Increasing the Potassium Use Efficiency of Crops

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Biology

September 2017

Abstract

Increasing the potassium use efficiency (KUE) of crops will be important in satisfying growing demand for food that is cost-effective and has minimal environmental consequences. The work presented in this thesis contributes to this aim by exploring the measurement of KUE and identifying drivers responsible for differences in KUE between rice genotypes at the physiological and genetic levels. In Chapter 2, a mathematical model was constructed based on growth experiments using *cv. IR64* rice plants. Subsequently, sensitivity analysis was used to assess how best to measure KUE experimentally, showing how KUE could be comprehensively measured using a small selection of metrics. KUE was then quantified in over 300 rice genotypes in Chapter 3. From this, high performing rice genotypes were identified along with associations between KUE metrics and physiological traits. While key physiological drivers of differences between genotypes differed with metrics, the replacement of potassium by sodium was consistently found to aid KUE. In Chapter 4, differences in KUE between rice genotypes were then explored at the genetic level, using genome-wide association studies (GWAS) to describe the genetic underpinnings of KUE for the first time. This resulted in the identification of KUE-relevant quantitative trait loci (QTLs) and candidate genes. Both novel and previously described QTLs for KUE were found and links between the sub-population structure present in rice and the genetic architecture of KUE were uncovered. Furthermore, several genes coding for proteins with regulatory functions were identified in GWAS analyses, as were genes for sodium transport proteins. Taken together, the findings of the work presented in this thesis could be used to inform future KUE studies as well as providing candidate physiological traits and genetic loci for crop improvement.

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List of Accompanying Material

This thesis is accompanied by a compact disc which contains the supplementary files outlined below:

- **sensitivityAnalysis.py**: Python code that solves ordinary differential equations and carries out the sensitivity analyses described in Chapter 2
- **riceGenotypes.xlsx**: a spreadsheet summarising the rice genotypes used in Chapters 3 and 4
- **gwasPlots.pdf**: Manhattan plots and QQ plots for each phenotype analysed using genome-wide association studies (GWAS) as described in Chapter 4
- **genesList.xlsx**: spreadsheet summarising the genes found within QTLs from Chapter 4

The disc can be found at the back of the thesis.

Acknowledgements

I would first like to thank my supervisors Frans Maathuis and Jon Pitchford. Our conversations over the years were not only crucial to the advancement of this project but have served as an education that I am sure will benefit me for years to come. Thank you both for giving me this opportunity and for all your help. Thanks must also go to my advisory panel, Angela Hodge, Michael Schultze, and Mike Haydon. It was a great benefit to receive your thoughts on my project as it progressed. I would also like to express my appreciation to Mike Blatt for agreeing to examine this thesis. Furthermore, I am grateful for the funding I have received for this project from the BBSRC.

The help of several other colleagues cannot be ignored. My attempts to grow rice benefited greatly from the input of the horticultural technicians at the University of York. The lab groups I was a part of were a constant source of advice and support. Furthermore, it has been a great pleasure to get to know so many fellow students during my studies; the future of science is bright.

I would also like to thank Lorna Warnock and Amanda Barnes for helping to set up my placement. I am hugely grateful to the team at Man GLG Japan Core Alpha for letting me spend three months with you. I learned so much during my placement and felt like part of the team from the moment I joined. My placement was a real highlight in the middle of four happy years. All the best to you all.

Finally, I would like to acknowledge the importance of friends and family. Being able to spend time away from my research has resulted in so many happy memories. I am sure that many more are to come.

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 The Need to Improve Crop Potassium Fertiliser Use Efficiency

Each year, over 30 million tonnes of potassium fertilisers are applied to agricultural fields around the world (FAO, 2016). Commonly referred to as potash fertilisers due to the historic use of wood ash in their production, these fertilisers comprise several potassium salts including potassium sulphate (K_2SO_4) and potassium nitrate (KNO_3), as well as the most commonly applied potassium chloride (KCl). These salts are now predominantly produced by mining in the Northern hemisphere (Ciceri *et al.*, 2015). Global demand for potash fertilisers is expected to grow with a compound annual growth rate (CAGR) of approximately 2.5% between 2014 and 2019. This increased demand is strongest in Africa (8.6% CAGR), while Western and Southern Asia have predicted CAGRs of nearly 5% (FAO, 2016).

Part of the increasing demand for potash fertilisers is driven by population growth and the need to replenish potassium off-take from increasing output. At the same time, deficiency for potassium in agricultural soils is widespread around the world.

Over half of the Southern Australian wheat belt and three quarters of Chinese rice paddies have been described as lacking adequate potassium (Römheld and Kirkby, 2010). Many areas have endured historic under-fertilisation as well, but this varies geographically (Rengel and Damon, 2008). For example, under-fertilisation is commonly reported in Asia (Dobermann *et al.*, 1998; Sheldrick *et al.*, 2003; Pathak *et al.*, 2010; Timsina *et al.*, 2013) and a negative nutrient balance of approximately 20 kg ha^{-1} a^{-1} exists for potassium in African agriculture (Sheldrick and Lingard, 2002). At the same time, over-fertilisation has occurred in much of Europe (Bach and Frede, 1998; Foy *et al.*, 2002; Spiess *et al.*, 2011). Appropriate potash application will undoubtedly be important in dealing with these issues.

The use of potash is not without drawbacks, however. The purchase and application of potash presents a cost to agriculture. Furthermore, this cost is not constant and can rise rapidly. Potassium chloride prices peaked in February 2009 at over US$ 870 per tonne, having been under US$ 400 t^{-1} a year before and less than US$ 200 t^{-1} in early 2007. One tonne of potash fertiliser currently costs US$ 218 (World Bank, 2017), but the fact remains that price instability can be an issue with potash, as well as other fertilisers. Furthermore, over 95% of potash was produced in the Northern hemisphere in 2016 (USGS, 2017). This, combined with inefficient distribution, limits use in the Southern hemisphere (Manning, 2010; Ciceri *et al.*, 2015). The production and transportation of potash fertilisers also has implications for carbon emissions (Brentup and Palliére, 2008; Ciceri *et al.*, 2015) and further environmental concerns are linked with potash use. While understanding of the anthropogenic impacts of ecological potassium cycles is limited (Sardans and Peñuelas, 2015), potassium inputs can influence plant community composition (Chytrý *et al.*, 2009; Huang *et al.*, 2013).

In summary, there are clear incentives to increase crop potassium fertiliser use efficiency. The need for greater agricultural output to satisfy demand from a growing world population coupled with a legacy of under-fertilisation means that demand for potash fertilisers is increasing. This leaves farmers vulnerable to fertiliser price fluctuations, as have been observed in the recent past. At the same time, there is a need to decrease the environmental impact of agriculture. Increased fertiliser use conflicts with this aim through carbon emissions from production as well as transport and the ecological impacts of fertiliser application. Without improvements in crop potassium fertiliser use efficiency, it is difficult to envisage a way to achieve a sustainable agriculture that can meet global food demands.

1.2 Potassium in Plants

1.2.1 Roles of Potassium

Potassium has several inter-related roles in plants. It is the most abundant cation in cells, with tissue concentrations of approximately 100 mM in potassium replete plants (Clarkson and Hanson, 1980). Along with calcium, potassium is never incorporated into biological compounds and so is only present in its ionic form throughout plant tissues, in contrast to most other macronutrients including nitrogen and phosphorus (Amtmann and Blatt, 2009). Without adequate potassium supply, plants become impaired in many processes such as long-distance transport of nutrients, photosynthesis, and protein synthesis.

