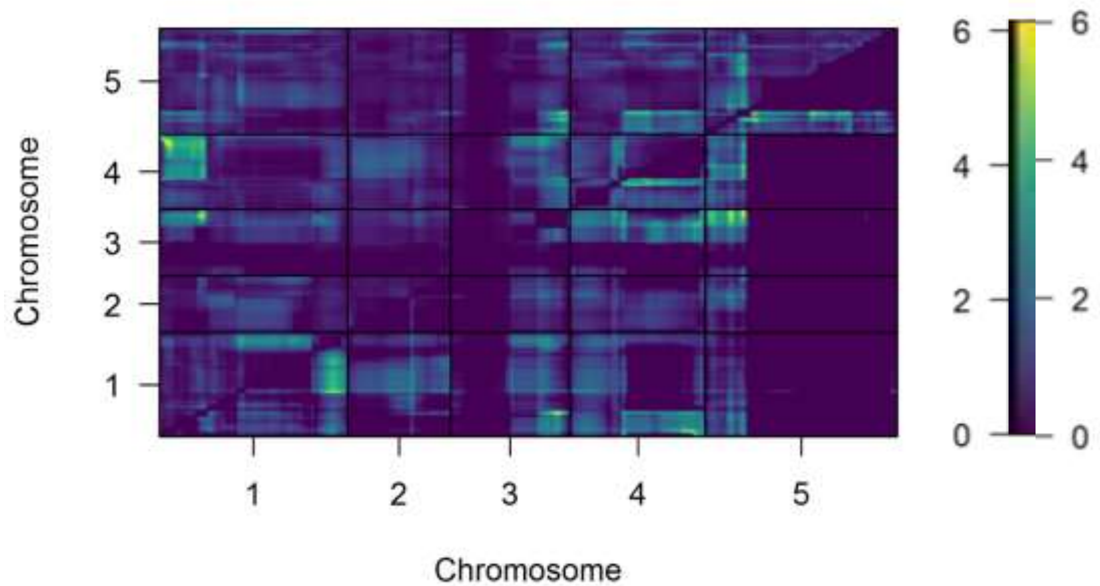



Figure 29: LOD scores from a two-QTL scan using the multiple-imputation method of the standard deviation of mean hypocotyl length with exogenous auxin. The lower right triangle contains $LOD_{fv1}$ scores showing the probability of one half of a pair of epistatic QTL existing at that position. The right side of the key indicates the magnitude of $LOD_{fv1}$ scores. The upper left triangle contains $LOD_i$ scores showing the probability of synergistic interactions as opposed to an additive relationship. The left side of the key indicates the magnitude of $LOD_i$ scores.

### Discussion

There are some similarities between the pairs of QTL found in this analysis and the QTL found in the single-QTL scan. The QTL in the single-QTL scan is on chromosome 5 around position 21.5. The two-QTL scan found a QTL on chromosome 5 at position 20.5. The proximity of the two positions and the fact that a two-QTL merely estimates positions makes it likely that these are in fact the same QTL. This QTL easily falls within the region of high LOD score on chromosome 5 found in my single-QTL scan for variation with exogenous auxin. I previously hypothesised that this region of high LOD score was a compound peak containing multiple QTL. It now seems that at least one QTL under the peak has an epistatic interaction with at least one other QTL. The QTL it interacts with seem to be unique to affecting variation through exogenous auxin. They could be elements of an auxin signal transduction pathway or a growth regulatory pathway that can be altered by the action of auxin. These unique QTL build up the picture of interacting genes impacting growth and development.

## 5.7 QTL for standard deviation in wild-type and mutant populations

### Results of analysis

As explained previously (section 4.4), I investigated the effect of the *hsp90.2-3* mutation on variation in the presence of exogenous auxin by mapping the wild-type and mutant populations separately. Each population contained 44 genotypes. I mapped the standard deviation of the mean hypocotyl length of 20 individuals from each genotype grown on media containing 3µM picloram using the method in section 2.6. Figure 30 displays the QTL maps obtained from this analysis layered over one another for easy comparison. The wild-type population contains a region of high LOD score on chromosome 5 from position 0.52 to position 20.52. The width of this region and the pattern of LOD scores within it makes me believe that this is a compound peak composed of many smaller peaks. The mutant population contains a single peak within the same region, on chromosome 5 at position 14.5. It is possible that these regions of

high LOD score on chromosome 5 are QTL with a role in variation in the presence of exogenous auxin.

## Validity of test

Wild-type population

Variation:

Figure 31A displays the standard deviation of the mean hypocotyl length of each genotype in the wild-type population with exogenous auxin.

Distribution:

Figure 31B displays the distribution of the standard deviation of the mean hypocotyl length in the wild-type population with exogenous auxin.

Shapiro-Wilks: $W = 0.983$, $p = 0.769$

Mutant population

Variation:

Figure 32A displays the standard deviation of the mean hypocotyl length of each genotype in the mutant population with exogenous auxin.

Distribution:

Figure 32B displays the distribution of the standard deviation of the mean hypocotyl length in the mutant population with exogenous auxin.

Shapiro-Wilks: $W = 0.955$, $p = 0.099$

See section 2.7 for a full explanation of these statistics.

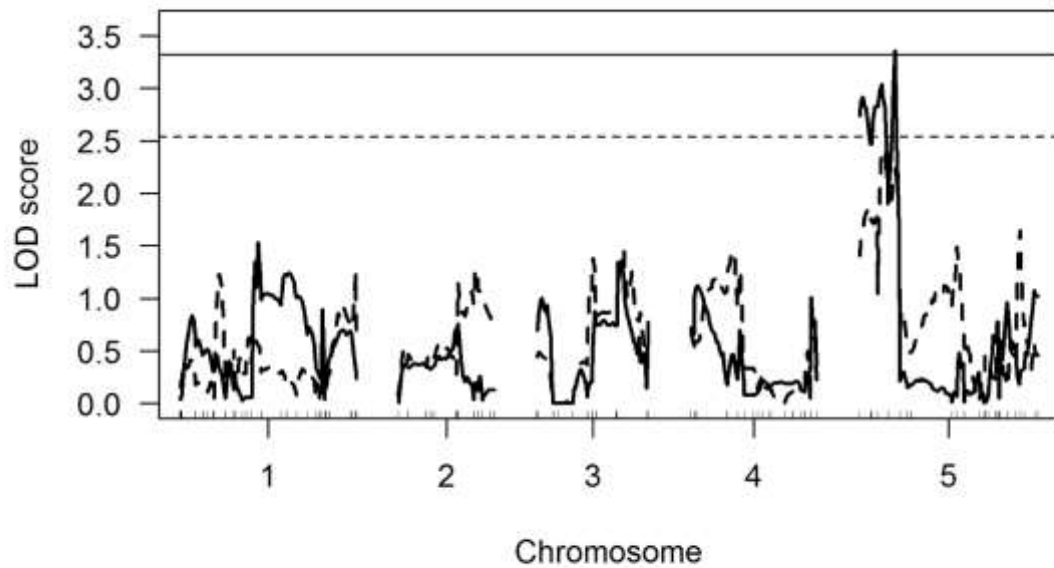



Figure 30: QTL map of the standard deviation of mean hypocotyl length in wild-type and mutant populations with exogenous auxin using the multiple-imputation method. The solid line shows LOD scores of the wild-type population. The horizontal solid line shows the 20% significance threshold for the wild-type population. The dotted line shows LOD scores of the *hsp90.2-3* mutant population. The horizontal dotted line shows the 20% significance threshold for the mutant population.

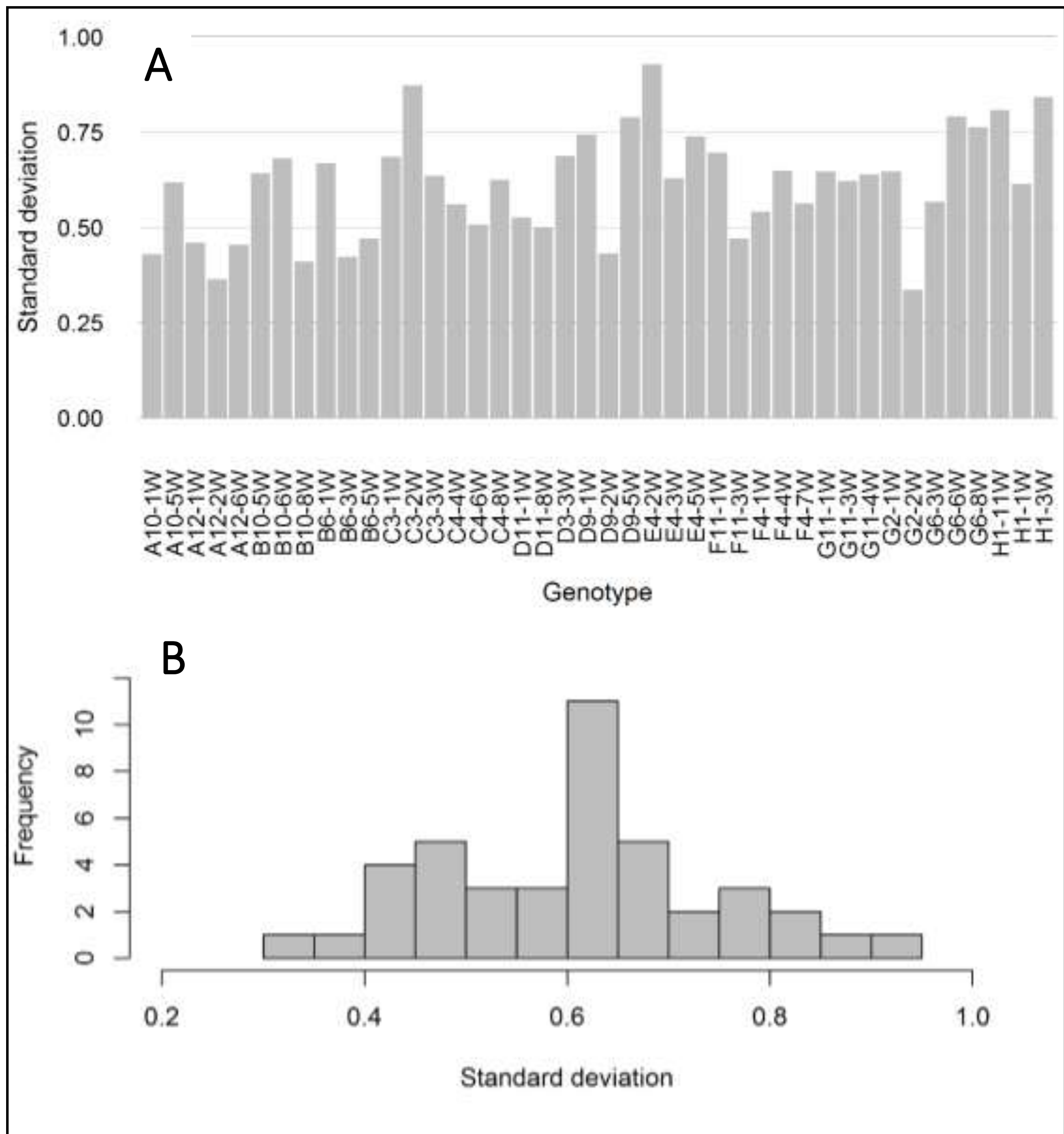



Figure 31: Validity of wild-type population standard deviation of hypocotyl length data with 3μM picloram media for QTL mapping.

A: Standard deviation of hypocotyl length of each genotype in the population.

B: Histogram of standard deviation of hypocotyl length of each genotype in the population.

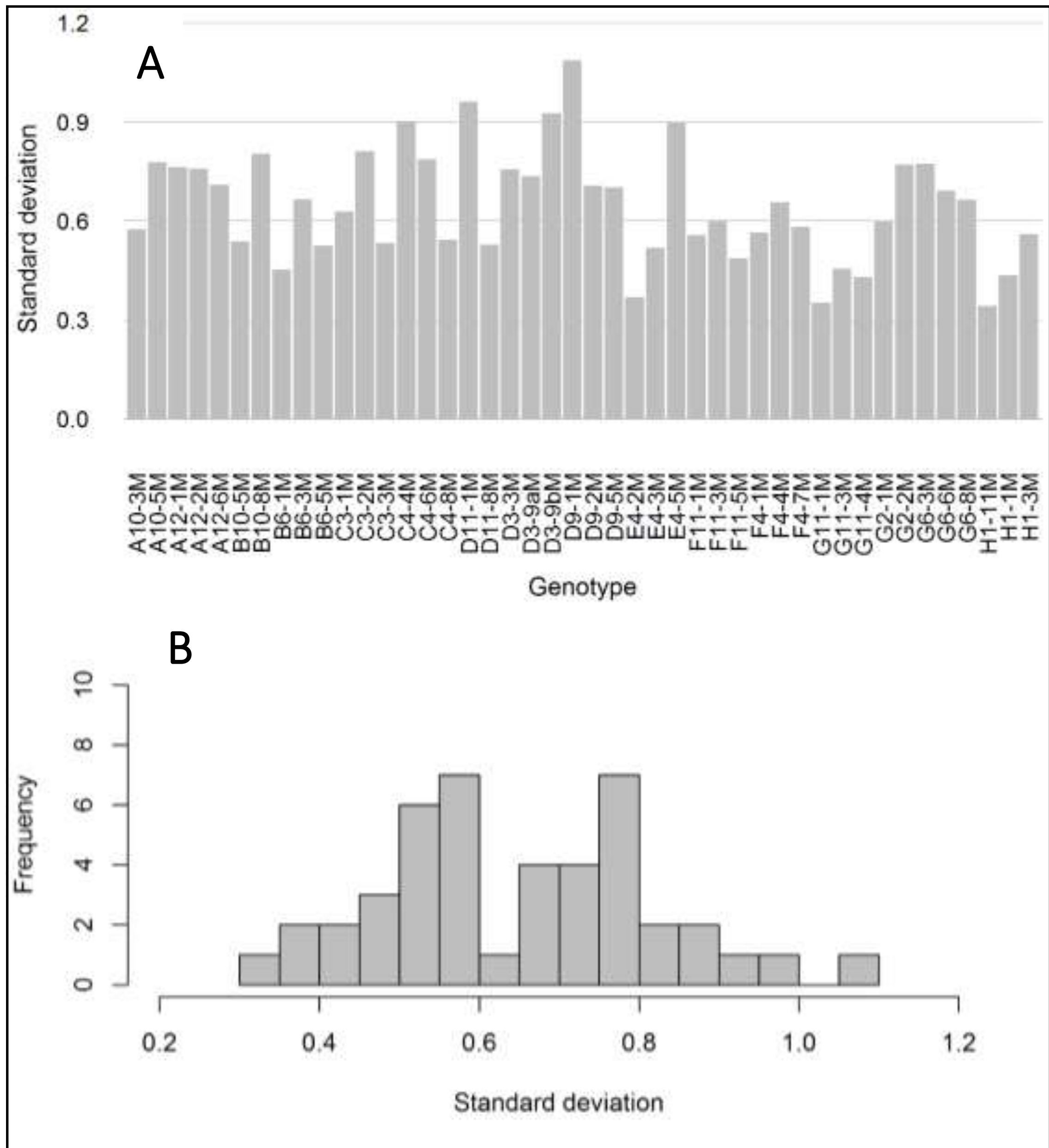



Figure 32: Validity of *hsp90.2-3* mutant population standard deviation of hypocotyl length data with 3µM picloram media for QTL mapping.

A: Standard deviation of hypocotyl length of each genotype in the population.

B: Histogram of standard deviation of hypocotyl length of each genotype in the population.

### Discussion

Although both populations have regions of high LOD scores, the peaks in both populations are not statistically significant. The reasons why a QTL may not be statistically significant are discussed in section 4.4. In the case of the mutant population, the lack of significance could be due to the unsuitability of the data for QTL analysis. I concluded that the data are approximately normally distributed, but since the p-value comes close to my chosen significance threshold it could easily be argued that my data are unsuitable for the QTL mapping model I used. However, I think that both peaks represent QTL for variation with exogenous auxin and the relatively small population size and the low statistical power it caused are the reason for the low LOD scores.

The peaks in both populations fall within the region of high LOD score found when standard deviation in the whole population was mapped. This region contains QTL that were found in other QTL maps. I previously concluded that the compound peak in the standard deviation map represented several QTL and that some of them could be QTL from the other maps. These new results add more detail. The peak in the wild-type population is likely a compound peak – the pattern of high and low LOD scores is clear. The peak in the mutant population could be several QTL, but looking at the LOD scores I think it is more likely to only be one QTL. I think that the QTL from both the wild-type and mutant populations were under the single wide peak in the standard deviation map. However, the position of the highest LOD scores makes me think that they are not the same QTL as were found when growth was mapped. These results have built up a complex picture of several QTL important to growth and variation on chromosome 5.

## 5.8 Two-QTL scan of standard deviation with exogenous auxin in mutant population

My single-QTL analysis did not reveal all significant QTL in my mapping population. As explained previously (section 4.3) single-QTL mapping rarely reveals every significant

QTL, making further analysis using two-QTL scans necessary. I analysed my data this way to reveal QTL with epistatic interactions that were invisible to a single-QTL scan.

## Results of analysis

I performed a two-dimensional, two-QTL scan on the same mapping data using the method in section 2.6. I found no significant QTL in the wild-type population. Figure 33 displays the results of the analysis of the *hsp90.2-3* mutant population. The high $LOD_{fv1}$ scores in the lower right triangle show evidence for pairs of QTL if interaction is allowed, particularly on chromosome 5. The high $LOD_i$ scores in the upper left triangle show clear evidence for epistatic interactions rather than an additive relationship.

I found two pairs of QTL with LOD scores high enough to be considered statistically significant in the mutant population. One pair is on chromosome 3 position 67.3 and chromosome 5 position 21.5. These positions can only be considered QTL in a model that allows interaction. The other pair is on chromosome 5 position 21.5 and chromosome 5 position 58.5. These positions can only be considered QTL in a model that allows interaction. All of these positions are likely to be pairs of QTL which only show their effect when they are allowed to interact with each other.

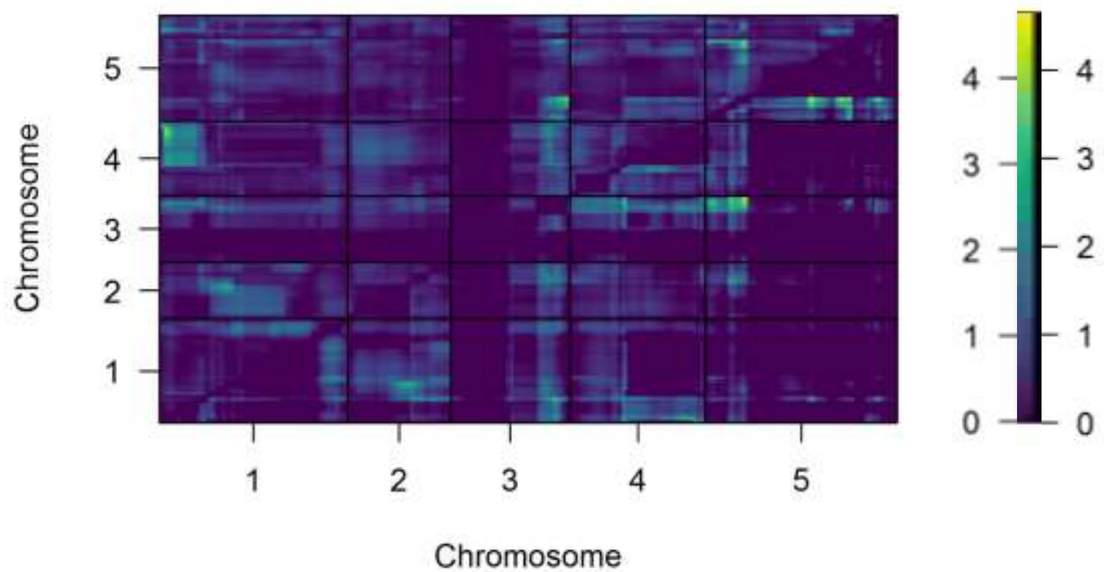



Figure 33: LOD scores from a two-QTL scan of standard deviation with 3µM picloram in a population containing the *hsp90.2-3* mutation using the multiple-imputation method. The lower right triangle contains $LOD_{fv1}$ scores showing the probability of one half of a pair of epistatic QTL existing at that position. The right side of the key indicates the magnitude of $LOD_{fv1}$ scores. The upper left triangle contains $LOD_i$ scores showing the probability of synergistic interactions as opposed to an additive relationship. The left side of the key indicates the magnitude of $LOD_i$ scores.

### Discussion

All of the QTL found in this analysis are shared with other QTL maps, specifically the two-QTL analysis of variation with exogenous auxin in the whole mapping population. Additionally, the QTL on chromosome 5 at position 21.5 looks similar to the QTL was found in the mutant population when standard deviation with exogenous auxin was mapped. Although the positions are not exactly the same, two-QTL scan positions are only estimates, and the two QTL look reasonable close together. It is not unreasonable to think that the same gene may be responsible, but in my opinion the positions are too far apart to argue this case strongly. I think that the QTL found in this analysis is not the same as any QTL found previously. It is clear that the QTL found in this analysis have an effect on variation in the presence of exogenous auxin.

The lack of QTL in the wild-type population in the two-QTL scan is an interesting result. There was a region of high LOD score in the single-QTL scan, although it was not a statistically significant QTL. Because of the lack of QTL in the two-QTL scan, it appears that the gene under this peak acts independently of any other QTL. It is also possible that the peak in the single-QTL scan was a false positive and there were no QTL in this population for this trait. However, the data was suitable for this analysis and there was variation in the trait. I think it is more likely that the peak did not reach statistical significance due to a small sample size. Possible reasons for negative results are discussed in more detail in section 4.4. This result makes me think that there is only one QTL for variation in the wild-type population.

## 5.9 QTL mapping of change in growth due to auxin

When mapping growth in two different conditions, some QTL could be QTL for general growth and not related to the effect of the condition. I wanted to investigate QTL specific to an increase in growth with added auxin. To do this I calculated the change in hypocotyl length due to auxin using the method described in section 2.6 and used this value as a trait in QTL mapping. Using this method, I found QTL that have a role in changing growth in response to increased auxin levels, rather than general growth QTL.

## Results of analysis

I calculated the difference in height due to added auxin and used this value as a trait in QTL mapping. I mapped the change in mean hypocotyl length due to auxin of 20 individuals from 85 genotypes at using the method in section 2.6. Figure 34 displays the QTL map obtained from this analysis. I found one significant peak on chromosome 5 at position 95.28 with a LOD score of 4.48. This peak is a possible QTL for the change in growth due to exogenous auxin.

## Validity of test

Distribution:

Figure 35A displays the distribution of the change in mean hypocotyl length due to auxin.

Shapiro-Wilks: $W = 0.857$, $p = 9.977e\text{-}08$

It is easy to see visually that the data were not normally distributed, and this is confirmed statistically. This distribution is due to some anomalous results in which seedlings grown with exogenous auxin were shorter than the same genotype grown in control conditions. On examining my raw data more closely, I found that certain genotypes had an unusually large number of sick and dead seedlings or seedlings that had failed to germinate, and the living seedlings were all unusually short. This could be due to contamination on the agar plate. I decided to exclude these anomalous results from my analysis.

The distribution of the corrected data are displayed in figure 35B.

Shapiro-Wilks: $W = 0.975$, $p = 0.101$

Variation:

Figure 36 displays the change in mean hypocotyl length due to auxin of each genotype in my mapping population. The data used for this figure does not include any anomalies.

ANOVA: $F = 10.67$, $d.f = 81, 1502$, $p = <2^{e-16}$

See section 2.7 for a full explanation of these statistics.

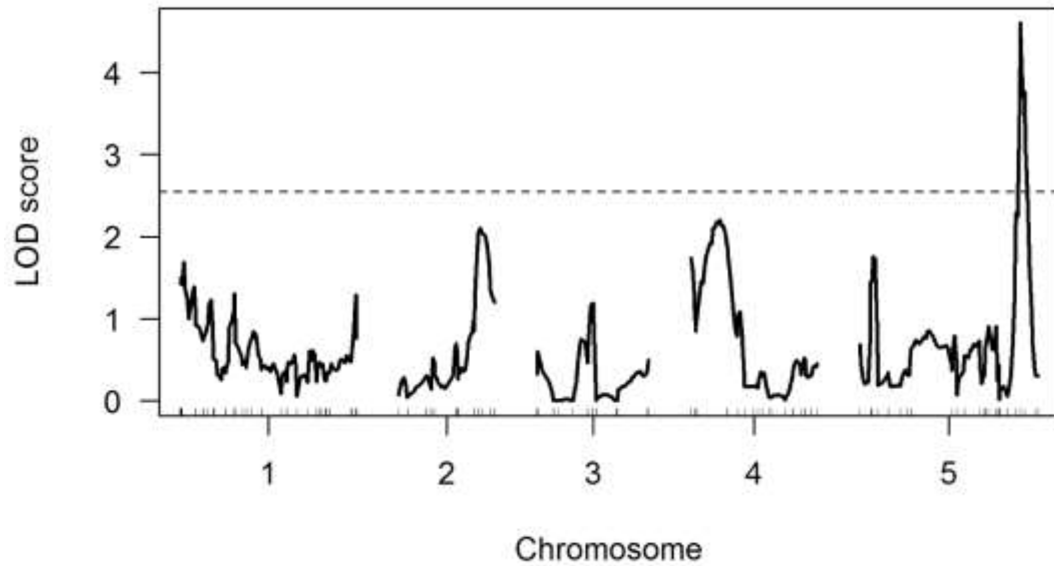



Figure 34: QTL map of the difference in mean hypocotyl length with exogenous auxin compared to control conditions using the multiple-imputation method. The dotted line indicates the 20% significance threshold.

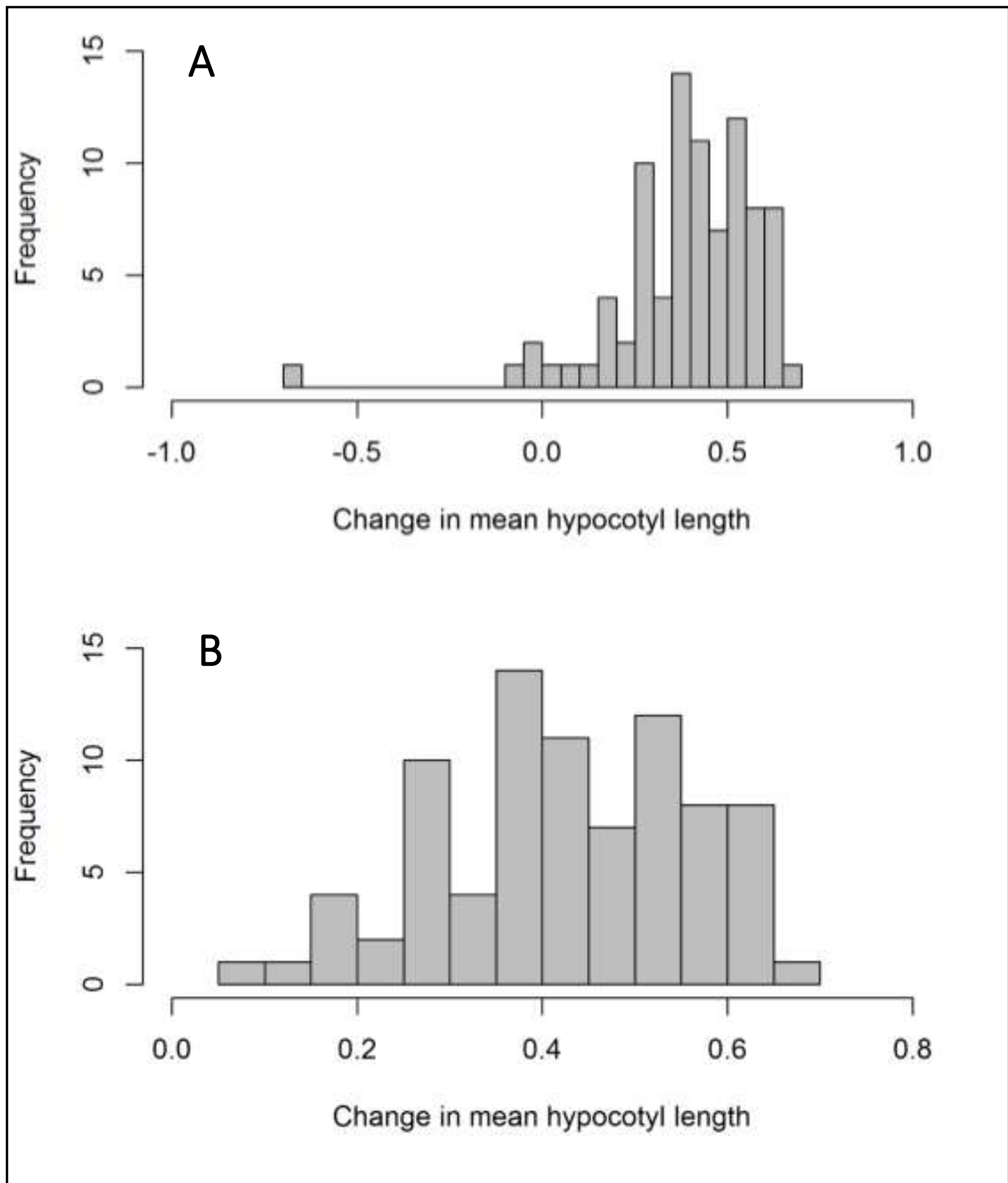



Figure 35: Distribution of the change in mean hypocotyl length due to exogenous auxin.

A: Distribution of all genotypes in the mapping population.

B: Distribution after anomalous results are removed from the data.

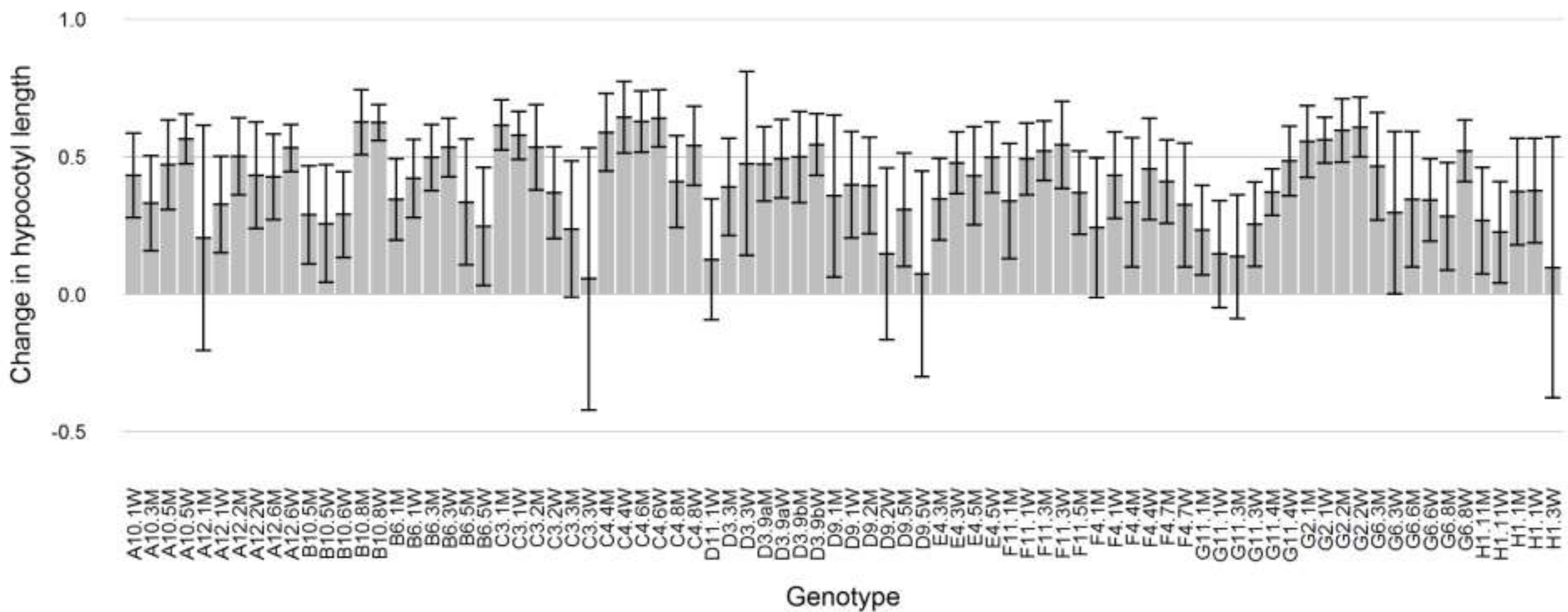



Figure 36: Change in hypocotyl length due to exogenous auxin in all genotypes used for QTL mapping.

## Discussion

The QTL found in this analysis is QTL-A, seen in several other QTL maps. It is surprising to see this results in an analysis designed to eliminate general growth QTL. Although QTL-A had an effect on growth with exogenous auxin, I assumed that its action was not specific to the condition because of its effect on growth in standard conditions. It is possible that this QTL not only affects growth generally, but affects the way growth changes under various conditions. Another possibility is that QTL-A interacts with other QTL specific to auxin-induced growth. The two-QTL scan of growth with exogenous auxin did not reveal any epistatic interactions involving QTL-A, but by focusing only on QTL that result in a change in growth new QTL may be revealed. This interesting result needs more investigation before conclusions can be drawn.

# 5.10 Two-QTL scan of change in growth due to auxin

My single-QTL analysis did not reveal all significant QTL in my mapping population. As discussed previously (section 4.3) single-QTL mapping rarely reveals every significant QTL, making further analysis using two-QTL scans necessary. I analysed my data this way to reveal QTL with epistatic interactions that were invisible to a single-QTL scan.

## Results of analysis

I performed a two-dimensional, two-QTL scan on the same mapping data using the method in section 2.6. Figure 37 displays the results of this analysis. The high $LOD_{fv1}$ scores in the lower right triangle show evidence for pairs of QTL if interaction is allowed, particularly on chromosome 1. The high $LOD_i$ scores in the upper left triangle show clear evidence for epistatic interactions rather than an additive relationship.

I found two pairs of QTL with LOD scores high enough to be considered statistically significant. One pair is on chromosome 1 position 1.38 and chromosome 1 position 8.38. There is some evidence for these QTL in both an additive and a full model, but LOD scores are maximized in the additive model. The other pair is on chromosome 1

position 100.38 and chromosome 4 position 1.47. There is some evidence for these QTL in both an additive model and a full model, but LOD scores are maximized in the additive model. All of these positions are likely to be pairs of QTL which only show their effect when they are allowed to interact with each other.

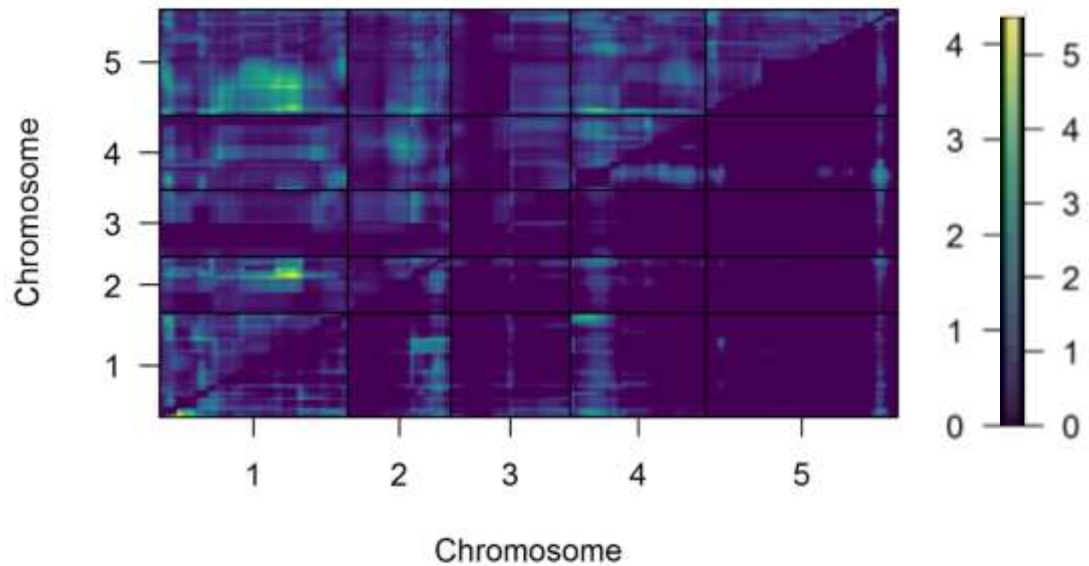



Figure 37: LOD scores from a two-QTL scan using the multiple-imputation method. The trait used was the difference in mean hypocotyl length with exogenous auxin compared to control conditions. The lower right triangle contains $LOD_{fv1}$ scores showing the probability of one half of a pair of epistatic QTL existing at that position. The right side of the key indicates the magnitude of $LOD_{fv1}$ scores. The upper left triangle contains $LOD_i$ scores showing the probability of synergistic interactions as opposed to an additive relationship. The left side of the key indicates the magnitude of $LOD_i$ scores.

### Discussion

There are some similarities and some differences between this analysis and others. Firstly, it should be noted that the QTL-A does not appear in the two-QTL scan despite being significant in the single-QTL scan. This means that QTL-A is likely to drive a change in growth due to increased auxin levels through its own action. One of the pairs of QTL seen in the two-QTL scan (chromosome 1 position 1.38 and chromosome 1 position 8.38) is also seen in the two-QTL scan for hypocotyl length with exogenous auxin (section 5.3). These QTL appear to be genes specific to auxin-induced growth, but only have an effect when both are present. The other pair from the two-QTL scan is unique to that analysis. These similarities and differences build up a picture of the roles of each QTL in growth.

## 5.11 Summary of results

The results in this chapter provide further evidence of the role of QTL-A. QTL-A still impacts growth when auxin levels are increased but no epistatic interactions ca be detected. Further discussion about QTL-A can be found in chapter 7.

There appears to be at least one, and probably multiple, QTL regulating variation in hypocotyl length in the presence of exogenous auxin. The QTL found when standard deviation was mapped was easily wide enough to assume that multiple QTL lie under it. It is likely that multiple genes work to regulate variation in the presence of exogenous auxin.

# Chapter 6: QTL analysis of growth and variation at warm temperatures

## 6.1 Overview

This chapter presents the results of my analysis of growth and variation at warm temperatures. I examined the mapping population at 27$^{o}$C, the upper limit of the range of temperatures *Arabidopsis thaliana* can grow at without obvious damage. I looked for QTL for hypocotyl length in three mapping populations: the full mapping population, the wild-type half of the mapping population and the half of the mapping population containing the *hsp90.2-3* mutation. I performed single-QTL and two-QTL scans to search for QTL that act on their own and those that act through epistatic interactions. I also analysed the change in hypocotyl length by comparing hypocotyl length at warm temperatures to the same genotype in standard conditions. I found several QTL using these methods, including QTL-A which is involved in growth in other conditions, and several QTL which appear to be unique to growth at warm temperatures. The results presented in this chapter provide an idea of what regions of the genome are important in regulating growth at warm temperatures.

## 6.2 QTL mapping of hypocotyl length at warm temperatures

I wanted to investigate QTL for growth at warm temperatures. I used hypocotyl length as a convenient way to measure growth in each genotype in my mapping population. I measured the hypocotyl length of each genotype in my mapping population at 27°C using the method in section 2.5. The full data can be seen in appendix 5. By using hypocotyl length as a trait in QTL mapping, I found QTL that are important in increasing or decreasing the growth of a seedling at the upper limit of the range of temperatures that *Arabidopsis thaliana* can grow normally at.

## Results of analysis

QTL mapping of growth at warm temperatures detected two significant QTL. I mapped the mean hypocotyl length of 20 individuals from 85 genotypes at 27$^{o}$C using the method in section 2.6. Figure 38 displays the QTL map obtained from this analysis. I found two significant QTL. One is on chromosome 1 at position 27.38 with a LOD score of 3.45. The other is on chromosome 4 at position 68.37 with a LOD score of 2.78. I concluded that these peaks are QTL for growth at warm temperatures.

## Validity of test

Variation:

Figure 39 displays the mean hypocotyl length of each genotype in my mapping population at 27$^{o}$C.

ANOVA: $F$ = 17.82, $d.f$ = 41, 767, $p$ = <$2^{e-16}$

Distribution:

Figure 40 displays the distribution of mean hypocotyl length at 27$^{o}$C.

Shapiro-Wilks: $W$ = 0.987, $p$ = 0.571

See section 2.7 for a full explanation of these statistics.

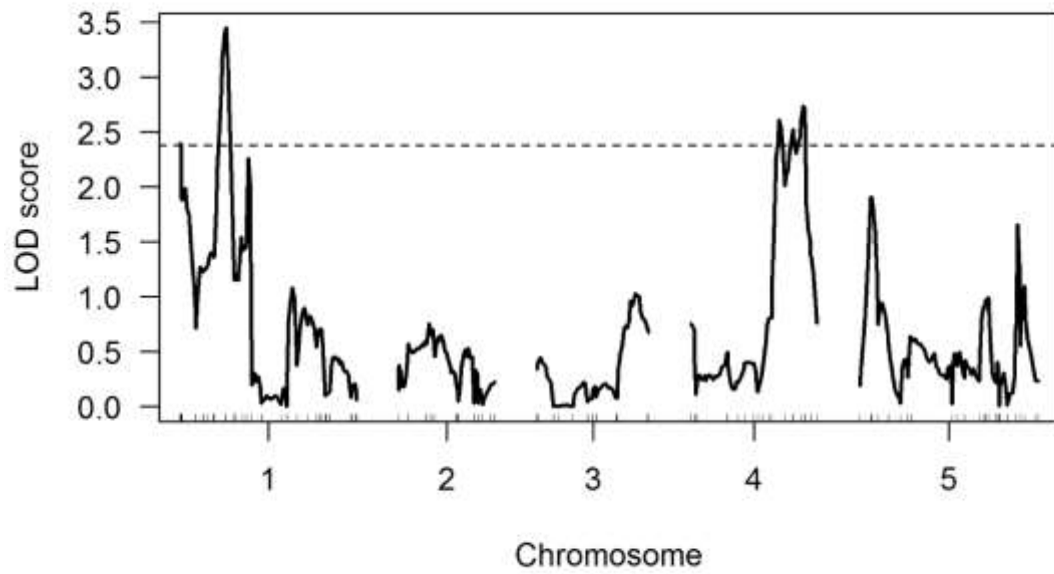



Figure 38: QTL map of mean hypocotyl length at 27°C using the multiple-imputation method. The dotted line indicates the 20% significance threshold.

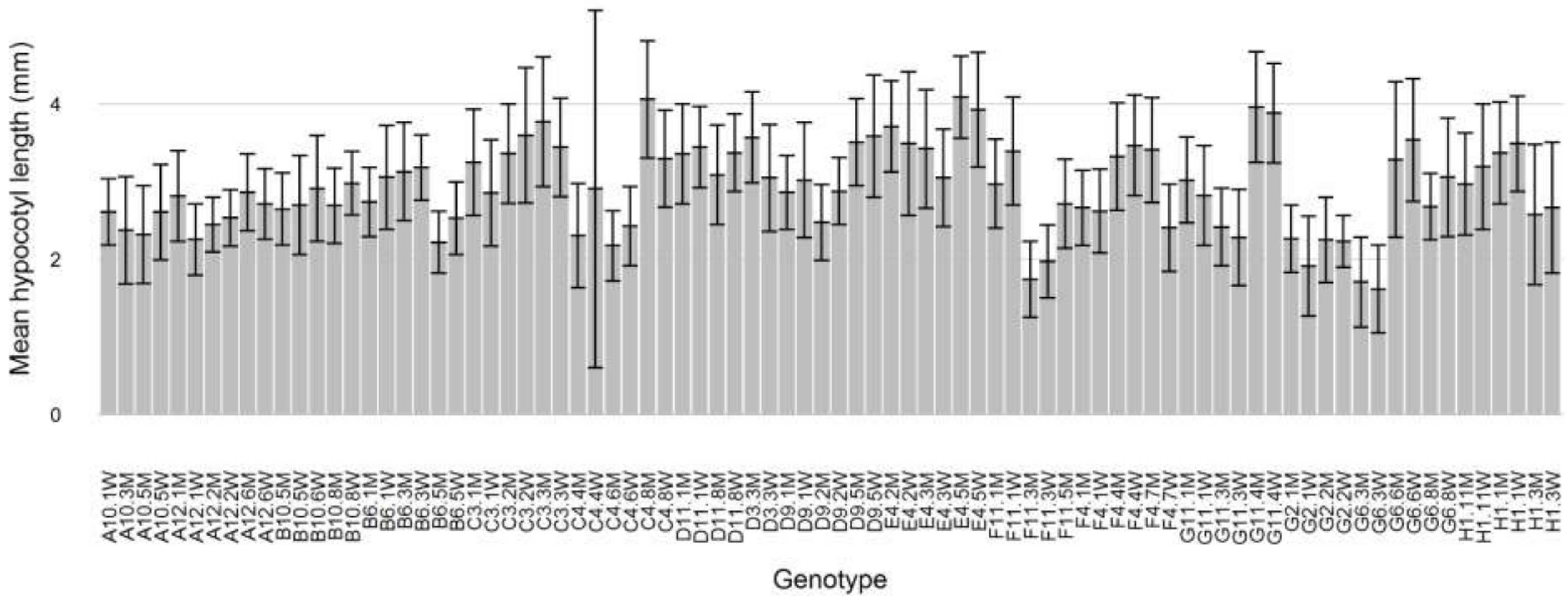



Figure 39: Mean hypocotyl length of each genotype in the mapping population at 27°C. Error bars show 1 standard deviation.

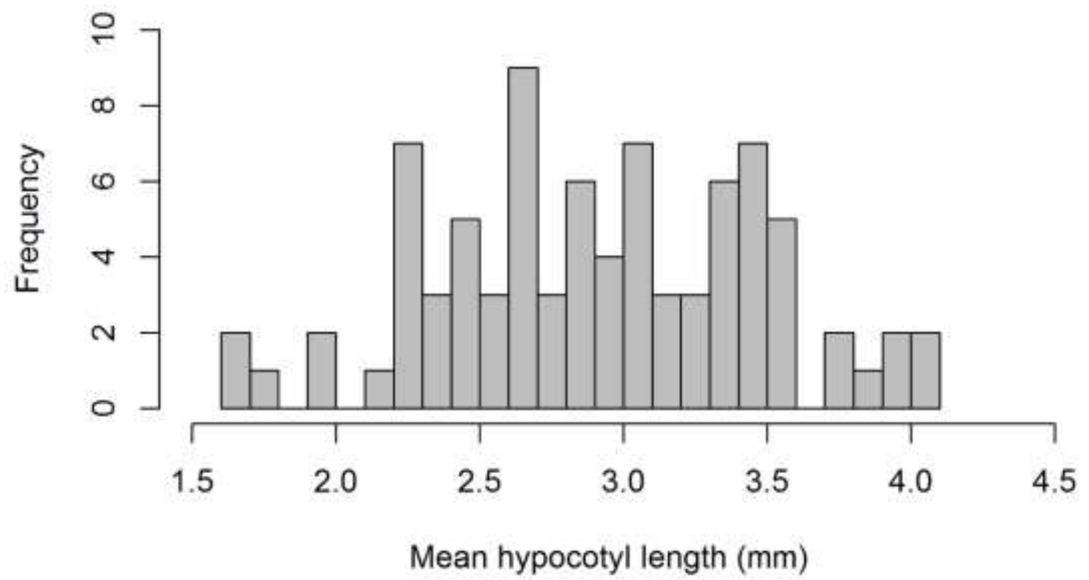



Figure 40: Histogram of mean hypocotyl length in the full mapping population at 27°C.

## Discussion

Previous work makes it possible to suggest what genes might be under the peaks on the QTL map. A study on *Arabidopsis* growth at 27°C found a similar peak on chromosome 4 and attributed it to the phytochrome PHYE (Box et al. 2015). Phytochromes have a role in perceiving temperature (Penfield 2008) and are key to thermomorphogenesis (Wigge 2013, Quint et al. 2016). A change in a phytochrome gene could lead to variation in temperature sensitivity, which in turn would lead to variation in growth at warm temperatures. PHYE is not the only candidate gene for this QTL. It is possible that multiple genes lie under the peak; its jagged appearance means it could be a compound peak made up of several individual peaks. It is more difficult to propose candidate genes for the peak on chromosome 1. Although no conclusions can be drawn, I think it is likely that QTL I found are phytochrome genes or other genes with a role in temperature perception and signalling.

# 6.3 Two-QTL scan of hypocotyl length at warm temperatures

My single-QTL analysis did not detect every QTL relevant to growth at warm temperatures in my mapping population. As discussed previously (section 4.3) single-QTL mapping rarely reveals every significant QTL, making further analysis using two-QTL scans necessary. I analysed my data this way to reveal QTL with epistatic interactions that were invisible to a single-QTL scan.

## Results of analysis

I performed a two-dimensional, two-QTL scan on the same mapping data using the method in section 2.6. Figure 41 displays the results of this analysis. The high $LOD_{fv1}$ scores in the lower right triangle show evidence for pairs of QTL if interaction is allowed, particularly on chromosomes 1 and 5. The high $LOD_i$ scores in the upper left triangle show clear evidence for epistatic interactions rather than an additive relationship.

I found three pairs of QTL with LOD scores high enough to be considered statistically significant. One pair is on chromosome 1 position 37.4 and chromosome 1 position 76.38. There is significant evidence for this pair of QTL in both a full model and an additive model. The second pair is on chromosome 1 position 41.4 and chromosome 5 position 99.5. These positions can only be considered QTL in a model that allows interaction. The third pair is on chromosome 4 position 53.5 and chromosome 5 position 6.52. These positions can only be considered QTL in a model that allows interaction. All of these positions are likely to be pairs of QTL which only show their effect when they are allowed to interact with each other.

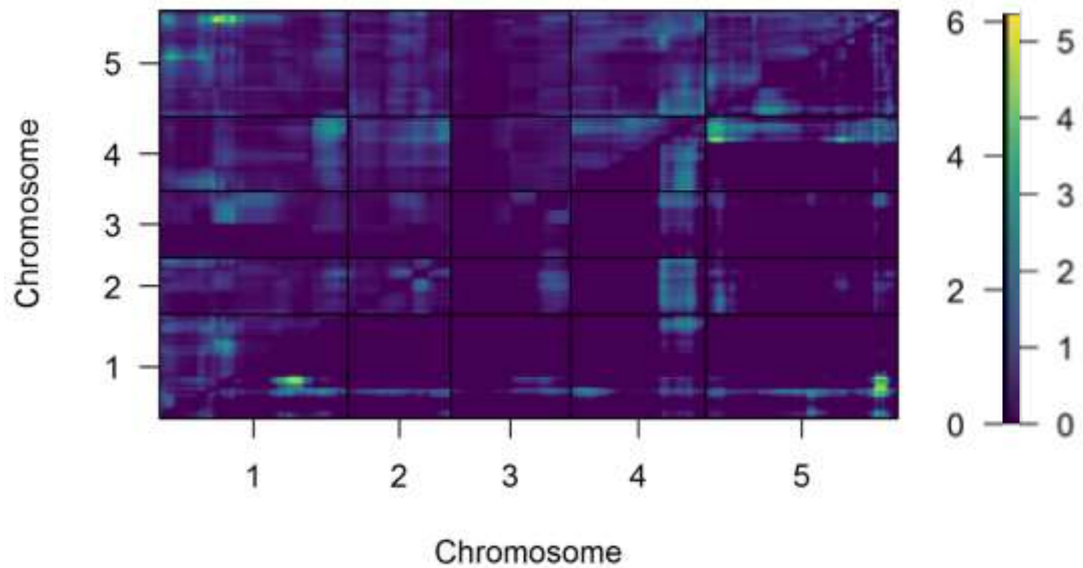



Figure 41: LOD scores from a two-QTL scan using the multiple-imputation method of mean hypocotyl length at 27°C. The lower right triangle contains $LOD_{fv1}$ scores showing the probability of one half of a pair of epistatic QTL existing at that position. The right side of the key indicates the magnitude of $LOD_{fv1}$ scores. The upper left triangle contains $LOD_i$ scores showing the probability of synergistic interactions as opposed to an additive relationship. The left side of the key indicates the magnitude of $LOD_i$ scores.

### Discussion

There are some key similarities between this two-QTL scan and other single-QTL scans. The first is the QTL on chromosome 5 at position 95.5, which I have called QTL-A. I found this QTL in several other QTL scans, including maps for growth in standard conditions with exogenous auxin. This new evidence supports my hypothesis that QTL-A is a QTL for general growth that has an enhanced effect when auxin levels are increased. The QTL that formed the other half of the pair, chromosome 1 position 41.4, was also significant in the two-QTL scan of growth in standard conditions interacting with its partner. This pair of QTL are likely to have a role in general growth and may be part of an auxin pathway.

The other pairs of QTL found in this analysis involve QTL near to those found in the single-QTL scan for growth at 27$^{o}$C. The positions are not exactly the same but since positions in a two-QTL scan are estimated, it could be argued that they represent the same gene. However, I think that the positions are too far apart to assume this, even knowing that they are an imperfect estimate. If the peaks do represent the same loci, both of the QTL found previously can have an increased effect by interacting with other QTL.