Ultimately, insufficient potassium supply limits yield (Sale and Campbell, 1986; Abbadi *et al.*, 2008; Ma *et al.*, 2013; Fageria and Melo, 2014). Yield gaps attributable to potash under-fertilisation have been described (Dai *et al.*, 2013; Xu *et al.*, 2015; Yousaf *et al.*, 2017) and maize yields in China were found to be decreased by 26% when potash was not applied (Dai *et al.*, 2013). Despite yield loss, the potassium concentrations of grains, such as wheat and barley, have been found to be maintained at species-specific levels, regardless of the potash fertilisation regime (Zörb *et al.*, 2014). However, potassium deficiency can still cause yield quality to suffer. A lack of potassium can result in shorter and weaker cotton fibres (Pettigrew, 1999; Perevz *et al.*, 2004; Yang *et al.*, 2016) while greater amounts of the carcinogen and neurotoxin acrylamide can form when potassium-deficient potatoes are fried (Gerendás *et al.*, 2007).

Yield can be compromised by abiotic and biotic stresses and potassium is greatly important in alleviating many of these. A strong supply of potassium can help protect plants against a range of abiotic stresses such as high salt, ammonium, cold, and drought (see reviews by Cakmak, 2005; Oosterhuis *et al.*, 2013; Wang *et al.*, 2013; Zörb *et al.*, 2014). For example, sodium can compete with potassium for transport and binding sites on enzymes and one approach for coping with salt stress is maintenance of high cytosolic ratios of potassium to sodium (Maathuis and Amtmann, 1999; Shabala and Cuin, 2007). Ammonium toxicity may be associated with several issues such as energetic costs arising from futile membrane cycling (Britto *et al.*, 2001; Kronzucker *et al.*, 2001), carbon costs from its assimilation (Schortemeyer *et al.*, 1997), and competition with other cations (ten Hoopen *et al.*, 2010). Potassium can compete with ammonium for uptake and an adequate supply of potassium is required for optimal function of enzymes involved in ammonium assimilation (Balkos *et al.*, 2010). The importance of potassium as an osmoticum as well as its relevance to stomatal opening (see below) help plants use water when it is limited (Zörb *et al.*, 2014) and potassium supply has a stimulatory effect on enzymes which help to reduce the oxidative damage which can result from chilling (Farooq *et al.*, 2008).

Potassium is also highly relevant to biotic stresses (reviewed by Amtmann *et al.*, 2008; Oosterhuis *et al.*, 2013; Wang *et al.*, 2013). Plants which are well supplied with potassium have lower concentrations of low molecular mass compounds, such as sugars, that can be targets for pests (Amtmann *et al.*, 2008; Römheld and Kirkby, 2010). Also, phytohormones, such as jasmonic acid, which increase under potassium deficiency, may prime plant defences (Amtmann *et al.*, 2008). While high plant tissue potassium concentrations are often found to increase resistance to pathogens and pests, this is not uniformly the case and some stresses, such as nematode and possibly virus attacks, may be more severe at high potassium supply (*e.g.* Table 1 in Perenoud, 1990).

The influence of potassium on yield and stress response stems from its many roles within plants and it should also be noted that potassium plays an important part of how plants manage other elements. For example, potassium ions are needed to chaperone nitrate (as well as other anions) in translocation from roots to shoots via the xylem (Maathuis, 2009). Potassium is also important in the long-distance transport of carbon. The loading of sucrose into the phloem is facilitated by the movement of potassium ions across phloem cell membranes to prevent depolarisation resulting from proton and sucrose symport into phloem (Deeken et al., 2002). In addition to energising the re-loading of photoassimilates into the phloem in the absence of sufficient ATP (Gajdanowicz *et al.*, 2011), potassium also balances the charges of organic and amino acids present in phloem (Ahmad and Maathuis, 2014).

Potassium is of great importance in regulating stomatal aperture, a process that influences both photosynthesis and transpiration (and therefore xylem-mediated translocation of compounds and ions as well as water content). Influx of potassium into guard cells surrounding stomata lowers their water potential resulting in an influx of water, increased guard cell turgidity, and opening of stomata (see reviews by Outlaw, 1983; Blatt, 2000; Schroeder *et al.*, 2001). This process allows carbon dioxide to enter the plant that can be incorporated into photoassimilates as well as water vapour to exit the plant which maintains a water potential gradient that drives xylem sap flow (Römheld and Kirkby, 2010).

As well as its role in guard cells, potassium is an important osmoticum in plant cells more generally. The typically high concentration of potassium relative to other osmotica means that it plays an important role in generating cell turgor and expansive growth (Leigh and Wyn Jones, 1984; Johnston and Milford, 2012; Oosterhuis, 2013). Furthermore, potassium is important in controlling turgor in cells as required for movement of plant organs, such as leaves (Satter and Galston, 1981; Ahmad and Maathuis, 2014).

While potassium can be replaced to some extent in its biophysical functions (*e.g.* turgor generation and charge balancing), it is not replaceable in enzyme activation. Over sixty enzymes require potassium for their activation, with concentrations in the tens of millimolar neccesary for optimal function (Evans and Sorger, 1966; Adams and Shin, 2014). While specificity for potassium varies among enzymes (Evans and Sorger, 1966), potential replacements, such as sodium or ammonium ions, are less efficient activators than potassium ions and can become toxic at relatively low cytosolic concentrations (Clarkson and Hanson, 1980; Benito *et al.*, 2014). Enzymes that require potassium include the vacuolar proton-pumping pyrophosphatase which transports protons into the vacuole (Davies *et al.*, 1991), pyruvate kinase, which catalyses the final step in glycolysis (Evans and Sorger, 1966), and starch synthetase (Murata and Akazawa, 1969; Nitsos and Evans, 1969). Protein synthesis by ribosomes requires high potassium concentrations in keeping with those observed in the cytosol (Flowers and Dalmond, 1992).

Taken together, potassium has several functions within plants and these can result in diminished quantity and quality of agricultural output when potassium is limited. The diversity of roles for potassium influences how plants can utilise other elements as well as osmotic regulation. Furthermore, many of the biochemical roles of potassium are not replaceable by other cations and underpin several crucial processes in plants. This therefore means that maintenance of potassium-dependent processes when this nutrient is in limited supply is an important part of ensuring plant growth and yield in agriculture.

1.2.2 Potassium Uptake

The potassium concentration of the soil solution generally ranges from approximately 0.1 to 1 mM (Maathuis, 2009; White, 2013) while plant tissue potassium concentrations are around 100 mM (Leigh, 2001). This accumulation of potassium against its concentration gradient is mediated by proteins in the membranes of root cells in exterior tissues, such as the cortex and epidermis (Ahmad and Maathuis, 2014; Nieves-Cordones *et al.*, 2016). This process can take place with external solution potassium concentrations that span several orders of magnitude, with evidence for plants being able to deplete solutions below 1 µM (Martínez-Cordero *et al.*, 2004).

Over the range of soil solution potassium concentrations that plants encounter, a biphasic pattern of influx has been described (Epstein *et al.*, 1963; Kochian and Lucas, 1982). This consists of a high-affinity system which approaches saturation as concentrations exceed 0.1 mM (Epstein *et al.*, 1963; Maathuis and Sanders, 1996) and a low-affinity system. The latter dominates influx at higher external potassium concentrations as well as exhibiting greater levels of efflux from roots (Szczerba *et al.*, 2006) and is believed to be carried out by ion channels (Epstein *et al.*, 1963; Kochian and Lucas, 1982; Maathuis and Sanders, 1995). Potassium-proton symporters from the KT/HAK/KUP family of proteins such as AtHAK5 (Rubio *et al.*, 2008) and OsHAK1 (Banuelos *et al.*, 2002) mediate high-affinity uptake. In rice, OsHAK1 has been found to be responsible for approximately half of the potassium uptake between 0.05 and 0.1 mM external potassium concentrations and this declined to nearly one-third of uptake at 1 mM (Chen *et al.*, 2015). Another transporter, OsHAK5, has also been identified as carrying out high-affinity potassium uptake in rice roots as well as aiding its export to the shoots (Yang *et al.*, 2014). The notion that channels are only relevant to low-affinity uptake is challenged by the shaker-type potassium channel AKT1, which can mediate potassium influx across external concentrations that span the high- and low-affinity range, *i.e.* 10 μM and above (Hirsch *et al.*, 1998).