## 6.4 QTL for growth at warm temperatures in mutant population only

### Results of analysis

As explained previously (section 4.4), I examined the effect of the *hsp90.2-3* mutation on growth in the presence of exogenous auxin by mapping the wild-type and mutant populations separately. Each population contained 42 genotypes. I mapped the average hypocotyl length of 20 individuals from each genotype at 27$^{o}$C using the method in section 2.6. Figure 42 displays the QTL maps obtained from this analysis layered over one another for easy comparison. I found no significant peaks in the wild-type

population. I found two significant peaks in the mutant population. One is on chromosome 1 at position 27.38 with a LOD score of 2.99. The other is on chromosome 4 at position 68.37 with a LOD score of 2.46. These peaks are possible QTL for growth at warm temperatures.

## Validity of test

Wild-type population

Variation:

Figure 43A displays the mean hypocotyl length of each genotype in the wild-type population at 27$^{o}$C.

ANOVA: $F$ = 14.53, $d.f$ = 431, 776, $p$ = <$2^{e-16}$

Distribution:

Figure 43B displays the distribution of mean hypocotyl length in the wild-type population at 27$^{o}$C.

Shapiro-Wilks: $W$ = 0.982, $p$ = 0.727

Mutant population

Variation:

Figure 44A displays the mean hypocotyl length of each genotype in the mutant population at 27$^{o}$C.

ANOVA: $F$ = 17.82, $d.f$ = 41, 767, $p$ = <$2^{e-16}$

Distribution:

Figure 44B displays the distribution of mean hypocotyl length in the mutant population at 27$^{o}$C.

Shapiro-Wilks: $W$ = 0.980, $p$ = 0.668

See section 2.7 for a full explanation of these statistics.

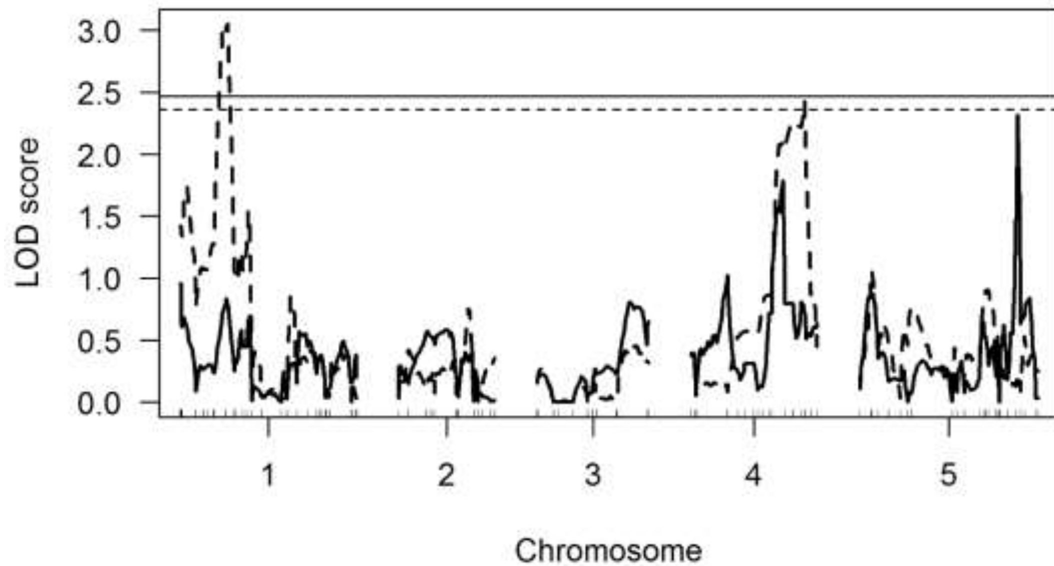



Figure 42: QTL map of mean hypocotyl length in wild-type and mutant populations at 27°C using the multiple-imputation method. The solid line shows LOD scores of the wild-type population. The horizontal solid line shows the 20% significance threshold for the wild-type population. The dotted line shows LOD scores of the *hsp90.2-3* mutant population. The horizontal dotted line shows the 20% significance threshold for the mutant population.

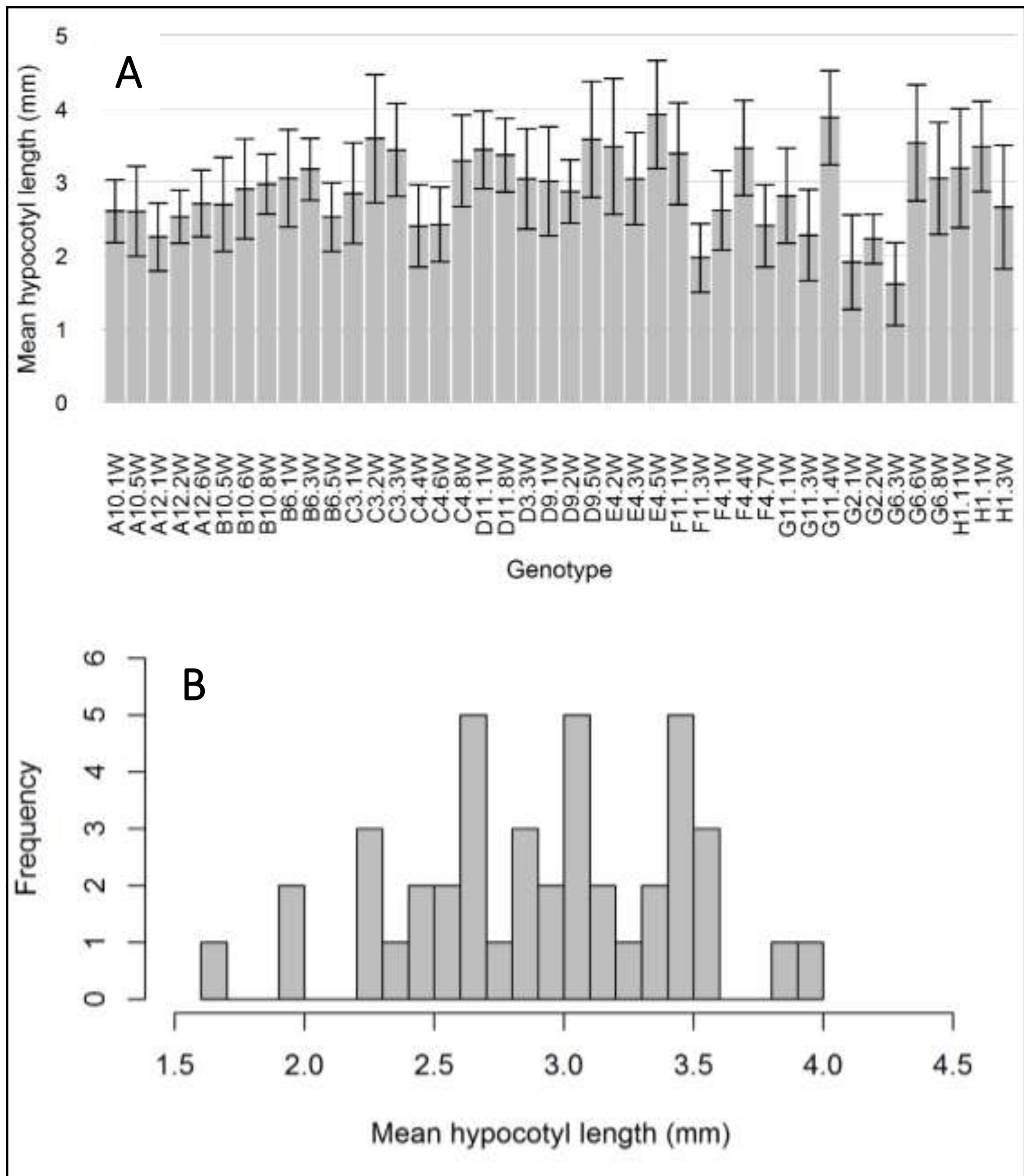



Figure 43: Validity of wild-type population hypocotyl length data at 27°C for QTL mapping.

A: Mean hypocotyl length of each genotype in the population. Error bars show 1 standard deviation.

B: Histogram of mean hypocotyl length of each genotype in the population.

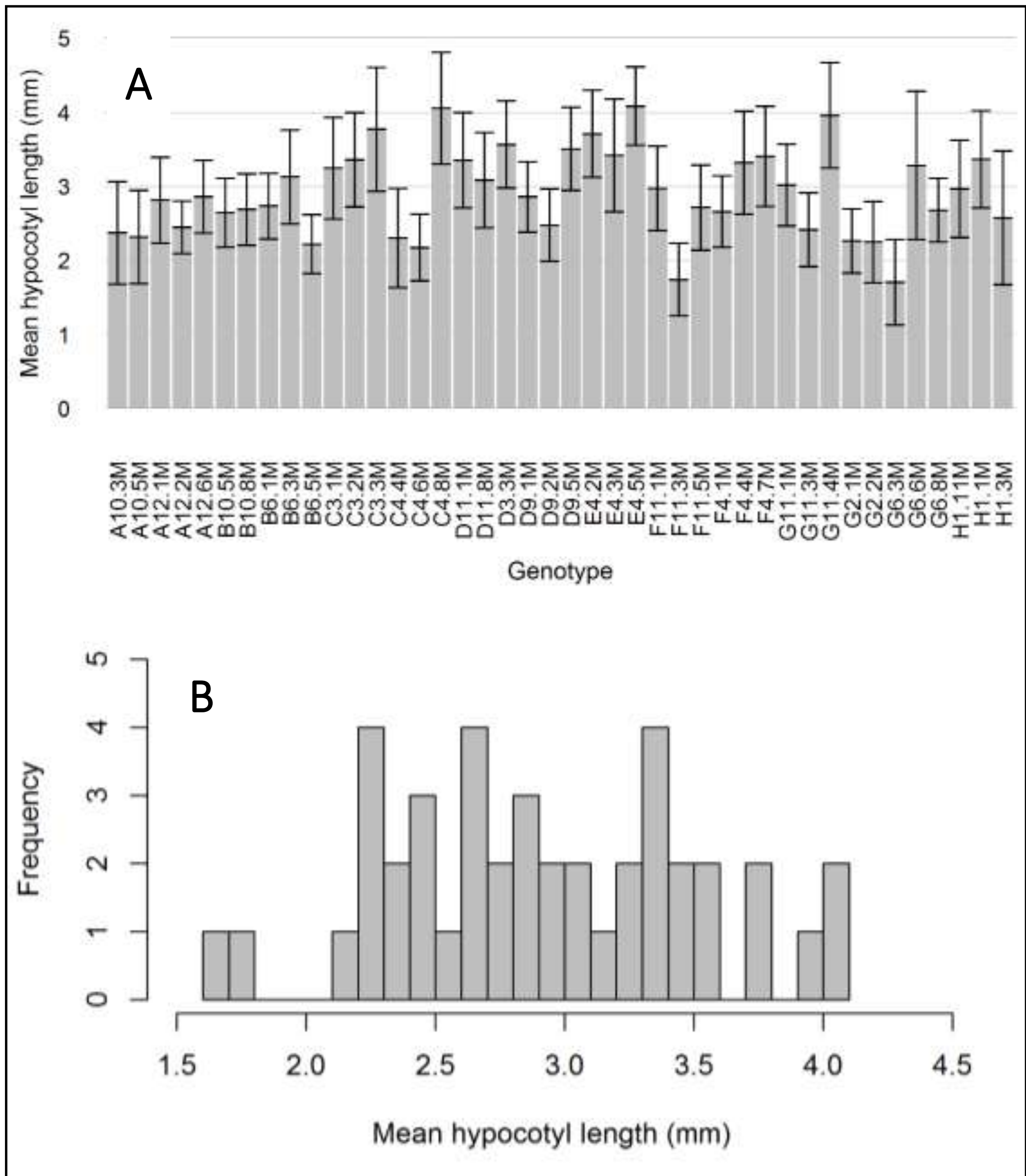



Figure 44: Validity of *hsp90.2-3* mutant population hypocotyl length data at 27°C for QTL mapping.

A: Mean hypocotyl length of each genotype in the population. Error bars show 1 standard deviation.

B: Histogram of mean hypocotyl length of each genotype in the population.

### Discussion

The lack of any significant peaks in the wild-type population could be due a variety of reasons. These reasons are discussed in detail in section 4.4. It is worth noting that mapping hypocotyl length of the wild-type population has returned negative results under all conditions tested. It is not certain that the cause of these similar results is the same, especially as the suitability of the data for QTL analysis has varied. As the quality of the data should have yielded positive results, I think it is fairly likely that growth at warm temperatures in the wild-type population is affected by several QTL of small effect, or QTL that are hidden by the action of HSP90 family proteins.

It is interesting to note the positions of the QTL found in the mutant mapping population. The QTL are in exactly the same positions as the QTL in the whole mapping population, although they have slightly lower LOD scores. The lower LOD scores are most likely a natural result of analysing only 44 genotypes instead of 85. As the QTL are in the same positions, it is likely that the genes under the peaks are also the same. The fact that these QTL do not appear when a wild-type population is mapped presents an interesting question. It is possible that the candidate genes, which I previously hypothesised are part of a temperature perception and signalling pathway, are cryptic variation in a wild-type population due to the action of heat shock proteins.

## 6.5 QTL mapping of change in growth due to warmth

When mapping growth in two different conditions, some QTL could be QTL for general growth and not related to the specific condition. I wanted to investigate QTL specific to an increase in growth when temperatures are raised. To do this I calculated the change in hypocotyl length due to warmth using the method described in section 2.6 and used this value as a trait in QTL mapping. Using this analysis, I found QTL that have a role in changing in growth in response to warmth, rather than general growth QTL.

## Results of analysis

I calculated the difference in height due to increased temperature and used this value as a trait in QTL mapping. I mapped the change in mean hypocotyl length due to warmth of 20 individuals from 85 genotypes at using the method in section 2.6. Figure 45 displays the QTL map obtained from this analysis. Although there are some regions of high LOD score, there are no peaks that are statistically significant.

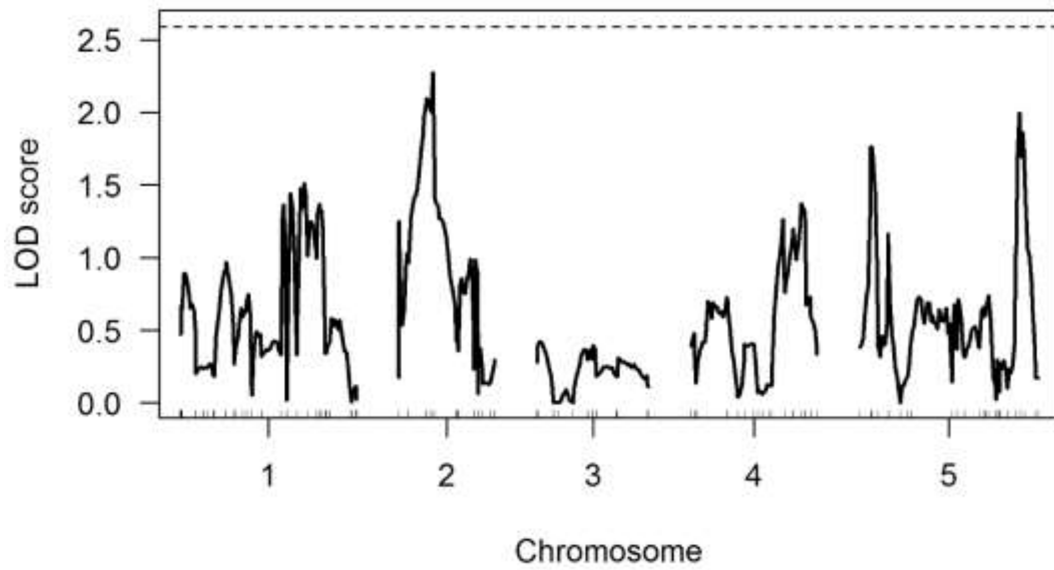



Figure 45: QTL map of the difference in mean hypocotyl length at 27°C compared to control conditions using the multiple-imputation method. The dotted line indicates the 20% significance threshold.

## Validity of test

Distribution:

Figure 46A displays the distribution of the change in mean hypocotyl length due to warmth.

Shapiro-Wilks: $W = 0.919$, $p = 6.163e\text{-}05$

It is easy to see visually that the data were not normally distributed, and this is confirmed statistically. This distribution is due to some anomalous results in which seedlings grown at warm temperatures were shorter than the same genotype grown at control temperatures. Upon careful examination of my raw data, I found that certain groups of genotypes grown on the same agar plate had reduced growth. This could be due to a number of environmental factors. I decided to exclude these anomalous results from my analysis.

The distribution of the corrected data are displayed in figure 46B.

Shapiro-Wilks: $W = 0.985$, $p = 0.453$

Variation:

Figure 47 displays the change in mean hypocotyl length due to warmth of each genotype in my mapping population. The data used for this figure does not include any anomalous results.

ANOVA: $F = 7.14$, $d.f = 80, 1468$, $p = <2^{e-16}$

See section 2.7 for a full explanation of these statistics.

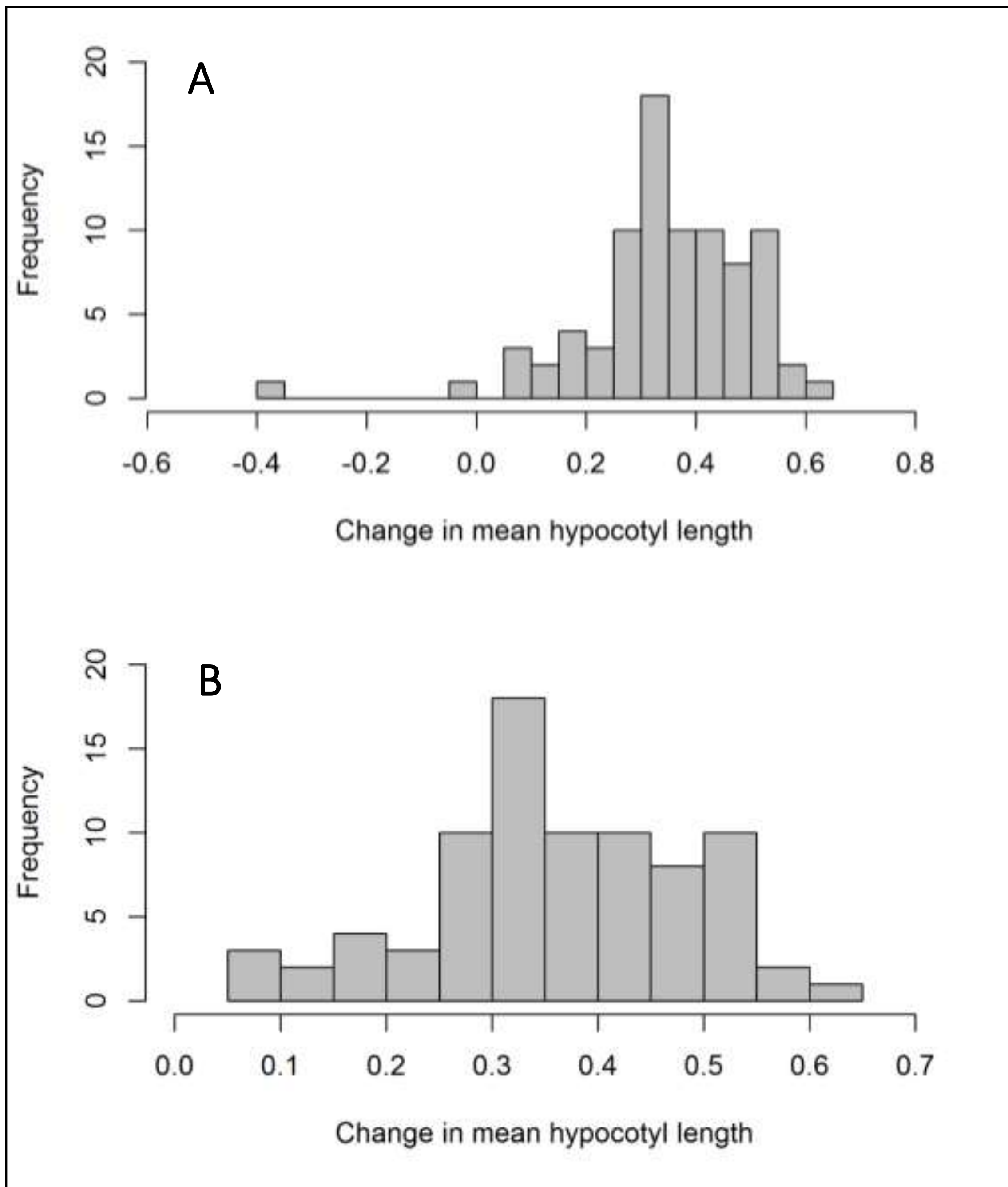



Figure 46: Distribution of the change in mean hypocotyl length due to warmth.

A: Distribution of all genotypes in the mapping population.

B: Distribution after anomalous results are removed from the data.

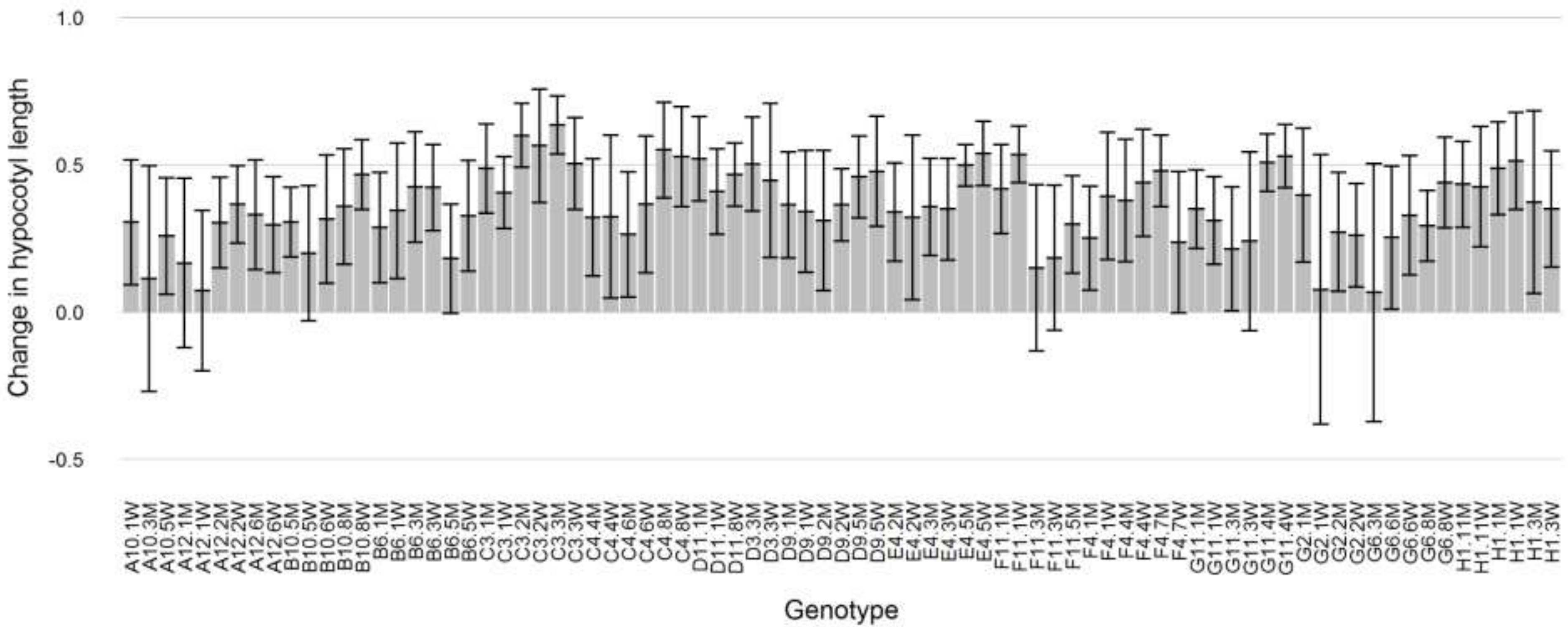



Figure 47: Change in hypocotyl length due to warmth in all genotypes used for QTL mapping.

### Discussion

The lack of significant QTL in this analysis was a surprising result. It is possible that there are relevant QTL that were not found, either because they only have a small effect or because they only seem relevant in a model which allows interaction between QTL. QTL like this would be found using a two-QTL scan. A more detailed discussion of why QTL mapping may yield no results can be found in section 4.4. Further analysis, including a two-QTL scan, is needed to fully understand this result.

## 6.6 Two-QTL scan of change in growth due to warmth

My single-QTL analysis did not detect every QTL relevant to growth in my mapping population. As discussed previously (section 4.3) single-QTL mapping rarely reveals every significant QTL, making further analysis using two-QTL scans necessary. I analysed my data this way to reveal QTL with epistatic interactions that were invisible to a single-QTL scan.

### Results of analysis

I performed a two-dimensional, two-QTL scan on the same mapping data using the method in section 2.6. Figure 48 displays the results of this analysis. The high $LOD_{fv1}$ scores in the lower right triangle show evidence for pairs of QTL if interaction is allowed, particularly on chromosomes 1 and 5. The high $LOD_i$ scores in the upper left triangle show clear evidence for epistatic interactions rather than an additive relationship.

I found four pairs of QTL with LOD scores high enough to be considered statistically significant. One pair is on chromosome 1 position 37.4 and chromosome 1 position 72.38. There is significant evidence for this pair of QTL in both a full model and an additive model, although the evidence is stronger if interaction is allowed. The second pair is on chromosome 1 position 66.4 and chromosome 2 position 20.51. These positions can only be considered QTL in a model that allows interaction. The third pair is on chromosome 1 position 61.4 and chromosome 5 position 98.52. These positions can

only be considered QTL in a model that allows interaction. The final pair is on chromosome 2 position 20.5 and chromosome 5 position 9.52. These positions can only be considered QTL in an additive model where no interaction is allowed. All of these positions are likely to be pairs of QTL which only show their effect when they are allowed to interact with each other or when both exist at the same time.