Proteins involved in the regulation of transport proteins have also been identified. When exposed to potassium deficient conditions, root cell membranes become hyper-polarised and calcium ions enter the cytoplasm (Nieves-Cordones *et* *al.*, 2014). This results in binding of the protein kinase CIPK23 with a calcium sensor (*e.g.* CBL1 or CBL9) and the resulting complex moves to the plasma membrane where it can increase AKT1 activity through phosphorylation (Li *et al.*, 2006; Xu *et al.*, 2006; Lee *et al.*, 2007). Such a complex can also phosphorylate AtHAK5, resulting in greater affinity for, and maximal uptake rate of, potassium (Ragel *et al.*, 2015). Signal cascades that result from low-potassium conditions, such as increased ethylene (Jung *et al.*, 2009), reactive oxygen species (Shin *et al.* 2005; Hernandez *et al.*, 2012), and jasmonic acid (Armengaud *et al.*, 2004), induce transcription factors which activate the transcription of genes for transporters such as AtHAK5 (Kim *et al.*, 2012; Hong *et al.*, 2013). Also, AKT1 proteins can form homotetramers or heterotetramers with AtKC1. Association with AtKC1 results in a more negative activation potential for AKT1, meaning that it is gated shut in low-potassium conditions and leakage out of the cell through AKT1 is prevented (Wang *et al.*, 2010; Wang *et al.*, 2016).

Other root physiological and morphological characteristics also influence potassium uptake. Exploiting a large soil volume through a greater root mass and volume aids potassium uptake (Mengel and Steffens, 1985; Chen and Gabelman, 2000; Høgh-Jensen and Perdersen, 2003; Jia *et al.*, 2008; White, 2013). Furthermore, over 90% of potassium in soils is in the non-exchangeable fraction, where it is held in soil minerals (Römheld and Kirkby, 2010). Plants can mobilise this potassium with organic acid exudates (Trehan *et al.*, 2005; Wang *et al.*, 2011). Transpiration also results in soil solution mass flow and this results in delivery of potassium to roots (Rengel and Damon, 2008; White, 2013). Interestingly, in contrast to responses for patchy availability of nitrogen or phosphorus, plant roots appear to be unable to preferentially proliferate in areas with high potassium supplies over those which lack potassium (Drew, 1975).

The uptake of potassium by crops is therefore a complex process mediated and influenced by processes at the molecular, tissue, and environmental level. While the above shows that much is known about how plants source potassium from the soil solution, many processes still require further investigation, particularly in the context of how cells sense and respond to low-potassium conditions. Such knowledge may play an important role in future crop improvement.

1.2.3 Potassium Distribution

Given the many roles of potassium in plants (Section 1.2.1), it is important that plants can effectively distribute this element among tissues and within cells. The long-distance transport of potassium through the plant vasculature is therefore of critical importance to move potassium to aerial tissues. Furthermore, potassium must also be moved between cell compartments. The key processes involved in this are outlined below and have also been reviewed by Ahmad and Maathuis (2014).

Absorbed potassium in roots can move symplastically and apoplastically towards the stele, although the symplastic route accounts for the majority of potassium loaded into xylem (White and Karley, 2010). Potassium enters the xylem from parenchymal cells via the action of outward rectifying channels, such as SKOR (Gaymard *et al.*, 1998). The concentration of potassium in the xylem sap can range from less than 10 mM to as high as approximately 50 mM (Jeschke *et al.*, 1997; White, 1997; Peuke, 2010). Potassium is then unloaded from the xylem into bundle sheath cells (Keunecke *et al.*, 2001) and potassium can move symplastically between adjacent cells (Botha *et al.*, 2008).

A high proportion of the potassium delivered to the shoot can return to the root via the phloem (Jeschke *et al.*, 1997; Marschner *et al.*, 1997; Peuke *et al.*, 2002). Furthermore, the phloem also allows potassium to be re-distributed to growing leaves, fruits, and seeds (White and Karley, 2010; Ahmad and Maathuis, 2014). This can help maintain growth and development even when potassium is limited. The potassium channel AKT2/3 is important for the movement of potassium into and out of the phloem (Deeken *et al.*, 2002). Further Shaker family potassium channels are believed to be involved in the entry of potassium into the phloem. For example, the channel KZM1 found in maize (which shows similarity to *Arabidopsis* to KAT2) has been characterised as mediating phloem potassium loading (Phillippar et al., 2003).

Potassium is also distributed on a sub-cellular scale. Vacuolar potassium concentrations can be greater than that of the cytosol in well-supplied plants, but low-potassium stress can lead to the cytosolic concentration exceeding that of the vacuole (Leigh and Wyn Jones, 1984; Walker *et al.*, 1996). Accumulation of potassium into the vacuole is believed to be mediated by CHX and NHX proton-cation exchangers (Cellier *et al.*, 2004; Ohnishi *et al.*, 2005; Barragán *et al.*, 2012). Channels, such as members of the TPK/KCO family (Gobert *et al.*, 2007) or TPC1 (Peiter *et al.*, 2005), can carry out the release of potassium from the vacuole into the cytoplasm. Transporters from the HAK/KUP/KT family may mediate the movement of potassium out of vacuoles when the vacuolar potassium concentration declines below that of the cytosol (Jaquinod *et al.*, 2007; White and Karley, 2010; Ahmad and Maathuis, 2014).

Taken together, these processes allow plants to distribute potassium sourced by roots from the soil solution around plant tissues. Furthermore, potassium can be moved between the vacuole and cytoplasm, allowing cytoplasmic processes with a strict requirement for potassium (Section 1.2.1) to be maintained. In the context of potassium use efficiency, the potential for plants to direct potassium to where it is required may present opportunities for crop improvement.

1.3 Potassium Use Efficiency in Crops

1.3.1 Definition of Potassium Use Efficiency

Potassium use efficiency (KUE) can be defined in several different ways. Part of this diversity is due to the complexity of KUE which subsumes the many roles of this nutrient within plants (Section 1.2.1) as well as its uptake (Section 1.2.2) and distribution (Section 1.2.3). For example, KUE can be dissected into the uptake (sometimes described as acquisition) and utilisation efficiency (Rengel and Damon, 2008; White, 2013; Wang and Wu, 2015). However, the ways they are calculated can differ between reports. For example, utilisation efficiency is often measured as the ratio of biomass to potassium content (*e.g.* George *et al.*, 2002; Yang *et al.*, 2003; White *et al.*, 2010), while other authors have reported the biomass divided by the tissue potassium concentration (*e.g.* Memon *et al.*, 1985; Ju *et al.*, 2014).

Metrics which are not exclusively uptake- or utilisation-related have also been used. The ability of plants to maintain important characteristics such as mass and tissue potassium concentrations have been reported (*e.g.* Yang *et al.*, 2003; Damon and Rengel, 2007; Damon *et al.*, 2007; Fan *et al.*, 2013) and can be influenced by both the uptake and utilisation of potassium. Furthermore, maximised plant biomass under low-potassium conditions can be considered to represent a high KUE (*e.g.* Guoping *et al.*, 1999; Liu *et al.*, 2009; Fageria and Melo, 2014). The identification of crop genotypes that can maintain adequate yield and quality in the face of limited potassium availability is a key concern when measuring KUE.

However, there are many potential pitfalls in the way KUE is determined. Screens typically take place on relatively young plants that are still in the vegetative phase of growth and evidence is mixed as to how well different KUE metrics link with economic output, such as grain yield. Using rice, Yang *et al.* (2003) found that the utilisation efficiency (mass divided by potassium content) at the vegetative stage of growth was good a predictor of grain utilisation efficiency across 134 genotypes.

In contrast, vegetative measures of KUE were not found to link with yield in wheat (Woodend and Glass, 1993; Damon *et al.*, 2007). It has been suggested that the reliability of KUE measured at the vegetative stage to yield may be compromised by genotypic differences in how the harvest index (grain mass divided by above ground mass) responds to potassium deficiency (Woodend and Glass, 1993; Rengel and Damon, 2008).

Nevertheless, vegetative screens facilitate high throughput assessment of many crop genotypes and can be used to identify important physiological drivers of differences in potassium use. Screening studies often report multiple metrics that relate to KUE to help address the complexity of this trait. However, the choice of metrics that best summarise KUE is not clear. Analyses based on mathematical models of potassium utilisation by Moriconi and Santa-Marìa (2013) were able to identify different strengths and weaknesses of utilisation metrics. For example, the commonly used formula of mass divided by potassium content was one of the more reliable measures of utilisation, but could be flawed when the initial potassium concentration of genotypes is an important source of variation.

The above suggests that the selection of KUE metrics in screening studies is not a trivial matter. While it is clearly important to measure KUE in a way that captures the complexity of the trait while remaining experimentally tractable, further research is required to properly inform metric choice. Furthermore, greater experimental probing of links between vegetative and yield measures is also required. At the same time, mathematical modelling poses an under-utilised method for exploring the measurement of KUE.

1.3.2 Potential for Improving Potassium Use Efficiency

To improve crop KUE, it is important to identify efficient crop genotypes and determine what causes efficiency differences at the genetic and physiological level.

Previous screening studies have been able to identify variation in KUE within and across crop species (see reviews by Rengel and Damon, 2008; White *et al.*, 2013). Such work, along with a knowledge of how potassium is sourced, distributed, and used by plants (see above), makes it possible to propose putative approaches for improving KUE (Rengel and Damon, 2008; White, 2013; Shin, 2014; Wang and Wu, 2015).

Variation in KUE across genotypes can be vast. Yang *et al.* (2003) reported approximately six-fold variation in utilisation efficiency (mass divided by potassium content) in 134 rice genotypes. Nearly twenty-fold variation has been described for the uptake efficiency (plant potassium content) across 343 *Brassica oleracea* genotypes (White *et al.*, 2010). In wheat, the loss of grain mass due to low-potassium stress was found to range from as low as approximately 30% to more than 60% (Damon and Rengel, 2007) and similar ranges were apparent for shoot biomass in canola, wheat, and watermelon (Damon and Rengel, 2007; Damon *et al.*, 2007; Fan *et al.*, 2013). In general, variation in KUE at the genotype level is consistently found regardless of the species or metric used (*e.g.* Table 1 in White, 2013 for a summary of uptake and utilisation efficiency).

Taken together, this provides strong evidence for a potential to improve crop KUE but to do so, the reasons why such differences in KUE exist need to be better understood. KUE is impacted by root potassium uptake from the environment, a process that can be influenced by many root characteristics. For example, greater transpiration rates can increase mass flow and delivery of potassium to roots (White, 2013). Strong proliferation of roots can result in large root masses that can exploit a large soil volume, increasing potassium uptake (Mengel and Steffens, 1985; Chen and Gabelman, 2000; Kellermeier *et al.*, 2013). The proliferation of root hairs in response to potassium deficiency has also been reported in several species to aid absorption of potassium (Høgh-Jensen and Pedersen, 2003). Another potentially important trait is the ability to mobilise the non-exchangeable pool of potassium in soil (Wang *et al.*, 2000; Trehan *et al.*, 2005). This could be achieved using plant root exudates such as organic acids (Rengel and Damon, 2008; White, 2013). Furthermore, the high-affinity potassium uptake system represents another target for KUE improvement and studies using over-expression of transcription factors that up-regulate *AtHAK5* expression have been shown to improve plant tolerance to low-potassium stress (Kim *et al.*, 2012; Hong *et al.*, 2013).

Engineering the activity of proteins involved in potassium distribution within plants, such as SKOR or AKT2/3 (Section 1.2.3), may aid KUE (Shin, 2014) by prioritising tissues which most require potassium. Effective distribution of potassium has been identified as an important determinant of potassium utilisation efficiency in rice, with potassium preferentially transferred to developing leaves (Yang *et al.*, 2004). Distribution of potassium at a sub-cellular level may also be highly relevant, with more efficient release of vacuolar potassium into the cytoplasm under potassium stress implicated in greater utilisation efficiency in barley (Memon *et al.*, 1985). Replacement of potassium by other cations and osmotica may be complementary to this. While the role of potassium in enzyme activation is not fully replaceable (Section 1.2.1), other functions, such as charge balancing and osmotic regulation, are less ion-selective. Large proportions of tissue potassium can potentially be replaced, for example with sodium or other cations (Leigh *et al.*, 1986; Subbarao *et al.*, 1995; Gattward *et al.*, 2012) and sugars can be used as replacement osmotica in many cells (White and Karley, 2010). The harvest index of plants, and its maintenance under potassium stress, is also an important trait, having been found to be a key determinant of potassium utilisation in economic output (Fageria *et al.*, 2001; Yang *et al.*, 2004; Damon and Rengel, 2007).

The genetic architecture of KUE has also received some attention. Quantitative trait loci (QTLs) in the context of potassium stress have been identified in *Arabidopsis* (Prinzenberg *et al.*, 2010), rice (Wu *et al.*, 1999; Miyamoto *et al.*, 2012; Fang *et al.*, 2015), and wheat (Guo *et al.*, 2012; Kong *et al.*, 2013; Zhao *et al.*, 2014). Co-localisation between potassium-relevant QTLs and genes involved in its uptake and distribution have also been described. For example, Harada and Leigh

(2006) found several potassium channels and transporters in the region of QTLs in *Arabidopsis* such as *AtAKT1*, *AtHAK5*, and *AtSKOR* (Sections 1.2.2 and 1.2.3) and an enrichment of transporters in the region of a marker identified in *Brassica oleracea* genotypes (White *et al.*, 2010). At the same time, the response of plants to low-potassium stress has been examined at a transcriptional level (*e.g.* Maathuis *et al.*, 2003; Armengaud *et al.*, 2004; Ma *et al.*, 2012; Shankar *et al.*, 2013; Zeng *et al.*, 2014). While genes encoding transport proteins, such as *OsHAK1* (Ma *et al.*, 2012; Shankar *et al.*, 2013) and its homologue *AtHAK5* (Ahn *et al.*, 2004; Armengaud *et al.*, 2004), are differentially expressed, evidence for a role of several other processes is apparent. For example, important roles of phytohormones, such as jasmonic acid (Armengaud *et al.*, 2004) or auxin (Ma *et al.*, 2012), has been described, as well as protein modification, such as phosphorylation by kinases (Ma *et al.*, 2012; Zeng *et al.*, 2014; Zhang *et al.*, 2017).

From the above, it is apparent that the complexity of KUE means that several credible mechanisms for improvement may exist. While the underlying mechanisms of the uptake, distribution, and use of potassium in plants is increasingly well understood, how this knowledge can best be used to optimise crop KUE is not clear. Understanding of the response of plants to low-potassium stress at the molecular level may provide key insights. Identification of candidate genomic regions and, potentially, genes for improvement will aid molecular-based breeding efforts. To this end, the relatively new technique of genome-wide association studies (GWAS) has not been utilised in the context of KUE. Given datasets now available in rice (Zhao *et al.*, 2011; Eizenga *et al.*, 2014; McCouch *et al.*, 2016), GWAS offers a new and potentially powerful approach to dissect the underlying genetic architecture of KUE.