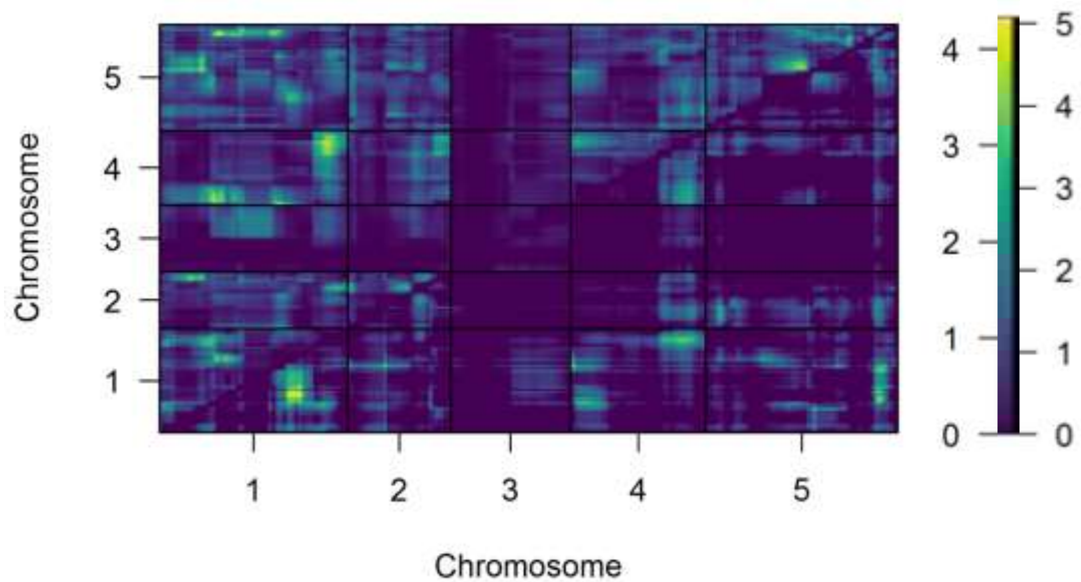



Figure 48: LOD scores from a two-QTL scan using the multiple-imputation method. The trait used was the difference in mean hypocotyl length with at 27°C compared to control conditions. The lower right triangle contains $LOD_{fv1}$ scores showing the probability of one half of a pair of epistatic QTL existing at that position. The right side of the key indicates the magnitude of $LOD_{fv1}$ scores. The upper left triangle contains $LOD_i$ scores showing the probability of synergistic interactions as opposed to an additive relationship. The left side of the key indicates the magnitude of $LOD_i$ scores.

### Discussion

This analysis found several QTL not seen in any other analysis as well as some QTL whose role in growth was established by my previous results. Among the established QTL was QTL-A. QTL-A has previously been seen to have an effect both on its own and in epistasis with other loci, as it does here. It was also established as having an important role in driving a change in growth due to exogenous auxin. This is supported by this result, as QTL-A appears to be important in auxin-driven growth whether the auxin is added artificially or produced naturally by the plant as a response to increased temperature. It seems likely that QTL-A is part of an auxin pathway with a possible role in detection, sensitivity or response pathways.

One of the pairs found in this analysis is the same as the pair found in the two-QTL scan of growth at warm temperatures. These QTL appear to be specific to driving the change in growth at warm temperatures, as opposed to general growth QTL.

This analysis found several QTL not seen in any of my other QTL maps. The QTL unseen before are likely to be genes which have a role in temperature perception or auxin signal transduction, since these pathways are the driving force behind the increase in growth seen at warm temperatures (McClung et al. 2016).

## 6.7 Summary of results

The results presented in this chapter provide a complex picture of the genetic basis of growth at warm temperatures. Many of the QTL involved seem to be unique to this condition, unshared by QTL maps for growth in standard conditions or growth with exogenous auxin. An increase in the complexity of signalling pathways is likely to lead to different genes having increased importance. This is discussed in more detail in chapter 7.

One QTL that appears to be important to many aspects of growth is QTL-A. QTL-A has previously been seen to have a role in general growth (section 4.8) and growth with exogenous auxin (section 5.11). The results in this chapter suggest that it is also

important for increasing growth in warm temperatures. The importance of QTL-A has been well established by my results, and its action and relationship with other QTL deserve more research. A full discussion of QTL-A can be found in chapter 7.

# Chapter 7: Discussion

## 7.1 The effect of *hsp90.2-3* on growth

I studied the effect of heat shock proteins through the mutation *hsp90.2-3*, which deactivates all other proteins in the HSP90 family (Hubert et al. 2003, Sangster and Quietsch 2005, Sangster et al. 2008). Plants with the mutation have a similar phenotype to plants growth at warm temperatures, including increased growth, early flowering and greater variation within a population (Sangster and Quietsch 2005, Sangster et al. 2008). I wanted to study the effect of the mutation on hypocotyl growth.

In my preliminary experiments I found that the effect of the mutation seems to vary depending on genetic background and environment (section 3.3). In standard conditions, Col-0 plants with the mutation were significantly taller than wild-type Col-0 plants, but there was no significant difference in WS-2 plants. This effect was reversed when auxin was added to the growth media. WS-2 mutants were significantly taller than wild-type WS-2 plants but the mutation had no significant effect on Col-0 plants. To complicate things further, at warm temperatures the mutation had no significant effect on either genotype. These results seem strange at first glance, but studying the standard deviation can provide some insight. In each case where the mutation increased growth, it also increased standard deviation. In each case where the mutation had no significant effect, the standard deviation was either very similar or decreased. I think that the reason for the decrease in standard deviation was that all the plants in the population were approaching their maximum possible height under the circumstances which led them to be quite similar. This is not the maximum possible height for an *Arabidopsis* seedling, so something must have been limiting further growth.

If this hypothesis is true, it may indicate a flaw in my method. Differences in hypocotyl length could be seen if seedlings were measured earlier than 10 days. Examining my results under this hypothesis, it appears that the mutation has no effect in Col-0 when

auxin levels increase, either naturally by warmth or by adding auxin to the growth media. Both of these conditions increase the growth of Col-0 and appear to do so to the point where the mutation cannot increase growth any further. In contrast, the mutation only has an effect on WS-2 seedlings when auxin in added to the growth media. It may be that after 10 days of growth with natural auxin levels WS-2 is at its maximum height whether it contains the mutation or not, and this can only be increased further by providing additional auxin. Future experiments could test this hypothesis by measuring hypocotyl length at a range of time points (Gendreau et al. 1997). There may be differences in growth earlier than 10 days that could provide additional insights into the part HSP90 proteins play in controlling growth.

## 7.2 The effect of auxin on growth

The phytohormone auxin is a major regulator of cell enlargement and cell division (Evans 1985), so it can have a dramatic effect on the hypocotyl (Smalle et al. 1997, Collett et al. 2000). However, this effect depends on a few things. Firstly, there are several different types of auxin. Auxins that occur naturally in the plant, most notably IAA, are polar. This means that they can only move one way through auxin transporters (Blakeslee et al. 2005) which prevents auxin from “flooding” the whole plant – it is localized in sink tissues which are actively growing such as the tips of the roots and stem (Ljung et al. 2001). This matters less in the hypocotyl, as nearly the whole seedling is actively growing when it is so small. However, a non-polar auxin like picloram will generally have a greater effect than IAA as picloram can be present in all cells. Secondly, the auxin signalling pathway is only one element in a very complex web of phytohormones and other factors controlling hypocotyl growth (Scheel and Wasternack 2002, Davies 2004). Natural auxin levels are at an optimum concentration (Collett et al. 2000). Although increasing auxin levels by a small amount results in an increase in growth, high concentrations of auxin will inhibit growth (Collet et al. 2000) due to the balance between different signalling pathways being disrupted. At the right concentration, adding auxin to growth media will increase hypocotyl length and standard deviation (section 3.5).

I chose to study the effect of auxin on growth in two ways. Firstly, I added synthetic auxin to the growth media, as shown previously. Secondly, I studied the role of auxin in a more natural process by increasing the temperature. Warm temperatures naturally increase auxin levels within the plant, so it is a useful way of observing the effect of auxin in nature. I found that warmth increased the hypocotyl length and standard deviation of Col-0 seedlings but not WS-2 seedlings. This could mean that WS-2 plants are naturally less sensitive to warmth. However, there is a decrease in standard deviation in WS-2 seedlings in warmth compared to control conditions. I think that this decrease means WS-2 seedlings are approaching their maximum possible height under the conditions, which makes all seedlings fairly similar to each other. Seedlings can grow taller than this if auxin is added to the growth media, suggesting that the seedlings capacity for auxin biosynthesis is limiting its growth. If this hypothesis is true, then measuring hypocotyl length before 10 days would reveal a difference between seedlings grown at warm temperatures and those grown in control conditions.

## 7.3 QTL for growth and variation

I aimed to find QTL for growth and variation in a range of conditions, and in this regard, I succeeded. Table 1 summarises all the QTL I detected. I found QTL for general growth and variation by performing QTL mapping under standard conditions. Interestingly, there were QTL for growth in the population containing the *hsp90.2-3* mutation but not a purely wild-type population. The mutation is used in QTL mapping studies to reveal cryptic variation (Sangster et al. 2007), which may be why there are no QTL in a wild-type population. The QTL for growth often acted through epistatic interactions. This is in contrast to QTL for variation in standard conditions, which only appeared in single-QTL scans. This suggests that the control of hypocotyl growth involves a complex network of interactions while the control of variation is relatively simple.

QTL mapping in warm temperatures and with added auxin produced broadly similar results. I found QTL for growth in the full mapping population and the population containing the *hsp90.2-3* mutation, but not a purely wild-type population. 2-QTL scans

suggest that these QTL for growth at warm temperatures act through epistatic interactions. Most of these growth QTL act through synergistic interactions, but there are some that have simple additive interactions. However, there are some differences in the QTL found in these two conditions. The QTL for growth with added auxin are similar to the general growth QTL in standard conditions, while the QTL for growth at warm temperatures tend to be different. Many of the QTL involved in growth at warm temperatures seem to be unique to this condition, unshared by QTL maps for growth in standard conditions or growth with exogenous auxin. Although warmth naturally increases auxin levels within the plant (Gray et al. 1998, Stavang et al. 2009, Quint et al. 2016), growth with exogenous auxin seems to have more in common at a genetic level with growth in standard conditions than growth at warm temperatures. This could be due to differences in the way the conditions are perceived and the signals needed to turn this perception into an increase in growth (Kamada et al. 1995, Orvar et al. 2000, Penfield 2008). Growth with exogenous auxin likely involves genes governing auxin sensitivity, while growth at warm temperatures involves a more complex web of temperature perception, auxin biosynthesis and multiple signal transduction pathways (Penfield 2008, Wigge 2013, Quint et al. 2017). This increase in complexity is likely to lead to different genes having increased importance. My results suggest that the pathways controlling growth in standard conditions mostly involve auxin, while the pathways active at warm temperatures involve different elements.

I found a difference when mapping variation in warmth and with added auxin. I found no QTL for variation at warm temperatures in any population, but I found several interesting results when I mapped variation with added auxin. There was a wide region of high LOD score which is likely to be a compound peak containing several QTL. There appears to be at least one, and probably multiple, QTL regulating variation in hypocotyl length in the presence of exogenous auxin. The QTL found when standard deviation was mapped was easily wide enough to assume that multiple QTL lie under it. This region was present in both the wild-type and mutant populations. This region encompasses loci where QTL were found on different maps and a region where very few QTL have been seen in my other analyses. This makes it difficult to draw any conclusions as to what genes might be under the peak and what action they may have. However, it is

clear that multiple genes work to regulate variation in the presence of exogenous auxin. 2-QTL scans revealed multiple pairs of epistatic QTL controlling variation in the full population and the mutant population. It is possible that increasing auxin levels activated a signalling pathway that influences variation.

One of my aims was to understand which QTL were needed to drive a change in growth due to auxin. To do this I calculated the change in growth using growth in standard conditions as a control and mapped the change as a trait. When I mapped the change in growth due to both warmth and added auxin I found that epistatic interactions are very important. Single-QTL scans produced limited results, but two-QTL scans were more detailed. The change due to warmth is mostly controlled by pairs of QTL with synergistic interactions, although there is one pair with an additive interaction. In contrast, the change due to added auxin appears to happen mostly through additive interactions. QTL-A has a role in both responses but otherwise not many QTL are shared between the two maps. Although warmth and picloram produce very similar phenotypes, they appear to do so through different genetic pathways.

| | | Chromosome 1 | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Population** | **Treatment** | 1.4 | 8.4 | 27.4 | 37.4 | 41.4 | 43.4 | 44.4 | 45.4 | 61.4 | 66.4 | 72.4 | 76.4 | 95.4 | 100.4 |
| Full | Control | | | | | | | ★ | | | | | | | |
| Wild-type | Control | | | | | | | | | | | | | | |
| Mutant | Control | | | | | | | | | | | | | | |
| Full | Auxin | + | + | | | | ✶ | ★ | ★ | | | | | ★ | X |
| Wild-type | Auxin | | | | | | | | | | | | | | |
| Mutant | Auxin | | | | | | | | | | | | | | |
| Full | Warmth | | | ✓ | ★ ★ | ✶ | | | | ✸ | ✶ | ★ | ★ | | |
| Wild-type | Warmth | | | | | | | | | | | | | | |
| Mutant | Warmth | | | ✓ | | | | | | | | | | | |

| | | Chromosome 2 | | Chromosome 3 | | | | | Chromosome 4 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Population** | **Treatment** | 20.5 | 35.5 | 36.4 | 56.4 | 58.4 | 63.4 | 67.3 | 1.5 | 42.5 | 53.5 | 68.4 |
| Full | Control | | | | | ✶ | | | | | | |
| Wild-type | Control | | ✓ | | | | | | | | | |
| Mutant | Control | | | | ★ | | | | | | | |
| Full | Auxin | | ★✸ | ✸ | ✶ | | ✶ | | X | ✸ | | |
| Wild-type | Auxin | | | | | | | | | | | |
| Mutant | Auxin | | | | | | | ★ | | | | |
| Full | Warmth | ✶+ | | | | | | | | | ✸ | ✓ |
| Wild-type | Warmth | | | | | | | | | | | |
| Mutant | Warmth | | | | | | | | | | | ✓ |

Table 1 continued on next page

| | | Chromosome 5 | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Population** | **Treatment** | 0.5 | 6.5 | 9.5 | 13.5 | 14.5 | 15.5 | 20.5 | 58.5 | 60.5 | 95.5 | 96.5 | 99.5 |
| Full | Control | | | | ✓ | ✸ | | | | | ✓★✶✸ | ✓ | |
| Wild-type | Control | | | | | | | | | | | | |
| Mutant | Control | | | | | | ✶ | | | | ✓★✶ | | |
| Full | Auxin | | | | | | | ✓✶✸✪ | | ✪ | ✓ ✓ | | |
| Wild-type | Auxin | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | | | | |
| Mutant | Auxin | ✓ | | | | ✓ | | ★✶ | ✶ | | ✓ | | |
| Full | Warmth | | ✸ | + | | | | | | | ✶ | | ✸ |
| Wild-type | Warmth | | | | | | | | | | | | |
| Mutant | Warmth | | | | | | | | | | | | |

Table 1: Summary of all QTL found. QTL for hypocotyl length are coloured green. QTL for standard deviation of hypocotyl length are coloured blue. QTL for change in hypocotyl length are coloured pink. QTL detected by single-QTL analysis are denoted by a tick mark. Pairs of QTL found in two-QTL analysis are denoted by matching symbols. Pairs of QTL found using a full model that allows interaction are denoted by star-shaped symbols. Pairs of QTL found using an additive model that does not allow interaction are denoted by cross-shaped symbols.

## 7.4 QTL-A and its role in growth

I produced QTL maps of hypocotyl length for three populations in three conditions. One QTL, which I have called QTL-A, appeared in more of these QTL maps than any other. The QTL is on chromosome 5 at approximately position 95. QTL-A is located very close to the *hsp90.2-3* mutation, which can appear as a QTL itself due to its role in growth. However, if QTL-A were the mutation, I would expect the highest LOD score to be either at the mutation or on one of the markers immediately next to it. At the position of QTL-A, there is no obvious linkage to the mutation, so I think QTL-A is a gene close to the mutation that is not linked to it.

By mapping different populations and conditions in different ways, the role of this QTL can be studied. In standard conditions, it is clear through mapping hypocotyl length and standard deviation in the full mapping population that QTL-A is important for both growth and variation. Two-QTL scans in standard conditions revealed that QTL-A interacts with several others. These other QTL were only detected in a full model that allowed QTL to truly interact, as opposed to an additive model where QTL simply co-exist. It is possible that QTL-A interacts with some heat shock proteins as I detected fewer pairs of QTL in a two-QTL scan of only mutant genotypes, which have deactivated heat shock proteins, compared to a two-QTL scan of the full population. QTL-A appears to play a major role in growth and a more minor role in regulating variation, as seen by its reduced LOD score when standard deviation was mapped. Any effect QTL-A has on variation appears to be through its own action alone, as two-QTL scans returned negative results.

With auxin added to the growth media, QTL-A still impacts growth but appears to do so independently, without any epistatic interactions. QTL-A also appears to impact growth at warm temperatures, although in this condition it appears to function mainly through epistatic interactions. QTL-A could be a gene whose action is altered or enhanced by auxin, leading its mode of action to change when exogenous auxin is added. Its role as a general growth QTL is challenged by analysing only the change in growth due to auxin, instead of overall growth. QTL-A appears to have a role in increasing growth in the presence of auxin. It is possible that QTL-A is part of an auxin pathway with a role in

perception or signal transduction. This would allow it to regulate growth and variation under standard conditions, as auxin is a major growth phytohormone (Collet et al. 2000), and have an enhanced effect when auxin levels increase. It appears to be able to drive growth through its own action as well as interacting with other QTL, depending on the environmental conditions.

I can draw three main conclusions about the nature of QTL-A from my data.

Firstly, QTL-A frequently appears in QTL maps for hypocotyl length but is only present in one map for standard deviation: the map of standard deviation in standard conditions. In this map there is a visible peak in LOD score at the position of QTL-A, but the LOD score does not pass the significance threshold calculated by a permutation test. QTL-A may have some role in regulating variation, but this role appears to be small and only relevant if no other factors, such as auxin, are controlling variation more strongly. The main role of QTL-A appears to be in growth.

Secondly, QTL-A is more commonly seen in 2-QTL scans than single-QTL scans. It interacts with a wide variety of other QTL. QTL-A only has a significant effect in a full model that allows synergistic interactions between QTL, not an additive model where QTL simply coexist. This suggests that QTL-A has a role in regulating the action of several genes. QTL-A could be a component of a signalling pathway that activates or de-activates other genes.

Thirdly, the role of QTL-A in growth is linked to auxin. As well as appearing in QTL scans for general growth, QTL-A appears in QTL scans for changes in hypocotyl length due to warmth and auxin. At warm temperatures, QTL-A acts through an epistatic interaction. With added auxin QTL-A appears in a single-QTL scan but not a 2-QTL scan, so it may also have an effect on its own. These results suggest that QTL-A is a component of an auxin signalling pathway, or is strongly regulated by auxin.

Overall, I can conclude that QTL-A may be part of an auxin signalling pathway that regulates growth through epistatic interactions. This hypothesis could be investigated further by using a bioinformatics approach to examine what genes are present in the region of QTL-A (Arcade et al. 2004). It is also possible to isolate a QTL through selective

breeding (Alonso-Blanco and Kornneef 2000). This approach would narrow the range of possible candidate genes (Paran and Zamir 2003). Additional information about the role of QTL-A and its effect on growth could be gained by mapping QTL in more environmental conditions that increase auxin, such as shade (Vandenbussche et al. 2003). No firm conclusions about QTL-A can be drawn from my current data, but there are many possibilities for further study.

## 7.5 Conclusions

I set out three aims at the beginning of this dissertation. I am satisfied that my results have accomplished these aims, although not all of my expectations were met.

Firstly, I aimed to investigate hypocotyl growth in a range of conditions. I have gained a greater understanding of the effect of warmth, the synthetic auxin picloram and the mutation *hsp90.2-3*. I would like to study these conditions further by measuring hypocotyl length at different time points as my experimental design seems limited in this regard. However, I have concluded that although warmth and the mutation increase growth, a plant's natural capacity for auxin biosynthesis may limit its growth after a certain point.

Secondly, I aimed to map QTL for growth and variation in three conditions: standard, warmth and with added auxin. I have accomplished this and produced QTL maps for all three conditions using three populations: the full RIL, a wild-type population and a population containing the *hsp90.2-3* mutation. I found several QTL, most notably one on chromosome 5 which I have called QTL-A. This QTL appears to have a role in growth in all three conditions and acts through epistatic interactions with other QTL. I have hypothesised that it is a component of an auxin signalling pathway. QTL-A could be studied further using bioinformatics or by cloning the QTL.

Thirdly, I aimed to investigate the similarities and differences between QTL maps for different conditions. I did this by mapping the change in growth due to warmth or auxin as a trait. Using this method, I have found that QTL-A is needed to produce a change in

growth in both warm temperatures and with added auxin, but that many other QTL involved are different.

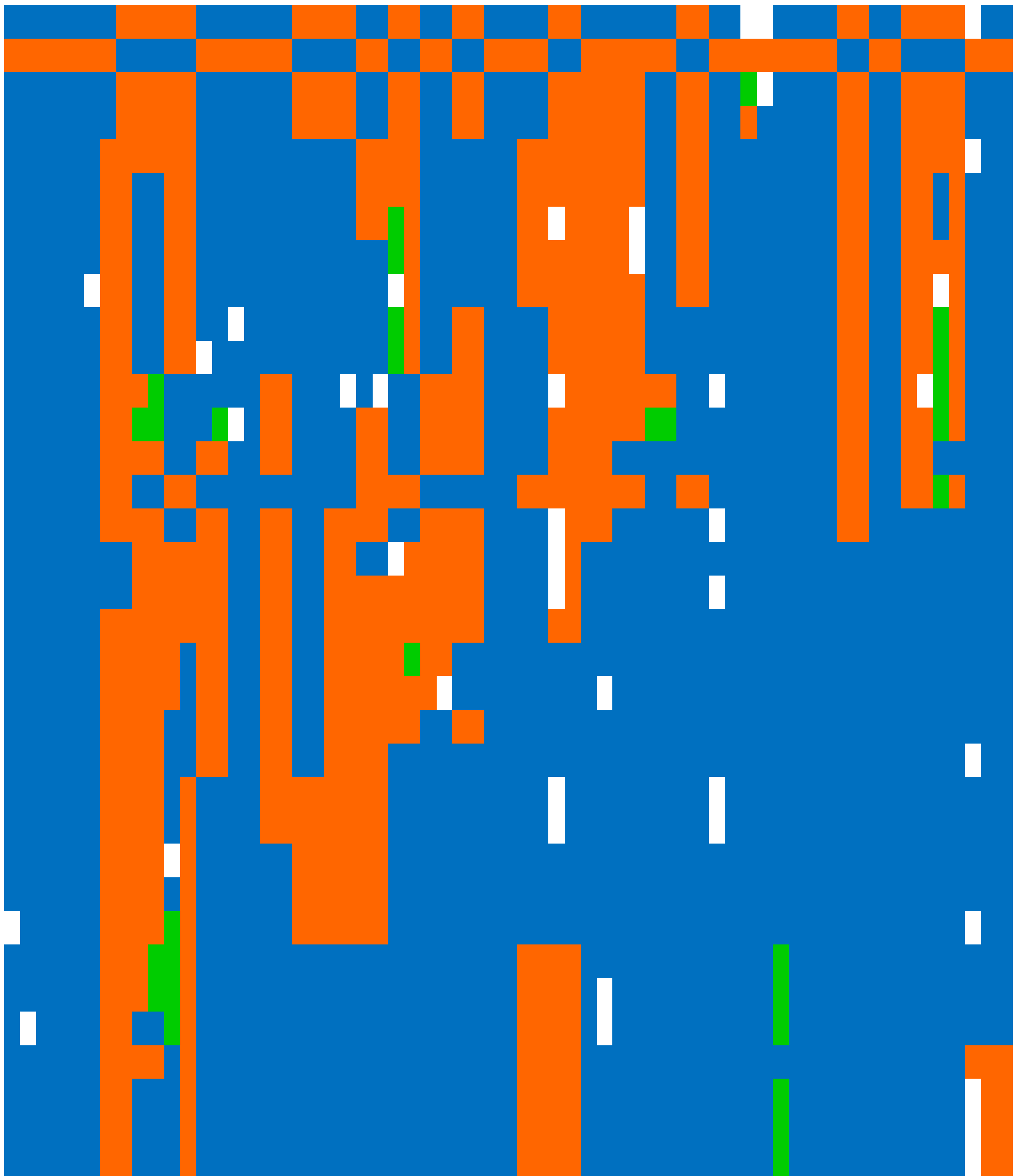
It must be noted that due to a small population size, the statistical power of my QTL analysis is low. Therefore, care must be taken when interpreting the results. It is possible that any positive results seen are statistical artefacts resulting from the natural stochastic variation in my data. It would be unwise to draw firm conclusions or hypothesise in too much detail about what may lie under any particular QTL. The small population size and lack of statistical power is the main flaw in my experimental design. This is especially true of the results of 2-QTL mapping, which requires especially high power to produce reliable results. This work could be built on and improved in the future by repeating experiments using a larger mapping population.

Overall, I am satisfied that I have accomplished the aims I set out with. My results confirm and provide additional details of previous hypotheses on auxin and hypocotyl growth, as well as providing new information into similarities and differences between QTL maps of different conditions. My results provide several new hypotheses and possibilities for further research.

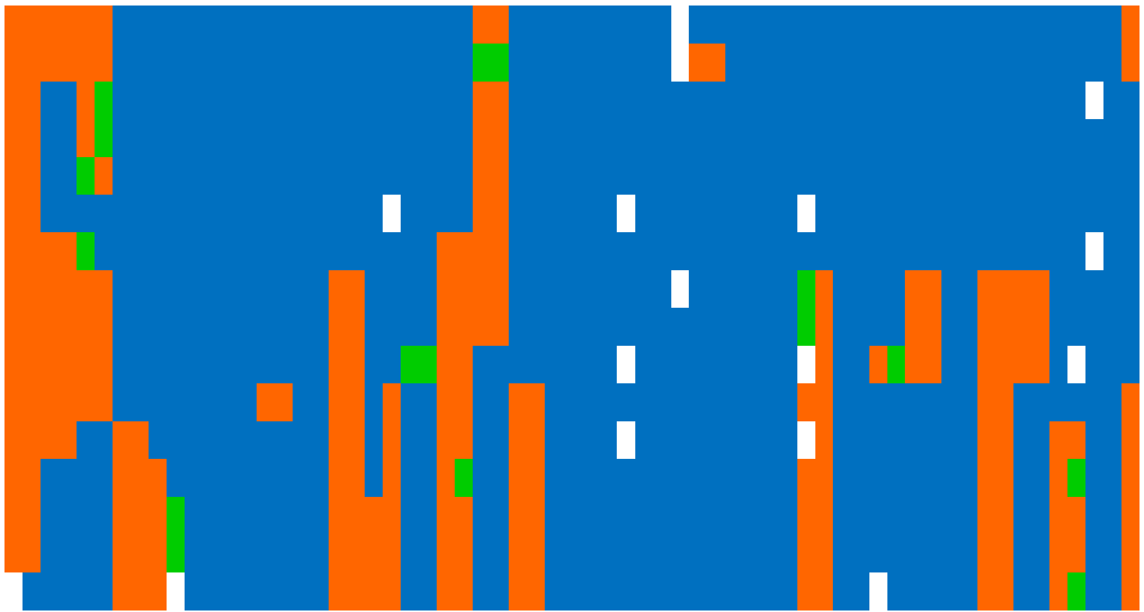
# Appendix 1: Graphical representation of mapping population

Graphical representation of the mapping population used for QTL analysis. Each column is an individual RIL. Each row is a genetic marker. WS-2 alleles are in blue, Col-0 alleles are in orange, heterozygotes are green and missing genotype information is white.