1.4 Rice as a Model Organism

Asian rice (*Oryza sativa*, L., henceforth 'rice') is used as the model plant organism for cereal crops. This species is of great importance to global agriculture. Each year, over 700 million tonnes of rice is produced, the third highest in terms of crop mass, behind only sugar cane and maize (FAO, 2017). Rice production is dominated by Asia, but occurs across the globe and the crop is largely consumed locally to where it is grown (GRiSP, 2013). Only 30 million tonnes of rice is traded internationally each year (GRiSP, 2013) and the prices are in the region of US$ 300 to 400 per tonne (FAO, 2017). Rice accounts for 19% of global human calorie intake (GRiSP, 2013) and is a staple food for half of the global population. It is of particular importance in many developing countries, such as Bangladesh, Cambodia, and Vietnam, where it is the source of over half of the consumed calories (IRRI, 2017).

Rice has been farmed for several thousand years (GRiSP, 2013). Over 40,000 varieties currently exist (Kushwaha, 2016) which comprise two sub-species (*Indica* and *Japonica*), each consisting of distinct sub-populations. While six sub-populations were identified on the basis of isozyme analysis (Glaszmann, 1987), rice is now commonly referred to as having five sub-populations on the basis of subsequent genetic work (Garris *et al.*, 2005; Ali *et al.*, 2011). The *indica* and *aus* sub-populations are grouped into the *Indica* sub-species while the *Japonica* sub-species includes the *aromatic*, *temperate japonica* and *tropical japonica* (Garris *et al.*, 2005; Ali *et al.*, 2011; Travis *et al.*, 2015). Admixtures between sub-populations and sub-species also exist.

Rice is also of great scientific importance since it is the model monocotyledonous plant species. It has a diploid genome made up of approximately 390 million base pairs (IRGSP, 2005; McNally *et al.*, 2009) and its genome was first sequenced in 2002 (Goff *et al.*, 2002; Yu *et al.*, 2002). More recently, genome-wide single nucleotide polymorphism (SNP) datasets have become available for rice genotypes that represent the breadth of rice diversity (Zhao *et al.*, 2011; McCouch *et al.*, 2016; https://ricediversity.org/). This means that rice is now ideally suited to agriculturally relevant analyses of how plant physiology and its genetic architecture underpins mechanisms of low potassium stress response.

1.5 Thesis Overview

This work aimed to explore and analyse the mechanisms that underlie KUE using rice as a model organism. The measurement of KUE is a crucial part of such studies and a lot of variety exists in previously used KUE metrics (Section 1.3.1). It is likely that a small subset of these metrics could be used to adequately summarise KUE in screening studies. Exploration of how KUE metrics respond to differences in plant physiology and how this could be used to find a suitable selection of KUE metrics for screening formed the focus of Chapter 2. Based on this, a selection of KUE metrics was used to quantify the variation in KUE present in rice as well as to identify which physiological plant characteristics give rise to genotypic differences in KUE (Chapter 3). The genetic underpinnings of differences between rice genotypes was then examined in Chapter 4.

Chapter 2

A Mathematical Model to Identify the Physiological Drivers of Potassium Use Efficiency in Crops

2.1 Introduction

Potassium use efficiency (KUE) is an important crop trait that must be optimised to secure adequate output in the future (Chapter 1). To this end, previous studies have examined variation in KUE among genotypes of a variety of plant species (*e.g.* Guoping *et al.*, 1999; Yang *et al.*, 2003; Trehan *et al.*, 2005; Fageria and Melo, 2014). It is notable that this literature uses a large variety of metrics to measure KUE. The use of several metrics which explore different aspects of this complex trait is recommended (Baligar *et al.*, 2001; White, 2013), but the fact remains that several measures exist for similar KUE-related traits, and that there is no clear rationale as to which metric should be preferred. For example, the production of biomass for a given amount of potassium, commonly referred to as utilisation, is often expressed as the shoot biomass divided by the potassium content measured at some fixed point in time (*e.g.* Guoping *et al.*, 1999; Yang *et al.*, 2003; White *et al.*, 2010). In other studies, the biomass is divided by the tissue concentration (*e.g.* Memon *et al.*, 1985; Ju *et al.*, 2014) and several somewhat similar quotients can also be proposed and it has been suggested that some metrics are more useful than others. For example, Moriconi and Santa-Marìa (2013) found the nutrient productivity, accumulative productivity, and utilisation ratio (see Table 2.1) were most appropriate for measuring utilisation efficiency in plants starved of potassium. Instances where metrics could provide misleading values were also described, such as when initial tissue potassium concentrations can influence the utilisation ratio.

A further frequently used class of metric compares performance under low and replete potassium supplies. Some studies have used the ratio of plant mass under two different potassium treatments (Yang *et al.*, 2003; Damon *et al.*, 2007), while others have reported the relative tissue potassium concentrations (Yang *et al.*, 2003; Fan *et al.*, 2013) or content (Guoping *et al.*, 1999; Yang *et al.*, 2004). While each of these ratios seeks to quantify how well plants cope with low potassium relative to non-stressed conditions, it is unclear how they inter-relate. Specifically, it may be the case that these metrics do not respond in the same way to differences in plant physiological traits present in screen panels. This ultimately means that important variation in KUE could be missed if an appropriate selection of metrics is not used.

Another important issue is how well KUE measured during vegetative growth relates to yield. Vegetative KUE measures have been reported as predictors of eventual yield or harvest index in sweet potato (George*et al.*, 2002) and rice (Yang *et al.*, 2003). However, Woodend and Glass (1993) found little correlation between vegetative utilisation efficiency measures with grain yield and negative correlation between the shoot mass of three-week-old plants and the grain mass was reported.

Damon and Rengel (2007) also found that the ratio of plant mass in two potassium treatments did not accurately predict the ratio of seed yield at maturity in wheat. Variability in the response of the harvest index (the proportion of above ground biomass that is seed) between crop varieties has been suggested as an important driver of a lack of correlation between vegetative- and grain yield-related measures (Damon and Rengel, 2008). Furthermore, the economic output of crops goes beyond grain yield, and may include the shoot biomass in forage crops and straw production.

Mathematical modelling of potassium in plants offers a novel method for examining these important questions related to KUE. Some effort has gone into modelling how plants source, distribute, and use nutrients (*e.g.* Thornley, 1969; Thornley, 1972; Ingestad and Ågren, 1988; Dewar, 1993; Roose *et al.*, 2001; Chen *et al.*, 2012; Hills *et al.*, 2012) and models offer a framework within which to explore nutrient use efficiency. Mathematical exploration can facilitate the identification of plant physiological characteristics that are principal drivers of differences KUE metrics between crop genotypes.

In this study, a mathematical model of potassium uptake and distribution together with the response of growth to tissue concentrations was constructed. Growth experiments using *IR64* cultivar rice plants were used to explore the dynamics of potassium in plants in response to the external potassium supply, and to inform model parametrisation. From this, the sensitivities of metrics from the KUE literature to model parameters were calculated. This was used to assess how different metrics responded to differences in plant physiological traits and how these metrics relate to each other and to yield. Key sensitivities of KUE metrics that would theoretically be most relevant to crop improvement efforts were also identified.