Chromosome 1

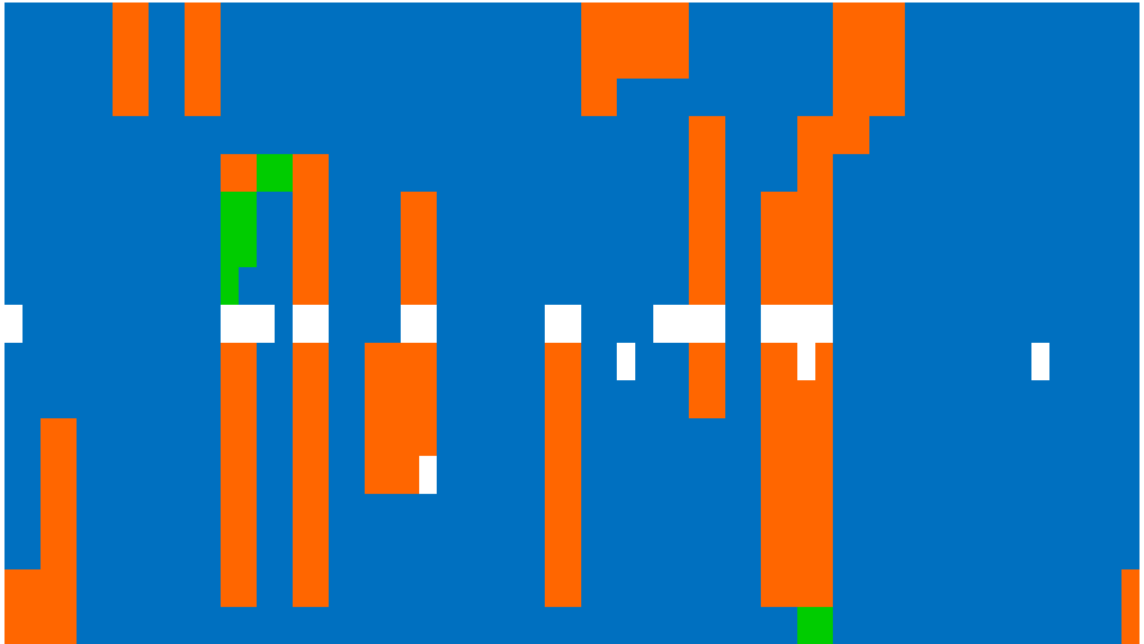


Chromosome 2

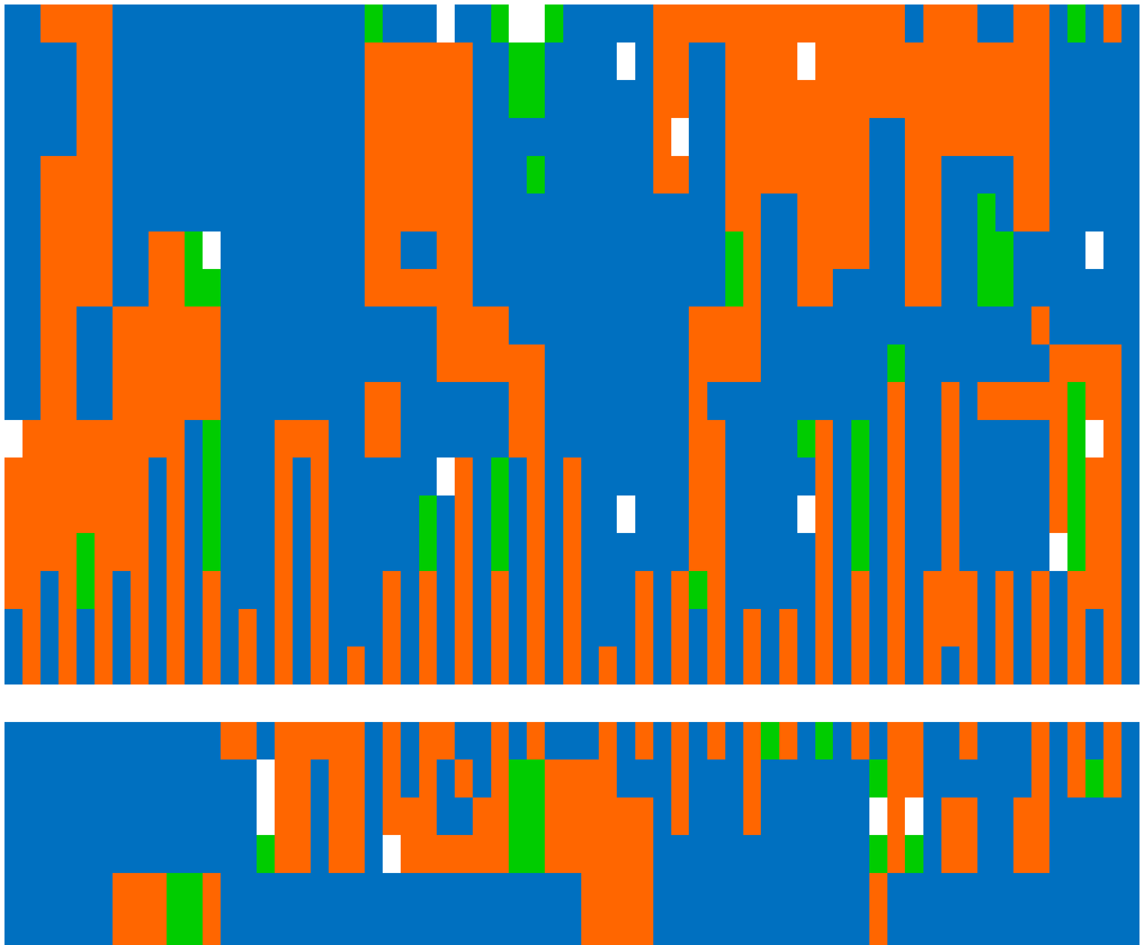


Chromosome 3

Chromosome 4



Chromosome 5



# Appendix 2: Marker information for mapping population

| Marker | % WS-2 | % Col-0 | % heterozygous | % missing |
|---|---|---|---|---|
| AtMSQTsnp2 | 59.6 | 37.2 | 0.0 | 3.2 |
| AthACSmod | 33.0 | 67.0 | 0.0 | 0.0 |
| AtMSQTsnp4 | 60.6 | 37.2 | 1.1 | 1.1 |
| NT7 123 | 61.7 | 38.3 | 0.0 | 0.0 |
| AtMSQTsnp15 | 67.0 | 30.9 | 0.0 | 2.1 |
| JV28/29 | 71.3 | 28.7 | 0.0 | 0.0 |
| W9W i5 | 70.2 | 25.5 | 1.1 | 3.2 |
| F20D23ext | 72.3 | 25.5 | 1.1 | 1.1 |
| AtMSQTsnp31; assum. G-w | 71.3 | 24.5 | 0.0 | 4.3 |
| AtMSQTsnp40; assump. G-w | 73.4 | 23.4 | 2.1 | 1.1 |
| F12K8 | 73.4 | 23.4 | 2.1 | 1.1 |
| AtMSQTsnp47 | 64.9 | 25.5 | 2.1 | 7.4 |
| ciw12 | 66.0 | 26.6 | 6.4 | 1.1 |
| AthSO392mod | 70.2 | 29.8 | 0.0 | 0.0 |
| nga63mod | 70.2 | 28.7 | 1.1 | 0.0 |
| AtMSQTsnp60 | 70.2 | 26.6 | 0.0 | 3.2 |
| msat1.4modB | 75.5 | 21.3 | 0.0 | 3.2 |
| AtMSQTsnp67 | 72.3 | 24.5 | 0.0 | 3.2 |
| ciw1 | 72.3 | 27.7 | 0.0 | 0.0 |
| UA1.20.2 | 77.7 | 21.3 | 1.1 | 0.0 |
| UA1.20.1 | 74.5 | 20.2 | 1.1 | 4.3 |
| AtMSQTsnp76; assum. C-w | 77.7 | 21.3 | 0.0 | 1.1 |
| AtMSQTsnp88 | 84.0 | 13.8 | 0.0 | 2.1 |
| AtMSQTsnp91 | 80.9 | 16.0 | 0.0 | 3.2 |
| AtMSQTsnp92 | 80.9 | 16.0 | 0.0 | 3.2 |
| AtMSQTsnp97 | 81.9 | 17.0 | 0.0 | 1.1 |
| msat1.13mod | 84.0 | 16.0 | 0.0 | 0.0 |
| AtMSQTsnp100 | 78.7 | 14.9 | 1.1 | 5.3 |
| W9W i10 | 73.4 | 23.4 | 3.2 | 0.0 |
| W9W i12 | 70.2 | 19.1 | 3.2 | 7.4 |
| W9W i11 | 74.5 | 21.3 | 2.1 | 2.1 |
| UA1.30.3 | 73.4 | 25.5 | 0.0 | 1.1 |
| AtMSQTsnp114 | 72.3 | 23.4 | 2.1 | 2.1 |
| W9W ii1 | 88.3 | 10.6 | 0.0 | 1.1 |
| W9W ii2 | 86.2 | 10.6 | 2.1 | 1.1 |
| AtMSQTsnp123 | 86.2 | 8.5 | 1.1 | 4.3 |
| LUGSSLP41 | 89.4 | 9.6 | 1.1 | 0.0 |
| AtMSQTsnp128; assum. G-w | 86.2 | 11.7 | 1.1 | 1.1 |

| Marker | % WS-2 | % Col-0 | % heterozygous | % missing |
|---|---|---|---|---|
| AtMSQTsnp129 | 86.2 | 9.6 | 0.0 | 4.3 |
| AtMSQTsnp130 | 79.8 | 13.8 | 1.1 | 5.3 |
| W9W ii4 | 67.0 | 30.9 | 1.1 | 1.1 |
| W9W ii3 | 68.1 | 29.8 | 2.1 | 0.0 |
| AtMSQTsnp145 | 63.8 | 28.7 | 3.2 | 4.3 |
| AtMSQTsnp159 | 69.1 | 29.8 | 1.1 | 0.0 |
| AtMSQTsnp164 | 67.0 | 28.7 | 1.1 | 3.2 |
| UA2.15.1 | 70.2 | 27.7 | 2.1 | 0.0 |
| nga168ext | 66.0 | 33.0 | 1.1 | 0.0 |
| AtMSQTsnp173 | 64.9 | 29.8 | 1.1 | 4.3 |
| UA3.1.1 | 88.3 | 10.6 | 1.1 | 0.0 |
| nga172mod | 89.4 | 10.6 | 0.0 | 0.0 |
| AtMSQTsnp194 | 100.0 | 0.0 | 0.0 | 0.0 |
| nga162mod | 100.0 | 0.0 | 0.0 | 0.0 |
| W9W iii2 | 100.0 | 0.0 | 0.0 | 0.0 |
| ciw11a | 95.7 | 4.3 | 0.0 | 0.0 |
| msat3.23mod | 91.5 | 8.5 | 0.0 | 0.0 |
| AtMSQTsnp220 | 80.9 | 17.0 | 0.0 | 2.1 |
| W9W iii6 | 83.0 | 17.0 | 0.0 | 0.0 |
| W9W iii7 | 83.0 | 17.0 | 0.0 | 0.0 |
| AtMSQTsnp237 | 70.2 | 19.1 | 1.1 | 9.6 |
| F27K19 | 68.1 | 25.5 | 4.3 | 2.1 |
| SD4-12 | 64.9 | 31.9 | 3.2 | 0.0 |
| SD4-13 | 64.9 | 33.0 | 2.1 | 0.0 |
| AtMSQTsnp249 | 73.4 | 25.5 | 1.1 | 0.0 |
| nga8 | 79.8 | 18.1 | 2.1 | 0.0 |
| AtMSQTsnp263; assum. T-w | 77.7 | 18.1 | 3.2 | 1.1 |
| AtMSQTsnp266; assum. G-w | 75.5 | 20.2 | 3.2 | 1.1 |
| AtMSQTsnp278 | 76.6 | 20.2 | 3.2 | 0.0 |
| SD3-1 | 78.7 | 20.2 | 1.1 | 0.0 |
| G3883 | 70.2 | 0.0 | 0.0 | 29.8 |
| AtMSQTsnp286 | 71.3 | 20.2 | 3.2 | 5.3 |
| AtMSQTsnp288 | 72.3 | 21.3 | 3.2 | 3.2 |
| F26K10 | 70.2 | 27.7 | 2.1 | 0.0 |
| F6E21 | 67.0 | 27.7 | 3.2 | 2.1 |
| AtMSQTsnp304 | 69.1 | 23.4 | 3.2 | 4.3 |
| AtMSQTsnp306 | 70.2 | 26.6 | 1.1 | 2.1 |
| AtMSQTsnp310 | 74.5 | 22.3 | 0.0 | 3.2 |
| AtMSQTsnp315; assum. T-w | 84.0 | 11.7 | 3.2 | 1.1 |
| W9W v1 | 55.3 | 31.9 | 7.4 | 5.3 |
| AtMSQTsnp331 | 61.7 | 33.0 | 2.1 | 3.2 |
| MOJB | 63.8 | 34.0 | 2.1 | 0.0 |
| SD5-4 | 68.1 | 30.9 | 0.0 | 1.1 |
| nga151a | 68.1 | 29.8 | 1.1 | 1.1 |

| Marker | % WS-2 | % Col-0 | % heterozygous | % missing |
|---|---|---|---|---|
| AtMSQTsnp355 | 71.3 | 27.7 | 1.1 | 0.0 |
| AtMSQTsnp361 | 68.1 | 24.5 | 4.3 | 3.2 |
| nga139modB | 70.2 | 24.5 | 5.3 | 0.0 |
| SO191ext | 77.7 | 22.3 | 0.0 | 0.0 |
| AtMSQTsnp373; assum. G-w | 70.2 | 27.7 | 1.1 | 1.1 |
| ciw9 | 71.3 | 27.7 | 1.1 | 0.0 |
| AtMSQTsnp388 | 54.3 | 35.1 | 4.3 | 6.4 |
| AtMSQTsnp390 | 55.3 | 37.2 | 5.3 | 2.1 |
| AtMSQTsnp392 | 53.2 | 36.2 | 6.4 | 4.3 |
| AtMSQTsnp395 | 55.3 | 34.0 | 9.6 | 1.1 |
| AtMSQTsnp398 | 54.3 | 41.5 | 4.3 | 0.0 |
| AtMSQTsnp399 | 51.1 | 46.8 | 2.1 | 0.0 |
| 2022_6 | 51.1 | 48.9 | 0.0 | 0.0 |
| *hsp90.2-3* | - | - | - | - |
| MNC17 | 53.2 | 43.6 | 3.2 | 0.0 |
| AtMSQTsnp408; assum. G-w | 57.4 | 35.1 | 5.3 | 2.1 |
| AtMSQTsnp409; assum. T-w | 54.3 | 36.2 | 4.3 | 5.3 |
| AtMSQTsnp415 | 55.3 | 36.2 | 5.3 | 3.2 |
| W9W v13 | 79.8 | 18.1 | 2.1 | 0.0 |
| W9W v11 | 79.8 | 18.1 | 2.1 | 0.0 |

Total number of markers: 102

# Appendix 3: Parental genotypes growth data

| Genotype | Col-0 | | | Col-0 *hs90.2-3* | | |
|---|---|---|---|---|---|---|
| Treatment | Standard | Warmth | Auxin | Standard | Warmth | Auxin |
| Hypocotyl Length (mm) | 1.29 | 1.9703 | 2.549 | 2.148 | 2.5412 | 2.551 |
| | 1.581 | 1.9949 | 2.226 | 2.125 | 2.3583 | 3.315 |
| | 1.754 | 2.3076 | 3.994 | 1.772 | 2.2608 | 2.854 |
| | 1.638 | 2.0151 | 5.646 | 1.909 | 2.5915 | 2.39 |
| | 1.809 | 2.7272 | 3.177 | 2.159 | 3.1577 | 4.392 |
| | 1.344 | 1.6383 | 3.272 | 1.892 | 1.9176 | 3.879 |
| | 1.748 | 1.4862 | 2.164 | 2.535 | 1.6467 | 4.374 |
| | 1.369 | 2.4079 | 3.746 | 2.173 | 2.2191 | 3.114 |
| | 1.055 | 1.6462 | 2.383 | 1.668 | 2.4446 | 2.94 |
| | 1.368 | 3.0459 | 2.495 | 1.603 | 2.1116 | 3.35 |
| | 1.415 | 1.4142 | 3.546 | 1.719 | 1.925 | 4.797 |
| | 1.243 | 1.6388 | 3.24 | 1.845 | 2.2072 | 2.439 |
| | 1.206 | 2.282 | 2.661 | 2.118 | 2.5386 | 4.287 |
| | 1.269 | 1.8596 | 3.265 | 1.576 | 2.6403 | 2.826 |
| | 1.327 | 1.3669 | 3.116 | 1.875 | 4.363 | 3.835 |
| | 1.27 | 2.1401 | 3.374 | 1.313 | 2.5015 | 2.822 |
| | 1.744 | 3.0206 | 2.439 | 1.322 | 1.8558 | 2.881 |
| | 1.167 | 2.5691 | 3.21 | 1.758 | 2.1487 | 3.35 |
| | 1.627 | 2.1736 | 3.371 | 1.77 | 2.5301 | 3.506 |
| | 1.338 | - | 2.141 | - | - | 2.48 |

| Genotype | WS-2 | | | WS-2 *hsp90.2-3* | | |
|---|---|---|---|---|---|---|
| Treatment | Standard | Warmth | Auxin | Standard | Warmth | Auxin |
| Hypocotyl length (mm) | 2.659 | 3.0258 | 6.051 | 3.236 | 3.2676 | 4.311 |
| | 2.588 | 2.8407 | 4.454 | 2.755 | 3.1893 | 6.521 |
| | 2.457 | 2.9063 | 6 | 2.784 | 3.0871 | 6.299 |
| | 2.378 | 2.2986 | 4.006 | 3.116 | 3.1774 | 4.701 |
| | 2.866 | 2.4556 | 4.626 | 2.215 | 3.1857 | 6.063 |
| | 1.72 | 2.8003 | 3.085 | 2.567 | 3.6812 | 7.077 |
| | 3.26 | 3.0904 | 5.115 | 3.134 | 2.4697 | 6.592 |
| | 3.321 | 3.0455 | 5.014 | 2.419 | 3.1343 | 5.663 |
| | 2.459 | 2.2809 | 5.697 | 2.659 | 3.0501 | 5.932 |
| | 3.437 | 2.1084 | 5.067 | 2.969 | 2.6897 | 5.46 |
| | 2.976 | 3.0115 | 4.038 | 2.776 | 2.3617 | 3.861 |
| | 3.36 | 2.3433 | 3.313 | 3.323 | 2.6327 | 4.28 |
| | 3.022 | 3.3808 | 3.177 | 2.429 | 3.6241 | 5.666 |
| | 1.825 | 2.9023 | 2.959 | 3.026 | 3.1471 | 3.65 |
| | 2.585 | 1.6011 | 3.64 | 2.766 | 1.7614 | 4.854 |
| | 2.831 | 2.4612 | 2.662 | 2.808 | 2.8751 | 4.72 |
| | 2.044 | 2.7506 | 3.69 | 2.795 | 3.6014 | 6.206 |
| | 1.782 | 2.8097 | 4.336 | 3.039 | 3.2483 | 3.664 |
| | 2.626 | 3.2493 | 3.778 | 3.122 | 2.6494 | 6.342 |
| | 2.854 | 3.2197 | 3.776 | 3.166 | 1.9098 | 6.201 |

# Appendix 4: Hypocotyl length of RIL set in standard conditions

| Genotype | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Hypocotyl length (mm) | 1.55 | 2.51 | 1.43 | 2.82 | 1.46 | 2.52 |
| | 1.58 | 1.63 | 1.90 | 2.59 | 1.45 | 2.31 |
| | 1.31 | 2.51 | 2.45 | 1.57 | 2.04 | 2.64 |
| | 2.07 | 1.53 | 1.50 | 2.60 | 2.31 | 2.51 |
| | 1.59 | 1.72 | 1.76 | 1.99 | 2.12 | 1.84 |
| | 2.35 | 1.57 | 1.92 | 3.25 | 1.91 | 2.60 |
| | 2.27 | 2.15 | 2.40 | 2.50 | 1.63 | 2.08 |
| | 1.82 | 1.68 | 1.75 | 1.81 | 1.95 | 2.53 |
| | 1.52 | 2.04 | 2.03 | 2.41 | 2.14 | 2.04 |
| | 2.26 | 1.50 | 2.13 | 1.63 | 1.79 | 1.93 |
| | 2.03 | 2.12 | 1.76 | 1.52 | 2.54 | 1.76 |
| | 1.25 | 2.59 | 1.83 | 2.02 | 2.16 | 2.13 |
| | 1.37 | 1.92 | 1.60 | 1.09 | 2.31 | 2.20 |
| | 1.61 | 2.21 | 1.61 | 1.52 | 1.59 | 2.13 |
| | 1.93 | 1.55 | 2.36 | 2.25 | 1.98 | 1.53 |
| | 0.67 | 1.74 | 1.67 | 2.39 | 1.99 | 2.33 |
| | 2.14 | 1.59 | 1.54 | 2.31 | 1.82 | 2.39 |
| | 2.10 | 2.17 | 1.98 | 1.87 | 2.10 | 1.76 |
| | 2.07 | 1.52 | 2.10 | 2.38 | 2.19 | 2.90 |
| | 1.78 | 1.59 | - | 2.00 | - | 2.99 |

| Genotype | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|
| Hypocotyl length (mm) | 2.08 | 1.41 | 1.86 | 1.94 | 2.12 | 1.66 |
| | 1.29 | 1.20 | 1.55 | 2.15 | 1.97 | 1.80 |
| | 1.71 | 1.49 | 1.60 | 1.91 | 1.65 | 1.74 |
| | 1.65 | 2.24 | 1.96 | 1.57 | 1.46 | 1.23 |
| | 1.77 | 1.65 | 1.88 | 1.89 | 1.72 | 1.44 |
| | 1.65 | 1.49 | 1.89 | 1.61 | 2.05 | 2.13 |
| | 1.29 | 1.40 | 2.57 | 1.84 | 1.96 | 1.64 |
| | 2.27 | 1.63 | 1.96 | 2.05 | 1.85 | 2.08 |
| | 1.61 | 1.88 | 2.31 | 1.65 | 1.58 | 1.90 |
| | 1.74 | 1.74 | 1.70 | 2.88 | 2.05 | 2.31 |
| | 1.38 | 1.74 | 1.47 | 1.58 | 1.77 | 1.75 |
| | 1.84 | 1.25 | 2.34 | 1.73 | 1.30 | 2.03 |
| | 1.36 | 1.66 | 1.54 | 1.94 | 2.40 | 1.80 |
| | 1.46 | 1.85 | 2.30 | 1.67 | 1.80 | 1.57 |
| | 1.43 | 1.61 | 1.60 | 1.56 | 2.26 | 2.20 |
| | 1.31 | 2.08 | 1.76 | 1.94 | 1.68 | 2.50 |
| | 1.53 | 1.42 | 1.41 | 1.21 | 2.37 | 2.33 |
| | 1.41 | 2.31 | 1.54 | 1.70 | 2.27 | 2.23 |
| | 1.54 | 1.79 | 2.07 | 2.36 | 1.93 | 1.74 |

| Genotype | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|
| **Hypocotyl length (mm)** | 2.23 | 1.79 | 1.83 | 2.00 | 1.72 | 1.90 |
| | 1.24 | 2.18 | 1.90 | 1.70 | 1.64 | 1.78 |
| | 1.66 | 1.55 | 1.43 | 1.56 | 1.90 | 1.80 |
| | 2.04 | 1.56 | 1.54 | 1.52 | 2.15 | 1.67 |
| | 1.88 | 1.71 | 1.75 | 1.82 | 2.14 | 2.21 |
| | 2.18 | 1.94 | 2.25 | 1.73 | 2.19 | 1.46 |
| | 1.62 | 1.65 | 2.21 | 1.96 | 2.87 | 1.68 |
| | 1.99 | 2.32 | 1.49 | 1.80 | 1.76 | 1.91 |
| | 1.76 | 1.60 | 0.88 | 1.49 | 1.92 | 1.48 |
| | 1.65 | 1.27 | 1.23 | 1.64 | 1.95 | 1.69 |
| | 1.91 | 1.55 | 1.57 | 1.88 | 2.05 | 1.95 |
| | 1.61 | 1.95 | 1.63 | 1.46 | 2.32 | 1.87 |
| | 1.28 | 1.68 | 2.18 | 1.66 | 1.99 | 2.00 |
| | 1.94 | 1.46 | 1.75 | 1.85 | 2.16 | 2.16 |
| | 1.97 | 1.65 | 1.51 | 2.13 | 2.56 | 1.58 |
| | 1.47 | 1.84 | 1.50 | 1.56 | 2.10 | 1.61 |
| | 1.82 | 1.98 | 1.66 | 2.25 | 1.83 | 2.03 |
| | 1.54 | 1.66 | 1.26 | 1.48 | 1.69 | 1.58 |
| | 1.64 | 1.64 | 2.00 | 2.24 | 1.83 | 1.53 |
| | 2.50 | 1.25 | 1.32 | - | 2.42 | - |

| Genotype | 19 | 20 | 21 | 22 | 23 | 24 |
|---|---|---|---|---|---|---|
| **Hypocotyl length (mm)** | 1.77 | 1.81 | 1.78 | 1.62 | 2.00 | 1.32 |
| | 1.61 | 1.33 | 1.35 | 1.60 | 1.82 | 1.40 |
| | 1.09 | 1.47 | 1.72 | 1.53 | 1.87 | 1.85 |
| | 2.11 | 1.88 | 1.89 | 1.65 | 1.76 | 1.42 |
| | 1.63 | 1.17 | 2.04 | 1.87 | 1.59 | 1.09 |
| | 1.76 | 1.48 | 1.92 | 1.30 | 1.46 | 1.39 |
| | 1.34 | 1.32 | 1.98 | 1.34 | 1.99 | 1.22 |
| | 1.83 | 1.66 | 1.50 | 1.78 | 1.47 | 1.65 |
| | 2.60 | 1.27 | 1.40 | 1.61 | 1.41 | 1.51 |
| | 2.01 | 1.62 | 1.35 | 1.55 | 1.56 | 1.48 |
| | 1.84 | 1.91 | 1.60 | 1.31 | 2.09 | 1.73 |
| | 2.14 | 1.57 | 1.42 | 1.52 | 1.24 | 1.43 |
| | 2.10 | 1.53 | 1.65 | 2.11 | 1.50 | 1.23 |
| | 1.97 | 1.53 | 1.81 | 1.78 | 1.85 | 1.26 |
| | 2.92 | 1.72 | 1.72 | 1.62 | 1.81 | 1.50 |
| | 1.46 | 1.93 | 1.89 | 1.63 | 1.42 | 1.22 |
| | 2.17 | 1.51 | 1.69 | 1.44 | 1.36 | 1.60 |
| | 1.46 | 1.41 | 2.15 | 1.91 | 1.21 | 1.48 |
| | 1.87 | 1.40 | 0.77 | 1.90 | 1.49 | 1.74 |
| | - | 1.47 | 2.09 | 1.46 | 1.00 | 1.27 |

| Genotype | 25 | 26 | 27 | 28 | 29 | 30 |
|---|---|---|---|---|---|---|
| **Hypocotyl length (mm)** | 1.35 | 1.92 | 0.95 | 1.53 | 1.49 | 1.43 |
| | 1.35 | 1.63 | 1.31 | 1.91 | 1.18 | 1.30 |
| | 1.33 | 1.40 | 1.58 | 1.68 | 1.67 | 1.26 |
| | 1.24 | 1.77 | 1.31 | 1.62 | 1.51 | 0.89 |
| | 1.55 | 1.44 | 1.30 | 1.00 | 1.55 | 1.63 |
| | 1.46 | 1.99 | 1.20 | 1.59 | 1.49 | 1.60 |
| | 1.02 | 2.44 | 1.20 | 1.46 | 1.26 | 2.14 |
| | 1.13 | 2.01 | 1.41 | 1.64 | 1.28 | 1.42 |
| | 1.00 | 1.46 | 1.23 | 1.70 | 1.31 | 1.77 |
| | 1.44 | 1.34 | 1.20 | 1.43 | 1.33 | 1.30 |
| | 0.76 | 1.47 | 1.71 | 1.87 | 1.47 | 2.19 |
| | 1.28 | 1.23 | 0.96 | 1.95 | 1.40 | 1.26 |
| | 1.67 | 2.13 | 1.14 | 1.26 | 1.59 | 1.33 |
| | 1.25 | 0.81 | 1.55 | 0.87 | 1.69 | 1.03 |
| | 1.53 | 1.89 | 2.15 | 1.25 | 1.76 | 1.30 |
| | 1.34 | 1.26 | 1.09 | 2.23 | 1.27 | 1.73 |
| | 1.41 | 1.77 | 1.49 | 1.12 | 1.34 | 1.07 |
| | 1.22 | 1.29 | - | 1.47 | 1.53 | 1.67 |
| | - | 2.30 | - | - | 1.31 | 1.42 |
| | - | 1.56 | - | - | 1.50 | 2.05 |

| Genotype | 31 | 32 | 33 | 34 | 35 | 36 |
|---|---|---|---|---|---|---|
| **Hypocotyl length (mm)** | 1.53 | 1.98 | 1.62 | 1.04 | 1.59 | 2.16 |
| | 1.50 | 1.68 | 0.94 | 1.36 | 2.37 | 1.64 |
| | 1.68 | 1.74 | 1.76 | 2.34 | 1.13 | 1.79 |
| | 1.70 | 2.10 | 1.48 | 1.24 | 1.67 | 2.36 |
| | 1.71 | 1.77 | 1.35 | 1.50 | 1.74 | 1.73 |
| | 1.76 | 1.44 | 2.01 | 1.46 | 1.56 | 1.32 |
| | 1.54 | 1.55 | 1.81 | 1.15 | 1.28 | 2.09 |
| | 1.49 | 1.62 | 1.61 | 1.90 | 1.88 | 2.13 |
| | 1.50 | 1.51 | 1.71 | 2.57 | 1.79 | 1.67 |
| | 1.16 | 1.34 | 2.31 | 0.90 | 2.63 | 1.43 |
| | 1.14 | 1.42 | 1.65 | 1.92 | 2.50 | 1.83 |
| | 1.44 | 1.06 | 1.52 | 1.89 | 1.50 | 1.68 |
| | 1.30 | 1.18 | 1.21 | 1.00 | 1.70 | 1.77 |
| | 2.11 | 1.54 | 1.74 | 1.27 | 1.30 | 2.46 |
| | 1.56 | 1.34 | 1.51 | 1.45 | 1.79 | 1.55 |
| | 1.28 | 1.56 | 1.87 | 1.75 | 1.42 | 1.62 |
| | 1.35 | 0.85 | 2.45 | 1.46 | 1.40 | 1.50 |
| | 2.10 | 1.21 | 2.22 | 2.22 | 1.77 | 1.88 |
| | 1.24 | 1.53 | 1.97 | 1.40 | 1.72 | 0.91 |
| | 1.12 | 1.01 | - | 1.71 | 1.58 | 1.40 |

| Genotype | 37 | 38 | 39 | 40 | 41 | 42 |
|---|---|---|---|---|---|---|
| Hypocotyl length (mm) | 2.09 | 1.50 | 1.82 | 2.41 | 1.83 | 1.68 |
| | 1.82 | 1.26 | 1.54 | 2.31 | 1.78 | 1.95 |
| | 1.98 | 1.61 | 1.94 | 2.42 | 2.03 | 1.60 |
| | 1.68 | 1.55 | 1.62 | 1.58 | 1.94 | 1.94 |
| | 1.60 | 1.68 | 1.04 | 1.90 | 2.00 | 1.50 |
| | 1.33 | 1.31 | 1.46 | 1.42 | 1.53 | 2.24 |
| | 1.70 | 1.56 | 1.56 | 1.76 | 0.76 | 2.08 |
| | 1.97 | 1.61 | 1.63 | 1.53 | 1.50 | 1.97 |
| | 2.02 | 2.31 | 1.95 | 1.65 | 1.46 | 1.36 |
| | 1.72 | 1.35 | 2.18 | 1.71 | 1.45 | 1.42 |
| | 1.59 | 1.99 | 1.55 | 2.09 | 1.28 | 1.72 |
| | 1.48 | 1.78 | 2.00 | 1.83 | 2.41 | 1.47 |
| | 1.46 | 1.49 | 1.73 | 1.65 | 1.40 | 2.14 |
| | 1.55 | 1.63 | 1.51 | 2.80 | 1.71 | 1.67 |
| | 1.77 | 1.54 | 1.67 | 2.27 | 1.93 | 1.92 |
| | 2.76 | 1.46 | 1.51 | 1.84 | 2.01 | 1.97 |
| | 2.00 | 1.09 | 1.62 | 1.39 | 2.26 | 1.83 |
| | 1.68 | 1.51 | 2.17 | 1.88 | 2.16 | 1.96 |
| | 1.55 | 1.36 | 1.95 | 1.37 | 1.79 | 1.66 |
| | - | 1.75 | 1.16 | 1.92 | 2.01 | 1.97 |

| Genotype | 43 | 44 | 45 | 46 | 47 | 48 |
|---|---|---|---|---|---|---|
| Hypocotyl length (mm) | 1.40 | 1.88 | 2.39 | 1.70 | 1.32 | 1.32 |
| | 1.99 | 1.58 | 1.66 | 2.59 | 1.89 | 1.75 |
| | 1.57 | 1.63 | 2.20 | 1.50 | 1.48 | 2.12 |
| | 1.67 | 1.52 | 0.87 | 1.81 | 1.89 | 1.76 |
| | 1.86 | 1.29 | 1.68 | 2.29 | 1.07 | 1.75 |
| | 1.59 | 1.65 | 2.82 | 2.16 | 1.10 | 1.29 |
| | 1.71 | 1.75 | 1.74 | 2.02 | 1.45 | 1.76 |
| | 1.61 | 1.60 | 1.79 | 2.22 | 1.59 | 1.81 |
| | 1.96 | 1.94 | 2.23 | 2.56 | 1.80 | 1.72 |
| | 1.75 | 1.31 | 1.48 | 2.18 | 1.81 | 2.01 |
| | 1.76 | 1.71 | 1.68 | 2.15 | 1.96 | 1.97 |
| | 0.93 | 2.15 | 1.55 | 1.61 | 1.23 | 1.58 |
| | 1.52 | 1.78 | 1.69 | 1.65 | 1.44 | 2.09 |
| | 1.67 | 1.36 | 1.72 | 1.92 | 1.43 | 1.81 |
| | 1.47 | 2.86 | 2.46 | 1.49 | 1.46 | 2.05 |
| | 1.48 | 1.26 | 2.21 | 2.14 | 1.71 | 1.60 |
| | 1.87 | 1.78 | 2.00 | 2.09 | 1.83 | 1.41 |
| | 1.30 | 2.05 | 1.60 | 1.63 | 1.39 | 1.88 |
| | 1.98 | - | 1.63 | 1.94 | 1.45 | 1.72 |
| | 1.25 | - | 2.06 | 1.68 | 1.59 | 2.02 |

| Genotype | 49 | 50 | 51 | 52 | 53 | 54 |
|---|---|---|---|---|---|---|
| **Hypocotyl length (mm)** | 2.14 | 2.86 | 2.05 | 2.40 | 1.85 | 1.79 |
| | 1.96 | 2.25 | 2.22 | 1.93 | 1.72 | 2.03 |
| | 2.11 | 1.71 | 1.84 | 1.75 | 1.97 | 2.32 |
| | 2.07 | 2.84 | 1.88 | 2.18 | 1.88 | 1.96 |
| | 1.69 | 2.57 | 1.71 | 2.43 | 1.31 | 2.05 |
| | 2.51 | 2.82 | 2.04 | 1.87 | 1.91 | 1.94 |
| | 2.74 | 2.12 | 2.48 | 1.82 | 1.93 | 1.62 |
| | 2.33 | 1.87 | 1.86 | 1.99 | 1.77 | 2.03 |
| | 2.09 | 2.40 | 1.61 | 2.15 | 1.73 | 2.34 |
| | 2.30 | 2.36 | 1.63 | 1.87 | 1.59 | 2.01 |
| | 1.42 | 2.81 | 1.82 | 2.52 | 1.77 | 2.08 |
| | 2.42 | 3.07 | 1.88 | 2.26 | 1.96 | 2.12 |
| | 2.15 | 1.90 | 1.86 | 2.60 | 1.54 | 2.32 |
| | 3.03 | 1.71 | 1.54 | 2.35 | 1.61 | 2.50 |
| | 2.06 | 2.38 | 1.54 | 2.48 | 1.70 | 2.20 |
| | 1.95 | 2.55 | 2.40 | 1.22 | 2.11 | 1.82 |
| | 1.92 | 2.57 | 1.67 | 2.22 | 1.81 | 1.67 |
| | 1.86 | 2.23 | - | 2.05 | 1.52 | 2.09 |
| | 2.38 | - | - | 2.00 | 1.85 | 1.73 |
| | 2.05 | - | - | 2.66 | 1.52 | 2.52 |

| Genotype | 55 | 56 | 57 | 58 | 59 | 60 |
|---|---|---|---|---|---|---|
| **Hypocotyl length (mm)** | 1.28 | 1.71 | 1.97 | 1.46 | 2.17 | 2.09 |
| | 1.34 | 2.69 | 2.86 | 2.11 | 2.31 | 1.65 |
| | 1.52 | 1.55 | 2.30 | 1.83 | 1.38 | 1.80 |
| | 1.15 | 2.18 | 1.90 | 1.22 | 1.24 | 1.50 |
| | 1.39 | 1.65 | 2.52 | 2.13 | 2.18 | 1.22 |
| | 1.42 | 1.59 | 1.73 | 2.08 | 1.33 | 1.90 |
| | 0.88 | 1.87 | 1.24 | 1.21 | 2.09 | 1.23 |
| | 1.15 | 1.86 | 2.12 | 1.90 | 1.38 | 1.68 |
| | 1.97 | 1.99 | 1.79 | 1.68 | 1.96 | 1.77 |
| | 1.72 | 2.19 | 1.69 | 1.44 | 2.25 | 1.50 |
| | 2.54 | 1.98 | 1.77 | 1.76 | 1.38 | 1.76 |
| | 1.76 | 1.71 | 2.02 | 1.90 | 1.63 | 1.65 |
| | 1.55 | 1.79 | 1.80 | 1.90 | 2.06 | 2.06 |
| | 1.71 | 2.02 | 1.96 | 2.39 | 1.53 | 2.80 |
| | 1.54 | 2.59 | 1.14 | 2.72 | 1.31 | 1.51 |
| | 1.40 | 1.92 | 1.53 | 2.51 | 1.74 | 1.58 |
| | 1.45 | 1.69 | 1.22 | 3.07 | 1.89 | 1.08 |
| | 1.77 | 1.81 | 1.38 | 2.32 | 1.72 | 2.05 |
| | 1.41 | 1.80 | 2.25 | - | 1.44 | 2.27 |
| | 1.21 | - | 2.49 | - | 1.51 | 1.67 |

| Genotype | 61 | 62 | 63 | 64 | 65 | 66 |
|---|---|---|---|---|---|---|
| **Hypocotyl length (mm)** | 1.49 | 1.51 | 1.04 | 1.39 | 2.19 | 1.99 |
| | 1.39 | 1.73 | 1.19 | 1.69 | 2.03 | 0.98 |
| | 1.37 | 1.33 | 1.41 | 1.36 | 1.93 | 1.45 |
| | 2.15 | 1.62 | 1.30 | 1.15 | 1.80 | 1.60 |
| | 1.60 | 2.11 | 1.06 | 1.64 | 1.88 | 1.62 |
| | 1.49 | 2.19 | 1.43 | 1.54 | 2.26 | 1.39 |
| | 1.62 | 1.55 | 1.96 | 0.99 | 1.14 | 1.86 |
| | 1.85 | 2.61 | 2.10 | 1.51 | 1.69 | 1.50 |
| | 1.44 | 1.47 | 1.72 | 1.54 | 1.74 | 1.29 |
| | 1.68 | 1.86 | 1.52 | 0.97 | 1.83 | 1.34 |
| | 1.37 | 0.96 | 1.43 | 1.20 | 1.95 | 1.39 |
| | 1.12 | 1.56 | 2.07 | 1.14 | 1.82 | 1.87 |
| | 1.54 | 1.52 | 1.25 | 1.09 | 1.69 | 1.67 |
| | 1.67 | 1.49 | 1.81 | 1.88 | 2.08 | 1.42 |
| | 1.27 | 1.72 | 1.39 | 1.32 | 2.50 | 1.52 |
| | 1.16 | 1.50 | 1.57 | 1.51 | 1.44 | 1.61 |
| | 1.76 | 1.88 | 1.38 | 1.93 | 1.89 | 1.67 |
| | 2.01 | 1.57 | 1.40 | - | 1.60 | 1.50 |
| | 1.24 | 1.79 | 1.93 | - | 1.60 | 1.34 |
| | 1.50 | 1.83 | 1.27 | - | 1.74 | - |

| Genotype | 67 | 68 | 69 | 70 | 71 | 72 |
|---|---|---|---|---|---|---|
| **Hypocotyl length (mm)** | 1.34 | 1.72 | 1.96 | 1.28 | 1.57 | 2.06 |
| | 1.20 | 1.49 | 1.69 | 2.32 | 1.18 | 2.84 |
| | 1.81 | 2.02 | 1.46 | 2.48 | 0.96 | 1.92 |
| | 0.94 | 1.78 | 1.29 | 1.63 | 1.57 | 1.86 |
| | 1.18 | 1.53 | 1.15 | 1.62 | 1.43 | 2.20 |
| | 1.30 | 1.17 | 1.37 | 1.60 | 1.52 | 2.69 |
| | 1.45 | 1.62 | 1.83 | 1.92 | 1.22 | 2.62 |
| | 1.22 | 1.97 | 1.78 | 1.80 | 1.16 | 2.04 |
| | 1.74 | 2.15 | 1.74 | 1.80 | 1.39 | 1.92 |
| | 1.21 | 1.80 | 1.71 | 1.98 | 1.50 | 1.64 |
| | 1.58 | 1.48 | 1.65 | 2.13 | 1.08 | 3.18 |
| | 1.41 | 1.18 | 1.89 | 1.63 | 1.38 | 2.33 |
| | 1.40 | 2.12 | 1.33 | 2.12 | 2.25 | 1.95 |
| | 1.50 | 1.61 | 1.26 | 2.15 | 1.19 | 2.22 |
| | 0.75 | 1.39 | 1.22 | 1.57 | 1.51 | 2.88 |
| | 1.35 | 1.43 | 1.23 | 2.44 | 1.31 | 1.98 |
| | 1.37 | 1.40 | 1.18 | 1.47 | 1.12 | 1.67 |
| | 0.81 | 1.25 | 1.50 | - | 1.96 | 2.19 |
| | 1.12 | 1.32 | 2.02 | - | - | 1.90 |
| | - | 1.89 | 1.75 | - | - | - |

| Genotype | 73 | 74 | 75 | 76 | 77 | 78 |
|---|---|---|---|---|---|---|
| Hypocotyl length (mm) | 2.24 | 1.75 | 1.82 | 1.76 | 1.56 | 1.83 |
| | 2.04 | 1.60 | 1.73 | 2.09 | 1.90 | 1.64 |
| | 1.45 | 1.86 | 1.72 | 1.91 | 1.81 | 1.52 |
| | 1.81 | 1.86 | 1.79 | 2.31 | 1.41 | 2.34 |
| | 2.03 | 1.57 | 1.70 | 2.54 | 1.48 | 1.09 |
| | 2.78 | 1.46 | 1.42 | 1.71 | 1.99 | 1.40 |
| | 1.92 | 1.72 | 2.32 | 1.94 | 2.06 | 2.19 |
| | 3.21 | 1.30 | 1.74 | 1.40 | 1.69 | 1.36 |
| | 2.06 | 1.58 | 1.74 | 1.61 | 1.90 | 1.17 |
| | 2.30 | 1.74 | 1.97 | 1.81 | 2.30 | 1.48 |
| | 2.48 | 1.36 | 1.87 | 2.00 | 2.12 | 1.67 |
| | 2.08 | 1.80 | 1.73 | 1.95 | 2.12 | 1.54 |
| | 2.78 | 1.55 | 2.39 | 1.80 | 2.06 | 1.25 |
| | 2.10 | 1.55 | 2.32 | 2.02 | 1.84 | 1.10 |
| | 3.27 | 1.08 | 1.65 | 2.03 | 2.27 | 1.22 |
| | 2.27 | 1.56 | 2.25 | 1.61 | 1.98 | 2.27 |
| | 2.13 | 1.88 | 1.68 | 2.24 | 2.01 | 1.64 |
| | 1.79 | 1.89 | 1.43 | 1.26 | 1.97 | 1.56 |
| | 2.02 | 2.20 | 1.78 | 1.78 | - | - |
| | 3.30 | 1.18 | 2.31 | 1.78 | - | - |

| Genotype | 79 | 80 | 81 | 82 | 83 | 84 |
|---|---|---|---|---|---|---|
| Hypocotyl length (mm) | 1.18 | 2.13 | 2.11 | 1.34 | 1.70 | 1.36 |
| | 1.28 | 2.03 | 1.61 | 1.16 | 2.09 | 1.99 |
| | 1.83 | 1.67 | 1.48 | 1.52 | 2.07 | 1.23 |
| | 1.67 | 1.64 | 1.74 | 2.43 | 1.57 | 1.52 |
| | 1.58 | 1.95 | 1.84 | 1.56 | 1.71 | 2.21 |
| | 1.99 | 1.60 | 1.88 | 2.06 | 1.59 | 1.50 |
| | 1.66 | 1.77 | 1.87 | 1.35 | 2.05 | 1.28 |
| | 1.95 | 1.66 | 1.71 | 1.89 | 2.04 | 1.40 |
| | 1.81 | 2.11 | 2.03 | 1.18 | 1.12 | 2.36 |
| | 1.77 | 1.81 | 1.63 | 1.87 | 1.80 | 1.30 |
| | 1.82 | 2.24 | 2.39 | 2.42 | 1.29 | 1.37 |
| | 1.74 | 1.35 | 1.80 | 1.20 | 1.35 | 1.45 |
| | 2.57 | 2.08 | 2.45 | 1.37 | 1.65 | 2.07 |
| | 1.80 | 1.88 | 2.15 | 1.86 | 1.51 | 1.95 |
| | 2.08 | 1.93 | 1.70 | 1.91 | 1.33 | 1.69 |
| | 1.66 | 1.73 | 1.67 | 1.25 | 1.45 | 1.47 |
| | 2.11 | 1.30 | 1.91 | 1.24 | 1.43 | 1.31 |
| | 1.44 | 1.82 | 1.84 | 1.23 | 1.59 | 1.44 |
| | - | 1.28 | 1.95 | 2.06 | 2.35 | 1.56 |
| | - | 1.65 | 2.29 | - | 1.33 | 1.60 |

| Genotype | 85 | 86 | 87 |
|---|---|---|---|
| Hypocotyl length (mm) | 1.69 | 1.87 | 1.36 |
| | 1.60 | 1.40 | 1.98 |
| | 1.38 | 1.59 | 1.38 |
| | 1.38 | 1.68 | 1.49 |
| | 1.39 | 1.35 | 1.90 |
| | 0.91 | 2.13 | 1.67 |
| | 1.34 | 1.87 | 1.87 |
| | 1.94 | 1.85 | 1.26 |
| | 1.23 | 1.63 | 1.37 |
| | 1.59 | 1.55 | 1.50 |
| | 1.56 | 2.68 | 1.63 |
| | 0.92 | 1.50 | 1.77 |
| | 1.15 | 1.49 | 1.89 |
| | 1.30 | 1.74 | 1.41 |
| | 1.59 | 1.52 | 1.79 |
| | 0.99 | 1.25 | 2.00 |
| | 1.12 | 1.67 | 1.65 |
| | 1.57 | 1.86 | 1.70 |
| | 1.67 | 1.84 | 0.91 |
| | 1.56 | 1.90 | 1.88 |

# Appendix 5: Hypocotyl length of RIL set with exogenous auxin

| Genotype | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Hypocotyl length (mm) | 4.95 | 3.49 | 4.33 | 4.25 | 3.36 | 2.01 |
| | 2.96 | 3.06 | 4.68 | 4.06 | 2.64 | 2.32 |
| | 3.13 | 3.08 | 4.98 | 4.43 | 2.90 | 1.14 |
| | 4.31 | 3.43 | 4.54 | 3.43 | 3.21 | 3.39 |
| | 2.34 | 3.39 | 4.16 | 2.90 | 2.66 | 3.57 |
| | 3.77 | 1.47 | 4.03 | 5.42 | 2.62 | 2.82 |
| | 4.13 | 2.55 | 4.36 | 4.52 | 3.20 | 3.18 |
| | 2.42 | 2.91 | 5.11 | 5.30 | 3.41 | 3.36 |
| | 2.83 | 3.42 | 4.82 | 3.34 | 3.37 | 2.64 |
| | 3.51 | 3.18 | 4.79 | 4.30 | 1.63 | 2.85 |
| | 2.86 | 3.07 | 5.45 | 4.18 | 2.53 | 3.80 |
| | 2.91 | 3.27 | 4.28 | 3.87 | 3.61 | 3.28 |
| | 2.94 | 2.04 | 4.37 | 4.34 | 2.83 | 4.51 |
| | 2.37 | 2.94 | 2.71 | 4.03 | 3.32 | 3.82 |
| | 2.74 | 2.19 | 3.94 | 3.41 | 3.58 | 2.88 |
| | 2.90 | 2.69 | 3.95 | 2.98 | 3.78 | 2.98 |
| | 3.29 | 3.75 | 4.81 | 4.69 | 3.20 | 3.45 |
| | 2.44 | 2.82 | 3.66 | 5.84 | 2.93 | 3.64 |
| | 4.15 | 2.28 | 4.38 | 4.27 | 2.65 | 2.95 |
| | 2.85 | 3.42 | 4.39 | 3.55 | - | 4.18 |

| Genotype | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|
| Hypocotyl length (mm) | 3.13 | 3.96 | 5.05 | 2.56 | 4.54 | 2.87 |
| | 4.58 | 3.24 | 4.03 | 3.27 | 2.56 | 3.02 |
| | 4.36 | 3.25 | 4.14 | 2.22 | 2.64 | 2.10 |
| | 2.40 | 4.85 | 4.52 | 3.23 | 3.44 | 2.79 |
| | 3.42 | 3.49 | 4.51 | 2.75 | 2.64 | 2.41 |
| | 2.66 | 3.85 | 4.34 | 2.96 | 4.25 | 2.67 |
| | 3.20 | 4.79 | 3.99 | 3.49 | 3.01 | 3.47 |
| | 4.25 | 3.47 | 4.28 | 5.11 | 3.50 | 3.84 |
| | 2.17 | 5.17 | 4.06 | 3.90 | 3.70 | 3.45 |
| | 4.05 | 3.38 | 3.96 | 4.05 | 3.96 | 2.94 |
| | 2.79 | 3.77 | 4.21 | 4.00 | 2.79 | 3.38 |
| | 2.10 | 3.20 | 3.48 | 3.68 | 3.39 | 2.97 |
| | 2.05 | 2.37 | 4.07 | 3.25 | 2.69 | 2.37 |
| | 3.30 | 3.14 | 3.72 | 4.45 | 3.97 | 3.52 |
| | 2.02 | 3.89 | 3.78 | 2.94 | 2.65 | 3.21 |
| | 2.77 | 2.42 | 3.57 | 3.38 | 3.06 | 2.41 |
| | 2.61 | 2.60 | 3.90 | 3.50 | 3.32 | 2.80 |
| | 1.99 | 3.21 | 3.92 | 2.67 | 3.34 | 3.02 |
| | 2.54 | 2.99 | 2.64 | 2.80 | - | 2.68 |

| Genotype | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|
| **Hypocotyl length (mm)** | 4.10 | 2.26 | 1.57 | 3.08 | 3.90 | 3.11 |
| | 4.49 | 4.13 | 3.16 | 2.85 | 2.92 | 3.15 |
| | 3.67 | 3.90 | 2.18 | 3.36 | 2.94 | 2.23 |
| | 4.28 | 2.93 | 2.47 | 2.30 | 3.25 | 2.91 |
| | 2.77 | 4.94 | 1.81 | 2.33 | 2.99 | 2.85 |
| | 4.17 | 3.99 | 2.03 | 3.05 | 3.68 | 3.46 |
| | 4.03 | 3.49 | 2.17 | 2.50 | 2.60 | 3.30 |
| | 3.94 | 3.69 | 2.43 | 3.41 | 3.55 | 2.44 |
| | 4.19 | 2.78 | 2.05 | 2.53 | 1.60 | 2.31 |
| | 4.49 | 3.13 | 2.06 | 3.28 | 2.10 | 2.68 |
| | 4.88 | 4.32 | 2.08 | 3.28 | 2.73 | 2.05 |
| | 3.90 | 2.99 | 2.31 | 2.23 | 2.27 | 2.41 |
| | 4.46 | 2.82 | 2.17 | 3.46 | 2.24 | 1.97 |
| | 4.03 | 3.59 | 1.98 | 3.25 | 3.93 | 2.00 |
| | 3.34 | 4.02 | 2.52 | 1.46 | 2.77 | 2.97 |
| | 2.54 | 3.53 | 2.71 | 2.52 | 2.67 | 1.87 |
| | 3.31 | 3.81 | 2.38 | 2.48 | 4.17 | 3.61 |
| | 4.33 | 2.50 | 2.08 | 3.36 | 2.26 | 2.10 |
| | 3.59 | 3.89 | - | 2.68 | 2.80 | 2.17 |
| | 4.12 | 3.26 | - | 2.91 | 3.09 | 2.89 |