2.2 Methods

2.2.1 Plant Growth Experiments

Rice (*Oryza sativa*, L. *cv. IR64*) seeds were germinated and grown in sand with distilled water for two weeks prior to transfer to hydroponic treatments. Seedlings were transferred to 335 x 210 x 140 mm black plastic boxes filled with 9 litres of an adapted Yoshida nutrient solution (Yoshida *et al.*, 1976). The culture solution consisted of the following: (in mM) 1.4 NH_4NO_3, 0.3 $NaH_2PO_4 \cdot 2H_2O$,1 $CaCl_2$, 1.6 $MgSO_4 \cdot 7H_2O$, and 0.2 $Na_2O_3Si \cdot 5H_2O$; (in µM) 9.5 $MnCl_2 \cdot 4H_2O$, 0.07 $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 18 H_3BO_3, 0.15 $ZnSO_4 \cdot 7H_2O$, 0.16 $CuSO_4 \cdot 5H_2O$, 35.8 $FeCl_3$ $\cdot 6H_2O$, 71 citric acid monohydrate. Potassium was added as KCl to give solutions with 10, 100, 500, 1,000, and 5,000 µM potassium concentrations. The solutions had an initial pH of 5.5 and solutions were changed twice per week. Plants were grown in a glasshouse with 12 hour day and night periods with temperatures of 32 and 28 °C in the day and night respectively and a relative humidity between 50 and 60%.

In the first growth experiment (henceforth Experiment 1), plants spent 103 days in culture with three plants from each treatment randomly sampled at 21, 42, 63, and 84 days after transfer to hydroponics. Seed development had begun by 69 days post-transfer, but plants died from diseases before harvesting was possible. Experiment 2 was carried out to measure how grain yield responded to potassium supply using the same external concentrations as in Experiment 1. Furthermore, the short-term dynamics of plant responses to external potassium concentration were quantified by sampling three plants from each concentration 2, 4, 6, and 9 days after transfer to hydroponics. Five plants in each treatment were then grown to maturity and seeds were harvested after 234 days. Flowering was first noted 104 days after transfer in this experiment. In both experiments, five plants were randomly sampled on the day of transfer to hydroponics.

Sampled plants were separated into roots, shoots, and (where applicable) seeds and their fresh weights were recorded before being oven dried at 80 °C for three days. Dried tissues were then re-weighed and potassium concentrations were determined for roots and shoots by suspending sub-samples of chopped tissues in 20 mM $CaCl_2$ for 24 hours and measuring the potassium concentration of the resultant solutions using a flame photometer (Sherwood Scientific, Cambridge, Cambridgeshire, UK).

2.2.2 Model Description

A system of coupled ordinary differential equations was used to model potassium uptake and distribution, as well as growth in response to tissue potassium concentrations. As an overview, plants were modelled as being made up of root and shoot compartments. The root compartment could take up potassium from a solution of fixed external potassium concentration. Translocation of potassium from roots to shoots was modelled as a net movement, and the growth rate of each compartment was governed by its internal potassium concentration. Yield was modelled as a function of shoot mass after 63 days of growth. Each part of the model is described in greater detail below.

The net uptake of potassium by plant roots has a saturating relationship with the potassium concentration (Besford and Maw, 1974; Fageria, 1976; Spear *et al.*, 1978b; Szczerba *et al.*, 2006). This can be captured using a Michaelis-Menten-type relationship:

$$\frac{dQ_{plant}}{dt} = \frac{\mu S}{\kappa + S} W_{root} \tag{2.1}$$

where Q_{plant} is the plant potassium content (µmol K^+), t is the time since transfer to hydroponics (d), W_{root} is the root mass (g fresh weight, henceforth FW), μ is the theoretical maximal net potassium uptake rate per gram of root (µmol K^+ (g root FW)$^{-1}$ d^{-1}), S is the fixed external solution potassium concentration (µM K^+), and κ is the external solution concentration at which the net uptake rate per gram of root is half of the maximum value μ (µM K^+).

The net translocation of potassium from root to shoot serves to maintain the shoot potassium concentration that would otherwise be diluted through growth. Shoot potassium concentrations remain relatively stable except when the root concentration has become depleted (Ahser and Ozanne, 1967; Spear *et al.*, 1978a; White, 1997). This suggests that net potassium translocation can be maintained across a wide range of relatively high potassium concentrations before declining as the root concentration becomes low. This relationship between the root potassium concentration and net root to shoot translocation was captured using a Hill-type function:

$$\frac{dQ_{shoot}}{dt} = \frac{\chi C_{root}^{\theta}}{\phi^{\theta} + C_{root}^{\theta}} W_{shoot} \tag{2.2}$$

where Q_{shoot} is the shoot potassium content (µmol K^+), W_{shoot} is the shoot mass (g FW), χ is the theoretical maximum net translocation rate from root to shoot per gram of shoot per day (µmol K^+ (g shoot FW)$^{-1}$ d^{-1}), C_{root} is the root potassium concentration (µmol K^+ (g root FW)$^{-1}$), θ is a Hill-coefficient that controls how rapidly the net translocation rate changes with C_{root}, and ϕ is the value of C_{root} at which the net translocation rate per gram of shoot is half of χ (µmol K^+ (g root FW)$^{-1}$).

The relationship between the growth rate of plants and their internal potassium concentration is also saturating (Hommels *et al.*, 1989; White, 1993; Trehan and Claassen, 2000; Jordan-Meille and Pellerin, 2008). Furthermore, tissue or vacuolar concentrations typically maintain a minimum level of approximately 10 mM, even under extreme potassium stress (Leigh and Wyn Jones, 1984; White, 1993; Walker *et al.*, 1998), suggesting that the growth rate may become extremely small in such conditions. The relationship between the growth rate and tissue potassium concentration can be represented using a Hill-type function:

$$\frac{dW_i}{dt} = \frac{\alpha_i C_i^{\sigma_i}}{\beta_i^{\sigma_i} + C_i^{\sigma_i}} W_i \tag{2.3}$$

where, for the ith tissue (*i.e.* root or shoot), W is the tissue mass (g FW), α is the theoretical maximum relative growth rate (d^{-1}), C is the tissue potassium concentration (μmol K^{+} $(\mathrm{g\ FW})^{-1}$), β is the tissue potassium concentration at which the relative growth rate is half of α (μmol K^{+} $(\mathrm{g\ FW})^{-1}$), and σ governs how quickly the growth rate declines with the internal tissue potassium concentration.

To explore how the grain yield relates to vegetative metrics, a Hill-type function is used to relate the grain yield to the shoot mass after 63 days of growth. A sigmoidal relationship between grain and shoot mass has been found in safflower, sunflower (Abbadi *et al.*, 2008), and wheat (Ma *et al.*, 2013) subjected to a range of potassium concentrations. Data consistent with a non-linear relationship have also been reported in rice (Brohi *et al.*, 2000; Yang *et al.*, 2004; Fageria *et al.*, 2013). Furthermore, increasing seed mass coincides with a near cessation in growth of rice shoot mass (Sheehy *et al.*, 2004) and so shoot mass at flowering time is likely to be a reliable indicator of seed yield. This relationship is described using:

$$Y = \frac{\eta W_{shoot}^{\lambda}}{\nu^{\lambda} + W_{shoot}^{\lambda}} \tag{2.4}$$

where Y is the grain mass (g), η is the theoretical maximum seed mass (g), W_{shoot} is the shoot mass after 63 days of growth (g FW), λ is a Hill-coefficient that governs how rapidly the seed yield changes with the shoot mass, and ν is the shoot mass at which the seed yield is half of η (g FW).

Simulations were initiated with a plant mass, ω, (g FW), a root to plant mass ratio, δ, and initial root and shoot potassium concentrations, γ_{root} and γ_{shoot} respectively, (µmol K^+ (g tissue FW)$^{-1}$). The model was written in Python 3.4.4 (Python Software Foundation, 2017) and differential equations were solved using Runge-Kutta fourth order approximation with the *scipy.integrate.ode()* (Jones *et al.*, 2001).