| Genotype | 19 | 20 | 21 | 22 | 23 | 24 |
|---|---|---|---|---|---|---|
| **Hypocotyl length (mm)** | 2.73 | 4.74 | 4.07 | 3.40 | 4.21 | 3.41 |
| | 2.19 | 3.93 | 4.09 | 4.93 | 4.65 | 3.25 |
| | 2.49 | 5.24 | 3.33 | 4.57 | 3.95 | 3.49 |
| | 3.67 | 4.50 | 5.47 | 3.76 | 3.93 | 1.58 |
| | 1.86 | 3.34 | 3.49 | 3.90 | 5.72 | 1.58 |
| | 2.50 | 4.52 | 3.92 | 4.67 | 3.64 | 2.89 |
| | 2.81 | 3.26 | 4.48 | 4.45 | 3.94 | 2.54 |
| | 2.98 | 5.40 | 4.56 | 4.32 | 3.65 | 2.50 |
| | 2.55 | 2.67 | 4.72 | 4.96 | 4.69 | 2.56 |
| | 3.88 | 3.50 | 5.89 | 3.15 | 4.26 | 2.09 |
| | 2.35 | 4.25 | 4.84 | 3.33 | 3.88 | 2.88 |
| | 2.69 | 4.21 | 5.34 | 3.46 | 4.23 | 2.38 |
| | 2.58 | 4.20 | 5.46 | 4.09 | 3.31 | 2.61 |
| | 2.97 | 5.71 | 3.20 | 4.10 | 3.84 | 2.74 |
| | 4.17 | 4.20 | 5.25 | 3.06 | 4.22 | 1.72 |
| | 2.52 | 4.77 | 5.34 | 3.37 | 4.05 | 1.90 |
| | 2.61 | 4.56 | 5.27 | 4.78 | 5.47 | 2.03 |
| | 1.82 | 4.30 | 4.07 | 3.33 | 3.38 | 1.92 |
| | 1.97 | 4.01 | 5.13 | 3.66 | 4.78 | 1.83 |
| | 2.14 | 2.87 | - | 3.40 | 4.71 | - |

| Genotype | 25 | 26 | 27 | 28 | 29 | 30 |
|---|---|---|---|---|---|---|
| Hypocotyl length (mm) | 3.15 | 2.80 | 1.82 | 3.79 | 3.03 | 3.90 |
| | 1.87 | 2.33 | 1.61 | 4.08 | 4.00 | 5.13 |
| | 2.87 | 1.51 | 1.87 | 5.64 | 4.08 | 3.95 |
| | 2.41 | 1.84 | 1.37 | 5.04 | 5.82 | 4.15 |
| | 2.80 | 1.21 | 3.51 | 5.33 | 3.92 | 4.15 |
| | 3.44 | 1.77 | 2.47 | 4.74 | 1.71 | 4.30 |
| | 3.29 | 2.09 | 2.53 | 4.22 | 3.96 | 4.59 |
| | 1.51 | 1.92 | 1.84 | 2.54 | 4.36 | 4.11 |
| | 2.57 | 2.00 | 1.82 | 5.60 | 4.39 | 4.55 |
| | 4.09 | 2.70 | 1.64 | 4.21 | 3.09 | 5.12 |
| | 4.24 | 1.65 | 1.50 | 5.49 | 3.22 | 3.97 |
| | 1.91 | 2.09 | 2.03 | 4.55 | 3.55 | 3.64 |
| | 2.94 | 2.57 | 1.53 | 3.87 | 2.56 | 3.81 |
| | 4.66 | 1.93 | 1.33 | 4.68 | 3.23 | 3.32 |
| | 2.74 | 1.79 | 1.98 | 5.07 | 4.53 | 4.40 |
| | 2.58 | 1.73 | 1.48 | 3.33 | 3.09 | 4.96 |
| | 3.93 | 0.67 | 1.45 | 3.65 | 3.29 | 4.10 |
| | 3.20 | 1.49 | 2.04 | 5.09 | 3.99 | 2.60 |
| | 3.18 | - | - | - | 4.59 | 4.48 |
| | 3.50 | - | - | - | 4.68 | 4.57 |

| Genotype | 31 | 32 | 33 | 34 | 35 | 36 |
|---|---|---|---|---|---|---|
| Hypocotyl length (mm) | 3.76 | 4.66 | 2.92 | 2.59 | 2.82 | 4.00 |
| | 4.08 | 3.07 | 3.12 | 3.33 | 3.32 | 4.05 |
| | 3.78 | 3.31 | 2.28 | 4.85 | 2.74 | 3.50 |
| | 4.25 | 2.69 | 2.89 | 3.04 | 1.69 | 4.68 |
| | 3.56 | 2.48 | 3.83 | 0.83 | 2.64 | 4.69 |
| | 3.45 | 2.36 | 3.68 | 3.57 | 1.76 | 3.72 |
| | 4.76 | 3.60 | 2.75 | 3.42 | 2.05 | 2.98 |
| | 5.36 | 2.58 | 3.46 | 4.70 | 3.16 | 3.81 |
| | 5.31 | 3.81 | 2.41 | 3.66 | 4.05 | 3.82 |
| | 2.95 | 3.14 | 3.34 | 2.86 | 3.45 | 3.05 |
| | 3.97 | 3.87 | 3.95 | 2.67 | 3.26 | 3.79 |
| | 3.81 | 3.50 | 2.69 | 4.48 | 3.86 | 4.24 |
| | 3.63 | 3.03 | 2.38 | 4.92 | 2.89 | 3.04 |
| | 2.98 | 3.43 | 2.95 | 2.77 | 2.53 | 2.63 |
| | 4.20 | 3.10 | 3.80 | 4.55 | 2.48 | 4.09 |
| | 4.58 | 2.90 | 2.02 | 3.07 | 3.97 | 2.63 |
| | 4.05 | 3.22 | 3.01 | 1.89 | 2.70 | 2.71 |
| | 4.93 | 3.08 | 3.02 | 3.80 | 2.42 | 3.41 |
| | 4.98 | 3.82 | 2.74 | 3.95 | 4.35 | 3.19 |
| | 5.82 | 4.81 | 3.31 | 3.85 | - | 2.52 |

| Genotype | 37 | 38 | 39 | 40 | 41 | 42 |
|---|---|---|---|---|---|---|
| **Hypocotyl length (mm)** | 4.30 | 4.41 | 2.44 | 3.34 | 3.67 | 2.22 |
| | 2.79 | 2.79 | 3.37 | 2.26 | 1.58 | 1.44 |
| | 3.85 | 4.12 | 1.93 | 3.47 | 2.69 | 1.38 |
| | 2.30 | 5.31 | 2.70 | 2.05 | 1.49 | 2.03 |
| | 3.31 | 2.40 | 3.48 | 2.24 | 3.09 | 1.86 |
| | 3.07 | 3.09 | 5.13 | 3.23 | 4.47 | 1.33 |
| | 2.84 | 4.23 | 2.85 | 3.50 | 2.50 | 3.37 |
| | 4.25 | 3.40 | 3.92 | 1.81 | 3.58 | 3.28 |
| | 3.79 | 4.92 | 3.43 | 2.60 | 1.96 | 1.76 |
| | 2.77 | 3.20 | 4.81 | 3.78 | 3.11 | 2.08 |
| | 4.52 | 3.67 | 2.76 | 3.06 | 3.66 | 2.45 |
| | 3.31 | 3.38 | 4.83 | 2.28 | 2.49 | 4.16 |
| | 3.78 | 2.34 | 3.36 | 6.04 | 2.96 | 1.85 |
| | 2.62 | 4.24 | 4.99 | 4.77 | 5.98 | 1.95 |
| | 4.35 | 3.67 | 3.59 | 4.02 | 2.73 | 1.96 |
| | 3.16 | 4.39 | 2.49 | 3.69 | 3.48 | 1.97 |
| | 3.83 | 2.45 | 3.75 | 3.43 | 4.29 | 4.67 |
| | 2.73 | 3.33 | 3.75 | 5.77 | 2.01 | 2.41 |
| | 4.71 | 3.59 | 4.62 | 2.85 | 2.68 | 2.53 |
| | 4.38 | 2.55 | 3.06 | 3.70 | - | 2.43 |

| Genotype | 43 | 44 | 45 | 46 | 47 | 48 |
|---|---|---|---|---|---|---|
| **Hypocotyl length (mm)** | 3.32 | 1.87 | 4.64 | 1.53 | 2.99 | 1.73 |
| | 2.96 | 1.98 | 2.94 | 2.02 | 1.15 | 1.15 |
| | 3.89 | 1.71 | 2.90 | 3.20 | 2.26 | 1.32 |
| | 3.12 | 1.70 | 3.67 | 1.45 | 1.13 | 1.58 |
| | 2.33 | 2.76 | 2.18 | 2.81 | 3.92 | 1.76 |
| | 3.77 | 1.12 | 2.80 | 2.57 | 1.21 | 1.52 |
| | 2.69 | 2.17 | 4.42 | 2.59 | 1.71 | 1.62 |
| | 1.90 | 1.77 | 3.11 | 3.18 | 2.12 | 1.03 |
| | 2.52 | 1.60 | 2.55 | 2.48 | 2.34 | 3.04 |
| | 3.65 | 3.49 | 2.29 | 3.01 | 0.72 | 3.40 |
| | 2.19 | 2.67 | 2.66 | 3.09 | 1.34 | 1.19 |
| | 2.42 | 1.47 | 2.81 | 2.41 | 0.85 | 2.14 |
| | 2.80 | 2.90 | 2.59 | 2.79 | 0.97 | 1.45 |
| | 2.13 | 1.86 | 2.78 | 2.11 | 1.18 | 1.80 |
| | 4.24 | 1.93 | 2.91 | 1.63 | 3.26 | 1.52 |
| | 1.58 | 2.61 | 2.17 | 1.85 | 2.41 | 2.28 |
| | 2.96 | 1.21 | 1.93 | 2.21 | 0.93 | 1.85 |
| | 2.94 | - | 2.22 | 1.76 | 0.78 | 2.22 |
| | 2.93 | - | 2.61 | 2.36 | 0.60 | 1.89 |
| | 2.09 | - | 2.43 | - | - | 1.77 |

| Genotype | 49 | 50 | 51 | 52 | 53 | 54 |
|---|---|---|---|---|---|---|
| Hypocotyl length (mm) | 1.75 | 3.68 | 1.27 | 2.90 | 3.51 | 3.84 |
| | 1.51 | 1.62 | 1.66 | 3.24 | 4.04 | 4.08 |
| | 1.97 | 1.78 | 1.65 | 5.05 | 3.71 | 4.46 |
| | 1.38 | 1.86 | 1.41 | 3.70 | 2.86 | 3.61 |
| | 1.42 | 1.26 | 1.27 | 4.10 | 3.22 | 5.33 |
| | 1.89 | 1.22 | 1.64 | 4.44 | 3.04 | 3.97 |
| | 2.11 | 4.25 | 1.29 | 4.64 | 2.61 | 2.59 |
| | 2.20 | 1.94 | 1.41 | 3.42 | 4.44 | 4.23 |
| | 1.71 | 2.39 | 2.33 | 3.11 | 3.11 | 4.67 |
| | 3.48 | 1.61 | 1.27 | 3.38 | 2.56 | 2.86 |
| | 1.41 | 3.37 | 2.09 | 3.87 | 2.99 | 3.31 |
| | 1.30 | 0.94 | 1.77 | 4.53 | 3.32 | 3.07 |
| | 2.14 | 1.85 | 0.91 | 2.41 | 3.06 | 3.33 |
| | 1.31 | 1.07 | 1.01 | 3.62 | 2.89 | 3.27 |
| | 1.70 | 1.27 | 1.66 | 3.36 | 3.08 | 3.72 |
| | 2.49 | 3.20 | 0.98 | 3.86 | 3.80 | 2.60 |
| | 1.83 | 1.60 | 1.14 | 3.12 | 3.40 | 4.21 |
| | 1.75 | 1.22 | 1.45 | 3.20 | 2.96 | 3.23 |
| | 1.33 | 2.17 | 1.20 | 2.57 | 4.14 | 2.83 |
| | 2.38 | - | - | 3.54 | - | 3.38 |

| Genotype | 55 | 56 | 57 | 58 | 59 | 60 |
|---|---|---|---|---|---|---|
| Hypocotyl length (mm) | 3.84 | 3.94 | 3.39 | 3.22 | 3.51 | 2.40 |
| | 2.84 | 2.69 | 1.90 | 4.18 | 2.52 | 3.18 |
| | 4.01 | 2.50 | 2.17 | 4.35 | 2.84 | 2.14 |
| | 2.87 | 2.36 | 2.92 | 4.41 | 1.85 | 3.30 |
| | 4.01 | 2.61 | 3.20 | 3.96 | 3.21 | 2.16 |
| | 3.73 | 2.48 | 2.15 | 3.23 | 3.28 | 3.01 |
| | 3.97 | 2.36 | 2.96 | 3.68 | 2.66 | 2.00 |
| | 5.27 | 2.12 | 2.39 | 3.68 | 2.60 | 4.19 |
| | 2.34 | 3.52 | 2.71 | 2.88 | 3.87 | 3.32 |
| | 5.16 | 2.89 | 3.05 | 4.33 | 4.38 | 2.44 |
| | 4.46 | 3.35 | 1.67 | 2.74 | 3.26 | 2.70 |
| | 3.15 | 3.45 | 3.05 | 3.76 | 2.64 | 1.64 |
| | 4.68 | 1.95 | 2.46 | 3.44 | 3.37 | 2.75 |
| | 4.00 | 1.82 | 2.01 | 4.41 | 4.08 | 3.13 |
| | 5.05 | 1.83 | 2.26 | 2.01 | 2.08 | 2.13 |
| | 4.85 | 3.15 | 3.26 | 3.61 | 2.93 | 2.96 |
| | 3.88 | 3.10 | 2.83 | 3.51 | 2.94 | 2.43 |
| | 2.59 | 2.78 | 3.76 | 3.72 | 3.94 | 3.54 |
| | 3.14 | 2.89 | 2.77 | 4.34 | 2.82 | - |
| | 2.76 | 2.84 | 2.06 | 2.08 | 3.46 | - |

| Genotype | 61 | 62 | 63 | 64 | 65 | 66 |
|---|---|---|---|---|---|---|
| **Hypocotyl length (mm)** | 3.29 | 4.38 | 1.85 | 3.89 | 3.35 | 2.64 |
| | 3.12 | 2.94 | 2.48 | 4.28 | 3.79 | 2.95 |
| | 3.08 | 2.47 | 2.86 | 3.29 | 3.30 | 3.53 |
| | 3.96 | 2.64 | 2.24 | 4.46 | 2.79 | 2.19 |
| | 3.40 | 2.71 | 2.11 | 2.91 | 3.20 | 3.14 |
| | 2.94 | 3.67 | 2.61 | 3.22 | 2.60 | 2.48 |
| | 2.04 | 2.33 | 3.26 | 2.46 | 2.12 | 2.59 |
| | 3.98 | 3.03 | 3.12 | 3.21 | 2.69 | 2.89 |
| | 3.58 | 2.58 | 1.94 | 4.00 | 3.02 | 3.26 |
| | 1.80 | 3.89 | 3.04 | 4.67 | 2.74 | 3.20 |
| | 2.38 | 4.39 | 3.85 | 4.28 | 3.78 | 2.82 |
| | 2.52 | 2.96 | 2.79 | 2.95 | 2.45 | 3.72 |
| | 2.98 | 3.24 | 2.97 | 3.60 | 2.92 | 3.28 |
| | 3.45 | 3.65 | 2.91 | 3.01 | 3.25 | 3.36 |
| | 2.76 | 3.02 | 2.99 | 2.56 | 2.28 | 3.54 |
| | 2.60 | 2.54 | 2.68 | 3.06 | 3.63 | 3.44 |
| | 3.11 | 3.28 | 2.45 | 3.64 | 2.58 | 1.98 |
| | 3.08 | 2.84 | 3.41 | 2.30 | 4.14 | 3.24 |
| | 2.70 | 3.07 | 2.60 | 3.28 | 3.13 | 2.31 |
| | 3.67 | 2.57 | 1.58 | 4.66 | 1.82 | 3.21 |

| Genotype | 67 | 68 | 69 | 70 | 71 | 72 |
|---|---|---|---|---|---|---|
| **Hypocotyl length (mm)** | 3.70 | 2.52 | 5.02 | 4.05 | 3.07 | 3.46 |
| | 3.38 | 2.44 | 3.78 | 4.30 | 3.21 | 3.11 |
| | 3.03 | 3.59 | 4.07 | 3.68 | 2.12 | 3.21 |
| | 4.12 | 3.60 | 4.64 | 4.05 | 3.74 | 2.68 |
| | 3.97 | 3.70 | 4.84 | 4.12 | 3.88 | 3.92 |
| | 2.62 | 2.35 | 3.69 | 3.99 | 3.50 | 3.59 |
| | 2.86 | 2.33 | 5.26 | 5.93 | 2.77 | 2.77 |
| | 3.21 | 2.44 | 2.88 | 4.38 | 2.74 | 2.90 |
| | 3.67 | 3.18 | 3.70 | 3.31 | 1.22 | 3.41 |
| | 3.45 | 2.17 | 4.72 | 3.04 | 4.05 | 1.52 |
| | 3.96 | 3.39 | 4.81 | 2.34 | 1.94 | 2.86 |
| | 3.65 | 2.16 | 4.51 | 3.51 | 2.50 | 2.57 |
| | 3.50 | 3.63 | 4.65 | 3.42 | 3.00 | 2.70 |
| | 3.43 | 3.73 | 3.89 | 4.35 | 2.89 | 2.54 |
| | 4.19 | 3.60 | 4.08 | 3.85 | 3.23 | 3.09 |
| | 3.47 | 2.73 | 3.53 | 3.36 | 4.20 | 1.48 |
| | 3.99 | 3.12 | 4.87 | 3.71 | 1.87 | 1.73 |
| | 3.33 | 3.86 | 3.46 | 5.11 | 2.85 | 2.94 |
| | 3.04 | 3.29 | 4.28 | 4.23 | 2.88 | 3.50 |
| | - | 2.62 | 3.56 | - | 3.11 | 4.64 |

| Genotype | 73 | 74 | 75 | 76 | 77 | 78 |
|---|---|---|---|---|---|---|
| Hypocotyl length (mm) | 3.44 | 4.17 | 4.03 | 3.31 | 3.02 | 2.39 |
| | 4.09 | 4.49 | 3.07 | 1.53 | 2.05 | 2.36 |
| | 3.14 | 3.06 | 2.74 | 2.74 | 1.79 | 2.23 |
| | 2.37 | 3.43 | 3.84 | 3.26 | 1.97 | 2.85 |
| | 2.82 | 3.65 | 2.53 | 2.70 | 2.38 | 2.93 |
| | 3.41 | 4.33 | 3.91 | 2.05 | 2.73 | 2.33 |
| | 3.67 | 4.76 | 2.39 | 3.19 | 2.03 | 1.97 |
| | 2.72 | 2.35 | 3.18 | 2.43 | 1.64 | 2.34 |
| | 4.60 | 2.89 | 2.79 | 2.35 | 2.71 | 3.14 |
| | 4.79 | 3.79 | 2.90 | 3.81 | 2.11 | 2.37 |
| | 3.89 | 3.04 | 3.63 | 2.49 | 1.85 | 3.00 |
| | 2.99 | 4.63 | 3.82 | 3.48 | 2.16 | 1.93 |
| | 3.68 | 2.66 | 3.97 | 2.09 | 2.46 | 3.23 |
| | 3.35 | 4.05 | 3.91 | 3.71 | 2.09 | 2.54 |
| | 3.06 | 3.23 | 2.80 | 2.56 | 2.28 | 2.66 |
| | 3.36 | 3.83 | 3.72 | 2.77 | 3.18 | 2.36 |
| | 2.77 | 3.33 | 3.99 | 1.71 | 2.30 | 2.69 |
| | 3.38 | 3.45 | 4.07 | 3.40 | 2.01 | 2.56 |
| | 4.05 | 4.52 | 4.18 | 1.99 | 2.47 | 2.59 |
| | 4.03 | 3.99 | 4.09 | 3.15 | 1.95 | 2.71 |

| Genotype | 79 | 80 | 81 | 82 | 83 | 84 |
|---|---|---|---|---|---|---|
| Hypocotyl length (mm) | 2.23 | 2.30 | 3.09 | 2.99 | 2.74 | 2.11 |
| | 1.98 | 1.48 | 4.03 | 3.34 | 2.53 | 3.19 |
| | 2.12 | 2.08 | 3.37 | 3.02 | 2.33 | 1.83 |
| | 2.49 | 1.86 | 4.42 | 2.37 | 2.51 | 2.78 |
| | 1.83 | 2.27 | 3.90 | 3.18 | 2.65 | 2.60 |
| | 1.47 | 1.37 | 4.14 | 3.15 | 3.17 | 2.20 |
| | 2.36 | 2.11 | 3.52 | 2.87 | 3.31 | 2.50 |
| | 1.98 | 2.04 | 2.92 | 2.73 | 2.41 | 3.31 |
| | 2.08 | 1.69 | 2.97 | 3.33 | 2.13 | 3.33 |
| | 2.80 | 2.20 | 4.55 | 3.25 | 2.66 | 2.55 |
| | 1.88 | 2.72 | 2.96 | 3.69 | 3.44 | 2.50 |
| | 1.81 | 1.82 | 3.81 | 2.52 | 2.78 | 3.01 |
| | 2.04 | 2.38 | 2.94 | 3.62 | 2.31 | 3.27 |
| | 1.95 | 2.33 | 3.12 | 4.14 | 2.35 | 2.37 |
| | 1.82 | 2.12 | 4.04 | 2.85 | 2.08 | 3.17 |
| | 2.97 | 1.77 | 4.46 | 2.68 | 2.47 | 2.92 |
| | 2.83 | 2.59 | 2.52 | 2.82 | 3.56 | 2.77 |
| | 1.61 | 3.35 | 3.06 | 2.65 | 3.23 | 3.13 |
| | 2.73 | 2.14 | 2.93 | 2.86 | 2.22 | 2.39 |
| | 1.70 | 1.63 | 4.42 | 2.89 | 2.97 | 2.54 |

| Genotype | 85 | 86 | 87 | 88 |
|---|---|---|---|---|
| Hypocotyl length (mm) | 3.02 | 1.59 | 1.62 | 1.95 |
| | 2.12 | 0.58 | 2.03 | 2.06 |
| | 1.73 | 0.84 | 2.26 | 2.72 |
| | 1.81 | 1.38 | 2.74 | 2.30 |
| | 2.26 | 0.93 | 1.66 | 1.71 |
| | 2.62 | 1.38 | 2.25 | 2.45 |
| | 2.37 | 2.15 | 2.21 | 2.62 |
| | 3.05 | 1.13 | 2.13 | 2.09 |
| | 2.54 | 0.61 | 2.14 | 2.38 |
| | 1.70 | 1.96 | 2.05 | 2.63 |
| | 0.85 | 1.13 | 3.08 | 3.11 |
| | 2.99 | 1.39 | 1.37 | 2.44 |
| | 2.39 | 2.43 | 2.79 | 1.97 |
| | 0.82 | 1.38 | 1.94 | 2.36 |
| | 3.17 | 2.03 | 3.33 | 1.91 |
| | 1.88 | 1.79 | 2.47 | 2.19 |
| | 0.89 | 1.68 | 2.50 | 2.11 |
| | 1.10 | 0.58 | 3.07 | 2.36 |
| | 1.70 | 0.75 | 2.04 | 2.22 |
| | 2.80 | - | 2.27 | 1.80 |

# Appendix 6: Hypocotyl length of RIL set at 27°C

| Genotype | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Hypocotyl length (mm) | 2.66 | 1.36 | 2.62 | 1.36 | 2.07 | 2.70 |
| | 1.72 | 3.58 | 3.25 | 2.18 | 2.17 | 2.05 |
| | 2.54 | 1.86 | 2.43 | 3.07 | 2.43 | 2.41 |
| | 2.69 | 2.10 | 3.10 | 1.26 | 2.18 | 2.55 |
| | 3.29 | 1.49 | 2.19 | 1.90 | 1.81 | 2.62 |
| | 2.50 | 2.48 | 2.40 | 2.63 | 2.46 | 1.48 |
| | 2.21 | 2.48 | 2.61 | 1.53 | 2.37 | 2.66 |
| | 2.92 | 1.85 | 1.41 | 2.30 | 1.99 | 3.31 |
| | 2.39 | 2.64 | 3.18 | 1.77 | 1.62 | 4.17 |
| | 2.16 | 3.32 | 3.78 | 2.86 | 1.49 | 3.43 |
| | 2.28 | 1.38 | 2.07 | 2.51 | 2.13 | 2.68 |
| | 2.65 | 1.91 | 2.10 | 2.48 | 2.84 | 2.57 |
| | 3.21 | 2.28 | 3.10 | 1.82 | 1.54 | 3.36 |
| | 2.15 | 3.02 | 2.11 | 2.32 | 3.11 | 2.32 |
| | 2.52 | 1.75 | 2.64 | 2.15 | 2.80 | 2.92 |
| | 2.43 | 2.54 | 2.21 | 2.47 | 2.68 | 2.84 |
| | 3.54 | 3.69 | 2.25 | 3.74 | 2.50 | 2.96 |
| | 2.55 | 2.62 | 2.38 | 2.30 | 1.71 | 3.02 |
| | 2.76 | 2.93 | 3.69 | 3.34 | 2.56 | 3.33 |
| | 2.91 | 2.13 | 1.71 | 2.31 | 2.56 | - |

| Genotype | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|
| Hypocotyl length (mm) | 2.68 | 2.39 | 2.61 | 3.06 | 4.46 | 2.49 |
| | 2.58 | 2.73 | 3.72 | 2.10 | 2.23 | 2.67 |
| | 3.01 | 2.26 | 2.31 | 2.89 | 3.24 | 2.41 |
| | 2.70 | 2.48 | 2.92 | 2.86 | 3.26 | 3.54 |
| | 2.68 | 2.29 | 2.53 | 3.50 | 4.32 | 3.23 |
| | 2.50 | 2.19 | 2.45 | 3.50 | 2.94 | 2.92 |
| | 2.35 | 1.97 | 2.96 | 2.72 | 2.79 | 2.36 |
| | 2.45 | 2.25 | 2.62 | 2.71 | 3.41 | 3.01 |
| | 2.79 | 2.65 | 2.94 | 2.87 | 3.51 | 2.89 |
| | 2.41 | 2.07 | 3.12 | 2.90 | 3.11 | 2.97 |
| | 2.25 | 2.63 | 3.10 | 2.50 | 3.01 | 2.73 |
| | 2.49 | 2.66 | 3.12 | 3.09 | 2.41 | 2.86 |
| | 3.04 | 2.08 | 2.46 | 2.01 | 2.70 | 2.39 |
| | 2.32 | 3.09 | 2.94 | 2.59 | 3.21 | 1.71 |
| | 1.91 | 2.88 | 2.36 | 3.68 | 3.14 | 2.46 |
| | 1.55 | 1.92 | 2.30 | 3.80 | 1.76 | 3.39 |
| | 2.31 | 3.09 | 1.81 | 2.55 | 2.42 | 2.44 |
| | 2.82 | 2.56 | 2.53 | 2.66 | 2.21 | 3.15 |
| | 2.89 | 2.19 | 2.06 | 2.18 | 3.62 | 2.27 |
| | 2.85 | - | 3.25 | 2.94 | 3.25 | - |

| Genotype | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|
| **Hypocotyl length (mm)** | 3.29 | 2.80 | 2.01 | 1.82 | 2.80 | 2.71 |
| | 3.28 | 2.03 | 1.85 | 2.77 | 2.65 | 3.17 |
| | 2.72 | 2.82 | 2.74 | 1.80 | 2.19 | 2.10 |
| | 3.16 | 2.87 | 2.71 | 2.44 | 3.89 | 2.33 |
| | 3.35 | 2.79 | 2.53 | 2.43 | 2.43 | 3.71 |
| | 2.98 | 4.00 | 3.57 | 1.70 | 3.46 | 2.79 |
| | 3.99 | 3.60 | 2.26 | 1.57 | 2.61 | 2.41 |
| | 3.55 | 2.25 | 2.44 | 2.25 | 3.10 | 2.73 |
| | 3.61 | 2.30 | 3.44 | 2.25 | 1.47 | 1.68 |
| | 3.29 | 3.63 | 2.44 | 2.38 | 2.64 | 2.78 |
| | 3.50 | 2.54 | 3.30 | 1.85 | 2.28 | 2.35 |
| | 2.23 | 3.57 | 2.32 | 1.96 | 1.91 | 2.89 |
| | 3.42 | 2.65 | 2.73 | 2.51 | 3.71 | 2.25 |
| | 2.75 | 3.38 | 2.68 | 2.10 | 2.50 | 2.95 |
| | 3.51 | 2.96 | 2.51 | 3.01 | 3.33 | 2.60 |
| | 2.88 | 3.48 | 2.08 | 1.97 | 2.38 | 2.24 |
| | 3.16 | 3.74 | 2.18 | 2.19 | 2.00 | 2.71 |
| | 2.91 | 4.53 | 2.09 | 2.54 | 2.64 | 3.16 |
| | 3.45 | 3.04 | 2.16 | 2.83 | 3.29 | - |
| | 2.48 | 3.48 | 2.42 | 1.92 | - | - |

| Genotype | 19 | 20 | 21 | 22 | 23 | 24 |
|---|---|---|---|---|---|---|
| **Hypocotyl length (mm)** | 3.37 | 2.50 | 2.98 | 2.89 | 2.37 | 5.06 |
| | 2.35 | 2.81 | 2.60 | 2.73 | 2.90 | 3.57 |
| | 2.73 | 2.94 | 2.54 | 2.25 | 2.44 | 1.61 |
| | 4.04 | 2.63 | 3.24 | 3.20 | 2.67 | 2.26 |
| | 4.55 | 2.84 | 2.42 | 3.95 | 4.32 | 3.90 |
| | 2.51 | 3.16 | 2.48 | 1.66 | 3.69 | 2.73 |
| | 3.34 | 3.35 | 2.39 | 3.21 | 2.58 | 3.63 |
| | 2.82 | 3.76 | 2.95 | 3.37 | 2.40 | 3.38 |
| | 2.42 | 2.66 | 2.72 | 2.59 | 3.63 | 3.14 |
| | 3.12 | 3.32 | 3.11 | 2.02 | 3.05 | 3.80 |
| | 3.26 | 2.88 | 3.89 | 4.07 | 4.49 | 3.99 |
| | 2.06 | 3.52 | 2.55 | 2.26 | 2.63 | 3.48 |
| | 3.51 | 3.38 | 2.98 | 3.66 | 3.16 | 3.96 |
| | 2.81 | 3.30 | 1.74 | 3.14 | 3.42 | 3.87 |
| | 2.96 | 2.42 | 2.55 | 2.36 | 3.94 | 4.79 |
| | 1.90 | 2.71 | 3.02 | 3.04 | 4.27 | 2.67 |
| | 2.21 | 2.31 | 2.64 | 1.85 | 3.00 | 3.01 |
| | 2.53 | 3.33 | 1.98 | 3.62 | 3.93 | 3.92 |
| | 2.18 | 2.68 | 2.20 | 2.60 | 3.27 | 5.04 |
| | 3.40 | - | - | 2.48 | 2.66 | 4.04 |

| Genotype | 25 | 26 | 27 | 28 | 29 | 30 |
|---|---|---|---|---|---|---|
| Hypocotyl length (mm) | 3.81 | 4.35 | 2.99 | 2.40 | 1.53 | 3.20 |
| | 2.94 | 4.91 | 5.87 | 1.46 | 1.70 | 3.46 |
| | 4.60 | 3.52 | 3.41 | 2.81 | 1.92 | 2.75 |
| | 4.20 | 4.26 | 3.15 | 2.14 | 1.90 | 2.35 |
| | 3.27 | 3.75 | 3.38 | 1.96 | 3.35 | 1.98 |
| | 2.42 | 3.74 | 2.74 | 2.43 | 2.07 | 2.48 |
| | 2.31 | 3.45 | 3.99 | 2.38 | 2.31 | 2.79 |
| | 3.87 | 3.43 | 5.05 | 3.98 | 2.52 | 2.89 |
| | 3.67 | 3.05 | 3.71 | 2.41 | 1.87 | 2.18 |
| | 2.89 | 3.72 | 3.67 | 3.13 | 3.25 | 2.43 |
| | 3.20 | 3.31 | 3.21 | 1.56 | 2.21 | 1.92 |
| | 3.54 | 3.57 | 3.91 | 2.07 | 2.47 | 2.15 |
| | 2.97 | 3.02 | 3.03 | 2.64 | 1.47 | 1.85 |
| | 2.67 | 3.06 | 3.59 | 2.82 | 2.13 | 1.81 |
| | 3.20 | 2.67 | 4.14 | 2.50 | 2.28 | 2.36 |
| | 3.82 | 2.93 | 5.20 | 2.08 | 1.51 | 1.47 |
| | 2.89 | 2.83 | 3.43 | 2.62 | 3.62 | 2.89 |
| | 4.14 | 3.92 | 3.33 | 2.30 | 3.28 | 2.39 |
| | - | 3.09 | - | 1.80 | - | 2.64 |
| | - | 2.15 | - | 2.53 | - | - |

| Genotype | 31 | 32 | 33 | 34 | 35 | 36 |
|---|---|---|---|---|---|---|
| Hypocotyl length (mm) | 3.13 | 2.57 | 3.24 | 2.55 | 3.27 | 3.26 |
| | 2.20 | 2.96 | 3.95 | 3.34 | 3.03 | 4.76 |
| | 2.41 | 1.95 | 3.05 | 1.66 | 4.41 | 2.30 |
| | 1.60 | 3.51 | 4.61 | 2.86 | 2.82 | 2.50 |
| | 2.04 | 3.85 | 4.48 | 2.73 | 3.10 | 2.04 |
| | 1.83 | 3.88 | 3.48 | 2.49 | 4.01 | 3.93 |
| | 2.16 | 3.95 | 4.76 | 3.32 | 3.92 | 3.19 |
| | 1.80 | 3.11 | 5.12 | 3.04 | 4.55 | 3.29 |
| | 2.55 | 2.97 | 5.44 | 3.69 | 3.86 | 3.09 |
| | 2.42 | 3.79 | 2.87 | 4.14 | 3.75 | 3.41 |
| | 1.95 | 3.12 | 4.39 | 3.31 | 2.79 | 3.45 |
| | 2.21 | 3.48 | 2.99 | 2.78 | 3.00 | 3.16 |
| | 1.46 | 3.25 | 4.59 | 2.42 | 3.35 | 2.52 |
| | 1.70 | 2.48 | 4.30 | 3.95 | 3.88 | 2.40 |
| | 1.93 | 2.69 | 3.97 | 3.89 | 3.30 | 4.03 |
| | 1.90 | 2.58 | 4.24 | 3.11 | 3.18 | 3.10 |
| | 3.09 | 4.35 | 3.46 | 1.74 | 3.32 | 2.49 |
| | 2.38 | 3.62 | 3.11 | 3.21 | 2.91 | 3.39 |
| | 2.46 | 3.72 | 4.45 | 3.56 | 4.60 | 1.78 |
| | - | 3.96 | 4.61 | - | 4.21 | 2.16 |

| Genotype | 37 | 38 | 39 | 40 | 41 | 42 |
|---|---|---|---|---|---|---|
| Hypocotyl length (mm) | 2.20 | 2.79 | 2.70 | 3.41 | 4.23 | 4.48 |
| | 3.11 | 2.68 | 2.43 | 4.53 | 2.15 | 3.91 |
| | 3.58 | 3.07 | 2.55 | 2.87 | 2.99 | 3.48 |
| | 2.20 | 3.18 | 2.38 | 3.75 | 3.60 | 4.26 |
| | 2.32 | 3.41 | 1.25 | 3.91 | 2.55 | 2.87 |
| | 2.74 | 2.51 | 3.17 | 3.44 | 4.12 | 2.30 |
| | 3.17 | 3.38 | 2.06 | 4.19 | 3.49 | 2.80 |
| | 2.96 | 2.90 | 2.74 | 2.89 | 4.16 | 3.65 |
| | 3.26 | 1.61 | 2.26 | 4.20 | 4.31 | 3.89 |
| | 2.64 | 2.93 | 3.16 | 3.57 | 3.39 | 3.21 |
| | 2.66 | 2.62 | 2.30 | 2.91 | 3.23 | 3.88 |
| | 2.64 | 2.52 | 2.17 | 2.57 | 3.97 | 3.17 |
| | 3.79 | 2.84 | 1.99 | 2.39 | 3.48 | 3.33 |
| | 2.35 | 3.42 | 2.12 | 4.84 | 3.58 | 3.53 |
| | 2.52 | 2.73 | 2.31 | 2.92 | 3.30 | 3.38 |
| | 3.14 | 2.62 | 2.80 | 3.77 | 3.51 | 3.00 |
| | 3.24 | 2.83 | 2.30 | 3.27 | 3.29 | 3.29 |
| | 3.51 | 3.26 | 3.51 | 3.27 | 3.29 | 3.71 |
| | 2.56 | 3.26 | 2.65 | 5.38 | 3.92 | 3.20 |
| | 2.50 | - | 2.55 | - | - | - |

| Genotype | 43 | 44 | 45 | 46 | 47 | 48 |
|---|---|---|---|---|---|---|
| Hypocotyl length (mm) | 2.26 | 4.22 | 4.46 | 4.08 | 3.27 | 3.32 |
| | 3.20 | 3.35 | 3.33 | 4.06 | 3.56 | 3.30 |
| | 4.86 | 3.48 | 3.34 | 2.73 | 4.04 | 2.82 |
| | 3.48 | 2.48 | 3.50 | 2.98 | 2.67 | 2.18 |
| | 3.66 | 3.55 | 2.88 | 3.57 | 5.24 | 3.00 |
| | 3.35 | 3.69 | 1.53 | 3.86 | 3.27 | 3.56 |
| | 2.40 | 3.56 | 2.28 | 2.14 | 3.17 | 3.25 |
| | 3.66 | 3.82 | 2.48 | 2.22 | 3.05 | 1.81 |
| | 2.83 | 2.82 | 3.19 | 4.40 | 3.55 | 2.22 |
| | 2.10 | 3.49 | 2.75 | 4.16 | 4.09 | 3.21 |
| | 3.75 | 2.93 | 3.82 | 3.60 | 3.43 | 3.70 |
| | 3.41 | 3.00 | 3.26 | 4.99 | 4.31 | 3.27 |
| | 3.49 | 3.85 | 2.43 | 2.71 | 4.26 | 2.42 |
| | 3.57 | 2.53 | 2.81 | 4.48 | 3.79 | 3.60 |
| | 3.94 | 3.80 | 2.89 | 1.46 | 3.68 | 4.16 |
| | 2.93 | 3.56 | 3.58 | 3.46 | 3.51 | 2.91 |
| | 3.38 | 2.73 | 3.92 | 3.25 | 4.19 | - |
| | 3.60 | 3.71 | 3.04 | 3.53 | 3.64 | - |
| | 3.80 | - | 2.95 | 4.53 | - | - |
| | - | - | 3.21 | - | - | - |

| Genotype | 49 | 50 | 51 | 52 | 53 | 54 |
|---|---|---|---|---|---|---|
| Hypocotyl length (mm) | 2.93 | 4.25 | 4.08 | 2.38 | 3.08 | 3.39 |
| | 3.62 | 3.58 | 4.29 | 2.68 | 2.92 | 3.23 |
| | 3.37 | 3.00 | 5.16 | 2.16 | 2.21 | 3.40 |
| | 3.35 | 3.58 | 4.41 | 2.00 | 3.32 | 3.33 |
| | 3.17 | 3.64 | 3.94 | 3.84 | 2.90 | 2.55 |
| | 3.09 | 4.75 | 4.58 | 2.14 | 1.98 | 3.45 |
| | 2.73 | 3.56 | 4.29 | 2.22 | 2.56 | 3.19 |
| | 4.29 | 4.63 | 3.77 | 2.12 | 2.00 | 5.13 |
| | 2.85 | 3.48 | 3.78 | 1.51 | 1.70 | 3.67 |
| | 3.69 | 4.13 | 3.52 | 3.00 | 2.48 | 3.38 |
| | 3.27 | 3.22 | 3.79 | 2.52 | 2.82 | 4.35 |
| | 6.01 | 5.51 | 4.64 | 3.27 | 2.87 | 3.43 |
| | 3.05 | 4.58 | 4.83 | 2.38 | 2.66 | 2.83 |
| | 3.37 | 4.10 | 4.00 | 3.02 | 2.43 | 3.85 |
| | 2.89 | 5.34 | 4.48 | 2.77 | 2.70 | 3.09 |
| | 3.72 | 2.87 | 3.26 | 2.93 | 2.46 | 4.64 |
| | 2.51 | 3.17 | 3.18 | 2.75 | 3.48 | 3.00 |
| | 3.14 | 3.56 | 3.55 | 3.06 | 3.24 | 3.00 |
| | 3.86 | 3.98 | 4.02 | 2.90 | - | 2.58 |
| | - | 3.46 | - | - | - | 3.75 |

| Genotype | 55 | 56 | 57 | 58 | 59 | 60 |
|---|---|---|---|---|---|---|
| Hypocotyl length (mm) | 3.80 | 2.00 | 3.05 | 2.44 | 3.20 | 3.24 |
| | 3.42 | 2.71 | 3.18 | 3.73 | 2.89 | 2.14 |
| | 3.88 | 1.90 | 2.46 | 3.47 | 2.34 | 2.47 |
| | 2.58 | 2.63 | 3.26 | 3.94 | 2.92 | 1.89 |
| | 5.16 | 2.39 | 3.47 | 4.04 | 3.84 | 1.70 |
| | 4.13 | 1.99 | 2.95 | 2.77 | 2.45 | 1.84 |
| | 3.34 | 2.31 | 2.82 | 3.17 | 2.07 | 1.75 |
| | 2.18 | 2.72 | 3.12 | 3.76 | 2.99 | 2.25 |
| | 3.19 | 2.78 | 2.42 | 3.87 | 3.00 | 1.46 |
| | 2.86 | 1.65 | 3.96 | 3.47 | 2.79 | 1.82 |
| | 2.47 | 2.20 | 4.67 | 3.26 | 2.85 | 1.54 |
| | 3.14 | 3.19 | 3.28 | 2.48 | 3.02 | 2.26 |
| | 3.85 | 3.46 | 3.95 | 4.31 | 3.67 | 2.33 |
| | 2.59 | 1.81 | 4.37 | 4.77 | 2.04 | 1.44 |
| | 3.22 | 2.08 | 4.07 | 1.88 | 4.03 | 1.56 |
| | 3.46 | 1.82 | 3.22 | 3.30 | 3.17 | 1.51 |
| | 2.76 | 3.32 | 2.58 | 3.29 | 2.89 | 1.55 |
| | 3.51 | - | 3.33 | 3.47 | 2.41 | 2.51 |
| | 3.48 | - | 4.50 | 2.92 | 3.81 | 2.11 |
| | - | - | - | - | - | - |

| Genotype | 61 | 62 | 63 | 64 | 65 | 66 |
|---|---|---|---|---|---|---|
| **Hypocotyl length (mm)** | 1.48 | 3.16 | 2.24 | 2.58 | 2.55 | 1.71 |
| | 1.28 | 2.74 | 2.21 | 2.32 | 2.83 | 1.91 |
| | 1.47 | 2.84 | 1.31 | 1.33 | 2.48 | 1.62 |
| | 1.19 | 2.31 | 1.33 | 1.95 | 2.73 | 1.39 |
| | 2.27 | 3.01 | 0.95 | 2.47 | 2.90 | 2.59 |
| | 1.75 | 2.43 | 1.50 | 2.17 | 2.00 | 1.38 |
| | 1.48 | 1.94 | 1.78 | 2.38 | 2.02 | 2.96 |
| | 1.55 | 2.18 | 2.32 | 1.59 | 1.95 | 2.27 |
| | 2.02 | 1.73 | 1.56 | 2.57 | 2.09 | 2.51 |
| | 2.09 | 2.85 | 1.98 | 2.50 | 2.10 | 2.82 |
| | 2.31 | 3.08 | 1.68 | 2.75 | 2.21 | 1.98 |
| | 2.63 | 2.41 | 0.81 | 2.28 | 2.13 | 2.42 |
| | 1.12 | 3.55 | 2.11 | 1.81 | 1.87 | 2.89 |
| | 1.30 | 3.18 | 2.39 | 2.67 | 2.08 | 1.96 |
| | 1.41 | 3.27 | 1.98 | 2.22 | 2.09 | 1.29 |
| | 2.45 | 3.21 | 2.85 | 2.47 | 2.35 | 2.37 |
| | - | 1.73 | 1.88 | 1.64 | 2.11 | 2.75 |
| | - | 2.50 | 3.45 | 2.19 | 1.54 | 2.87 |
| | - | 2.38 | - | 3.01 | 2.31 | 2.60 |
| | - | 3.70 | - | - | 2.16 | 2.62 |

| Genotype | 67 | 68 | 69 | 70 | 71 | 72 |
|---|---|---|---|---|---|---|
| **Hypocotyl length (mm)** | 0.88 | 1.94 | 4.33 | 3.90 | 2.88 | 2.31 |
| | 1.03 | 1.41 | 3.35 | 3.31 | 2.84 | 3.09 |
| | 0.75 | 1.09 | 3.88 | 4.24 | 2.90 | 2.45 |
| | 2.02 | 1.59 | 3.30 | 2.91 | 2.23 | 2.82 |
| | 0.93 | 0.67 | 2.13 | 2.90 | 2.52 | 2.67 |
| | 1.06 | 0.82 | 2.82 | 2.45 | 2.52 | 2.99 |
| | 2.40 | 2.81 | 4.16 | 3.25 | 3.31 | 2.95 |
| | 1.87 | 1.43 | 3.94 | 3.63 | 3.44 | 1.93 |
| | 2.00 | 1.19 | 2.56 | 2.49 | 2.38 | 2.43 |
| | 1.21 | 2.31 | 3.29 | 3.50 | 4.54 | 2.43 |
| | 2.80 | 1.55 | 4.20 | 2.56 | 2.94 | 2.96 |
| | 1.24 | 1.91 | 3.76 | 5.02 | 4.97 | 2.55 |
| | 2.14 | 1.71 | 3.50 | 2.49 | 3.17 | 3.72 |
| | 1.43 | 1.44 | 3.22 | 2.91 | 2.27 | 2.50 |
| | 1.78 | 2.21 | 2.55 | 2.53 | 2.84 | 2.59 |
| | 1.75 | 1.78 | 4.55 | 2.73 | 2.15 | 2.67 |
| | 1.72 | 2.17 | 3.85 | 3.37 | 3.06 | 2.61 |
| | 2.26 | 2.58 | 3.53 | 2.60 | 3.14 | 2.35 |
| | 1.18 | - | 2.47 | 2.42 | 2.66 | 2.04 |
| | 1.74 | - | 5.31 | 6.38 | 4.25 | 3.41 |

| Genotype | 73 | 74 | 75 | 76 | 77 | 78 |
|---|---|---|---|---|---|---|
| Hypocotyl length (mm) | 2.55 | 2.11 | 2.32 | 2.05 | 3.97 | 3.85 |
| | 3.25 | 2.42 | 2.88 | 2.92 | 3.24 | 4.95 |
| | 3.45 | 3.50 | 1.71 | 2.13 | 3.15 | 2.20 |
| | 2.77 | 3.69 | 1.95 | 1.76 | 4.03 | 3.58 |
| | 4.62 | 2.20 | 2.58 | 1.88 | 3.31 | 4.50 |
| | 2.10 | 2.92 | 1.90 | 2.10 | 4.20 | 4.75 |
| | 2.63 | 3.71 | 1.93 | 2.33 | 5.61 | 4.02 |
| | 2.43 | 3.01 | 3.03 | 2.23 | 3.22 | 3.73 |
| | 3.58 | 2.39 | 2.89 | 2.71 | 4.09 | 3.55 |
| | 2.44 | 3.19 | 2.14 | 2.56 | 4.03 | 3.15 |
| | 3.00 | 3.40 | 1.84 | 2.51 | 3.37 | 3.80 |
| | 2.03 | 3.47 | 2.40 | 2.73 | 4.43 | 3.53 |
| | 3.11 | 3.17 | 1.79 | 2.44 | 3.97 | 4.94 |
| | 2.88 | 2.62 | 2.21 | 3.55 | 4.62 | 3.70 |
| | 2.39 | 2.28 | 4.12 | 2.19 | 3.53 | 4.49 |
| | 2.28 | 3.83 | 1.71 | 1.87 | 4.08 | 4.92 |
| | 3.15 | 3.41 | 1.40 | 1.67 | 4.52 | 4.72 |
| | 3.34 | 3.10 | 1.81 | 2.53 | 3.13 | 3.33 |
| | 2.29 | 3.37 | 2.48 | 3.36 | 3.12 | 3.60 |
| | 1.97 | 2.45 | 2.40 | 2.65 | 3.92 | 3.82 |

| Genotype | 79 | 80 | 81 | 82 | 83 | 84 |
|---|---|---|---|---|---|---|
| Hypocotyl length (mm) | 3.85 | 4.25 | 2.47 | 2.32 | 3.77 | 2.60 |
| | 4.16 | 3.20 | 2.79 | 1.58 | 2.73 | 2.02 |
| | 3.98 | 3.04 | 4.38 | 2.35 | 3.11 | 2.51 |
| | 3.77 | 2.75 | 2.93 | 2.32 | 3.50 | 2.32 |
| | 4.04 | 2.87 | 2.41 | 2.13 | 5.45 | 3.35 |
| | 3.56 | 3.31 | 2.20 | 2.40 | 3.27 | 2.67 |
| | 3.33 | 2.77 | 2.80 | 3.13 | 3.15 | 4.13 |
| | 2.64 | 3.52 | 4.53 | 3.62 | 2.39 | 3.38 |
| | 2.85 | 3.42 | 2.91 | 0.93 | 3.51 | 1.94 |
| | 2.84 | 3.64 | 1.25 | 1.15 | 4.56 | 2.41 |
| | 3.27 | 2.39 | 1.79 | 2.28 | 2.13 | 3.42 |
| | 4.03 | 3.39 | 2.68 | 2.02 | 3.43 | 4.24 |
| | 2.66 | 2.46 | 2.66 | 2.75 | 2.15 | 3.50 |
| | 3.35 | 3.43 | 4.07 | 3.75 | 3.00 | 2.81 |
| | 3.10 | 4.29 | 2.34 | 4.17 | 3.78 | 3.21 |
| | 2.96 | 3.00 | 2.08 | 3.01 | 2.18 | 3.02 |
| | 3.96 | 3.73 | 2.03 | 3.82 | 3.06 | 2.76 |
| | 4.95 | 4.64 | 2.44 | 1.97 | 2.92 | 3.86 |
| | 2.77 | 2.69 | 2.65 | 2.01 | 2.93 | 2.65 |
| | 3.60 | 4.48 | 1.71 | 3.68 | 2.73 | 2.44 |

| Genotype | 85 | 86 | 87 | 88 |
|---|---|---|---|---|
| Hypocotyl length (mm) | 3.02 | 1.59 | 1.62 | 1.95 |
| | 2.12 | 0.58 | 2.03 | 2.06 |
| | 1.73 | 0.84 | 2.26 | 2.72 |
| | 1.81 | 1.38 | 2.74 | 2.30 |
| | 2.26 | 0.93 | 1.66 | 1.71 |
| | 2.62 | 1.38 | 2.25 | 2.45 |
| | 2.37 | 2.15 | 2.21 | 2.62 |
| | 3.05 | 1.13 | 2.13 | 2.09 |
| | 2.54 | 0.61 | 2.14 | 2.38 |
| | 1.70 | 1.96 | 2.05 | 2.63 |
| | 0.85 | 1.13 | 3.08 | 3.11 |
| | 2.99 | 1.39 | 1.37 | 2.44 |
| | 2.39 | 2.43 | 2.79 | 1.97 |
| | 0.82 | 1.38 | 1.94 | 2.36 |
| | 3.17 | 2.03 | 3.33 | 1.91 |
| | 1.88 | 1.79 | 2.47 | 2.19 |
| | 0.89 | 1.68 | 2.50 | 2.11 |
| | 1.10 | 0.58 | 3.07 | 2.36 |
| | 1.70 | 0.75 | 2.04 | 2.22 |
| | 2.80 | - | 2.27 | 1.80 |

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