2.2.3 Parameter Estimation

Data from the growth experiments were used to generate physiologically relevant estimates for model parameters. Net uptake rates per gram of root per day and translocation rates per gram of shoot per day were calculated for each treatment 21, 42, and 63 days after transfer to hydroponics using the net assimilate rate equation from Williams (1946). Relative growth rates were calculated using RGR = $(\ln(W_2) - \ln(W_1)) / (t_2 - t_1)$ (Hoffman and Poorter, 2002) using data from the same time points as described above for net uptake (*i.e.* 0, 21, 42, and 63 days post-transfer). Non-linear regression was used to fit model equations 2.1 to 2.4 to data using the *nls()* function in R 3.3.3. Relevant rates were calculated using data from Experiment 1 and time points were treated as independent samples in the non-linear least squares regressions.

Estimates of model constants are presented with 95% confidence intervals in the text below. For parameters from regression fits, confidence intervals were calculated using bootstrapping with the *bootCase()* function from the **car** R package (Fox and Weisberg, 2011).

2.2.4 Sensitivity Analysis

The purpose of constructing and parametrising the plant growth models was to allow the dependence of KUE metrics on the parameters governing plant growth to be be calculated. Explicitly, by integrating Equations 2.1 to 2.3 forward in time, and by incorporating yield via Equation 2.4 where appropriate, each KUE metric from Table 2.1 could be calculated. The response of these metrics to changes in plant physiology and experimental conditions could then be assessed using sensitivity analysis. Each model parameter in turn was altered ± 10% and corresponding changes in metrics were calculated. Experiments which lasted for 28 days with external solution potassium concentrations of 10 and 100 µM were simulated. Results for simulations at 950 µM external potassium are presented in the Appendix (Figure 6.1). Simulations were also carried out up to the 63 days time point to calculate grain yield long-term tissue mass. Separate simulations at 1,000 µM were used to calculate ratios between performance under potassium stressed and replete conditions.

Table 2.1: Summary of potassium use efficiency metrics used in this study. Values with a subscript *63* refers to a measurement taken after 63 simulated days, *init* denotes a value taken at the start of a simulation, and *HT* denotes values taken from the high treatment (1,000 µM external potassium concentration).

Metric	**Calculation**	**Units**	**Class**	**Source(s)**
Grain Yield, GY	W_{grain}	g	Yield	Yang *et al.* (2003), Quampah *et al.* (2011)
Harvest Index, HI	$\frac{W_{grain}}{W_{grain}+W_{shoot}}$	-	Yield	Xia *et al.* (2011)
Long-term plant mass, PL	$W_{plant,63}$	g FW	Yield	-
Long-term shoot mass, SL	$W_{shoot,63}$	g FW	Yield	Memon *et al.* (1985), Fageria and Melo (2014)
Long-term root mass, RL	$W_{root,63}$	g FW	Yield	-

Plant mass, P	W_{plant}	g FW	Mass	Spear (1978a)
Shoot mass, S	W_{shoot}	g FW	Mass	Damon *et al.* (2007), Fageria and Melo (2014)
Root mass, R	W_{root}	g FW	Mass	Fageria (2014), Krishnasamy *et al.* (2014)
Potassium utilisation ratio, KUtR	$\frac{W_{shoot}}{Q_{shoot}}$	g FW (µmol K^{+})$^{-1}$	Utilisation	Memon *et al.* (1985), Yang *et al.* (2003)
Potassium utilisation index, KUtI	$\frac{W_{shoot}}{C_{shoot}}$	g^{2} FW (µmol K^{+})$^{-1}$	Utilisation	Memon *et al.* (1985), Ju *et al.* (2014)
Nutrient productivity, NP	$\frac{\frac{dW_{shoot}}{dt}}{Q_{shoot}}$	g FW (µmol K^{+})$^{-1}$ d^{-1}	Utilisation	Moriconi and Santa-Marìa (2013)
Accumulative productivity, AP	$\frac{LN\left(\frac{W_{shoot}}{W_{shoot,init}}\right)}{C_{shoot}dt}$	g FW (µmol K^{+})$^{-1}$ d^{-1}	Utilisation	Moriconi and Santa-Marìa (2013), Yang *et al.* (2004)
Physiological efficiency, PE	$\frac{W_{shoot,HT}-W_{shoot}}{Q_{shoot,HT}-Q_{shoot}}$	g FW (µmol K^{+})$^{-1}$	Utilisation	Moriconi and Santa-Marìa (2013)

Shoot potassium concentration, CS	C_{shoot}	µmol K^+ (g shoot FW)$^{-1}$	Tissue Potassium	Trehan *et al.* (2005), Jiang *et al.* (2013)
Root potassium concentration, CR	C_{root}	µmol K^+ (g root FW)$^{-1}$	Tissue Potassium	-
Plant potassium content, QP	Q_{plant}	µmol K^+	Tissue Potassium	Guoping *et al.* (1999), White *et al.* (2010)
Shoot potassium content, QS	Q_{shoot}	µmol K^+	Tissue Potassium	-
Root potassium content, QR	Q_{root}	µmol K^+	Tissue Potassium	-
Relative shoot potassium concentration, RCS	$\frac{C_{shoot}}{C_{shoot,HT}}$	-	Ratio	Yang *et al.* (2003), Fan *et al.* (2013)
Relative root potassium concentration, RCR	$\frac{C_{root}}{C_{root,HT}}$	-	Ratio	-
Relative plant mass, RP	$\frac{W_{plant}}{W_{plant,HT}}$	-	Ratio	-
Relative shoot mass, RS	$\frac{W_{shoot}}{W_{shoot,HT}}$	-	Ratio	Damon and Rengel, 2007; Damon *et al.*, 2007

Relative root mass, RR	$\frac{W_{root}}{W_{root,HT}}$	-	Ratio	-
Relative plant potassium content, RQP	$\frac{Q_{plant}}{Q_{plant,HT}}$	-	Ratio	-
Relative shoot potassium content, RQS	$\frac{Q_{shoot}}{Q_{shoot,HT}}$	-	Ratio	-
Relative root potassium content, RQR	$\frac{Q_{root}}{Q_{root,HT}}$	-	Ratio	-

Standardised sensitivity scores for each metric for every model parameter in each potassium concentration were calculated using the equation:

$$s = \frac{a}{m}\frac{\Delta m}{\Delta a} \tag{2.5}$$

where s is the standardised sensitivity score of the KUE metric, a is the value of the unaltered parameter, m represents the value of each KUE metric when the constant is not changed, and Δa and Δm are the changes in a and m respectively when the constant was altered ± 10% (Sauvage *et al.*, 2003; Holt *et al.*, 2006).

Principal component analysis (PCA) was used on sensitivity scores from each external potassium concentration. The top two principal components (PCs) were used for visualisation and interpretation of results. The *prcomp()* function in R 3.3.3 (R Core Team, 2017) was used to carry out PCA.

2.3 Results

2.3.1 Plant Growth Experiments

The mass of *IR64* rice plants was influenced by the external solution potassium concentration and little growth occurred on average between 63 and 84 days after transfer to hydroponics (Figure 2.1). The plant mass on transfer to hydroponics had a mean average of 0.15 (0.13, 0.17; 95% confidence interval) g FW. The root to plant mass ratio approximately halved during the first 21 days of growth, declining from an initial value of 0.275 (0.266, 0.283) to an average across the five potassium treatments of 0.173 (0.165, 0.1825). After 21 days, ratios remained relatively stable (Figure 2.2). Root to plant mass ratios were statistically significantly different between potassium treatments at 63 days (ANOVA, $F_{4,10} = 8.15$, $P = 0.004$). Post-hoc testing using Tukey's range test revealed statistically significant ($P < 0.05$) lower root to plant mass ratios for plants in the 10 μM treatment compared to the 500 μM treatment.

The tissue potassium concentrations also varied in response to the external solution potassium concentration (Figure 2.3). Root and shoot potassium concentrations remained quite stable in the 500, 1,000, and 5,000 μM potassium treatments, but concentrations declined in the lower potassium treatments. Shoot (ANOVA, $F_{4,10} = 20.4$, $P = 8.5 \text{ x } 10^{-5}$) and root (ANOVA, $F_{4,10} = 22.6$, $P = 5.4 \text{ x } 10^{-5}$) potassium concentrations differed between treatments after 63 days of growth. Post-hoc testing using Tukey's range test revealed statistically significant ($P < 0.05$) lower shoot potassium levels in the 10 and 100 μM treatments compared to the highest three. For roots, the tissue potassium concentrations were statistically significantly lower when plants were grown with 10 μM external potassium compared to the

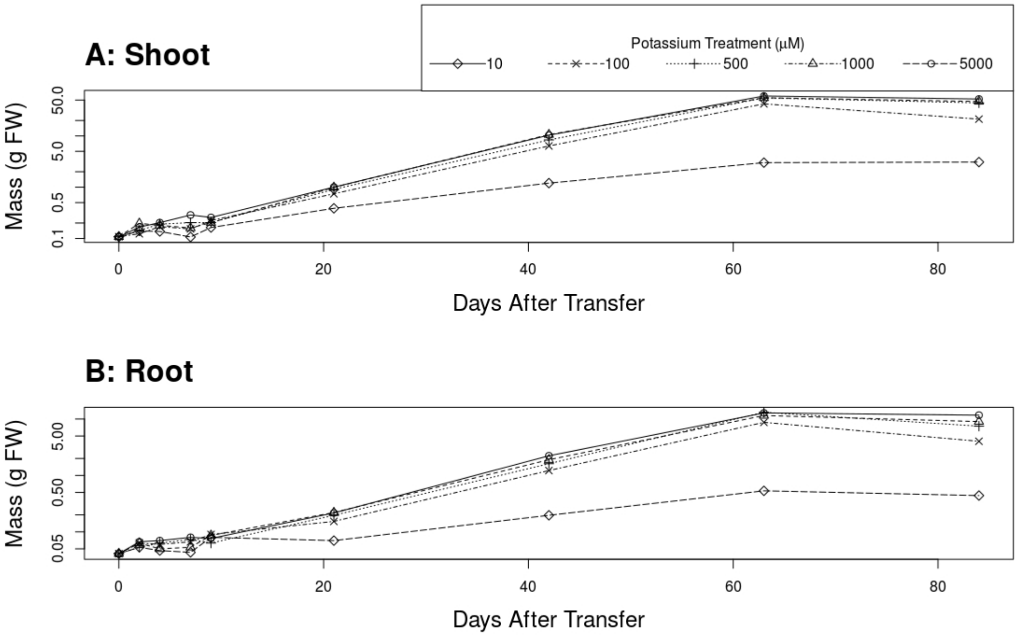


Figure 2.1: Shoot (A) and root (B) fresh weights from Experiments 1 and 2. Each point represent a mean of three plants. Error bars were not included on the plots to aid clarity. For reference, standard errors were on average 10.5% and 10.2% of means for shoots and roots respectively. Note the logarithmic scale on the vertical axis. Data are from Experiments 1 and 2 with points at the sampling dates outlined in Section 2.2.1.

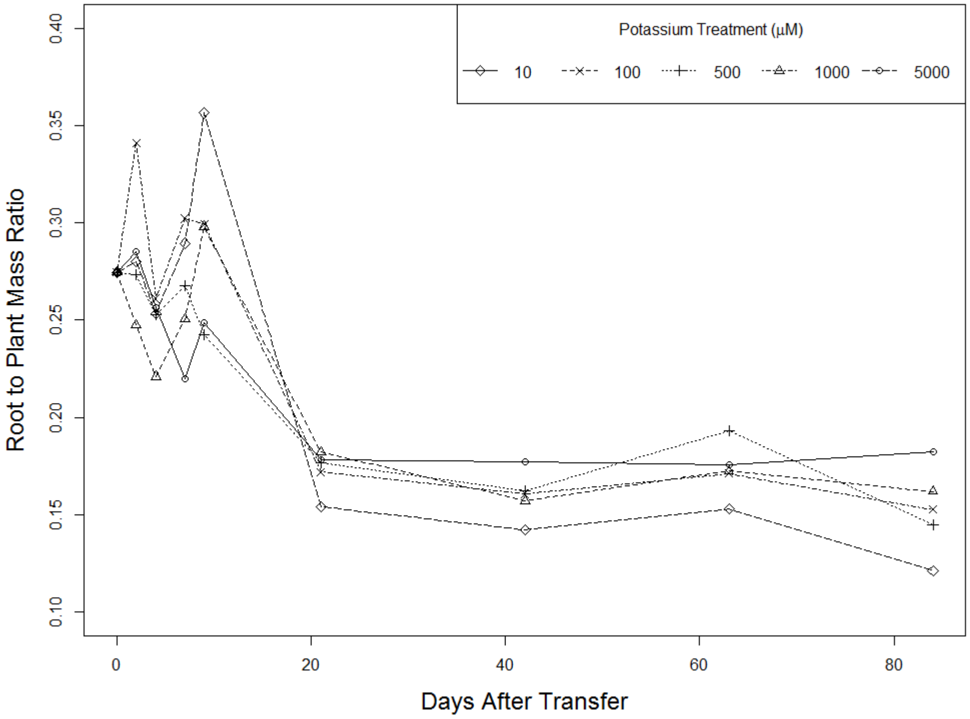


Figure 2.2: Root to plant mass ratio of IR64 rice plants grown in nutrient solutions with five different potassium concentrations. Each point represents a mean of three plants. Error bars were not included on the plots to aid clarity. For reference, standard errors were on average 7.0% of means. Data are from Experiments 1 and 2 with points at the sampling dates outlined in Section 2.2.1.

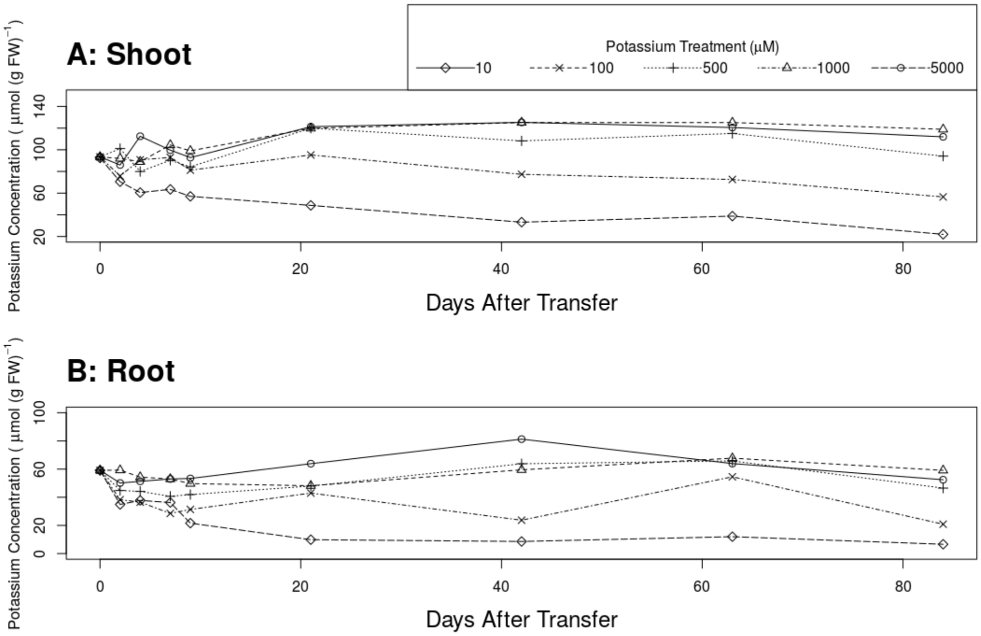


Figure 2.3: Shoot (A) and root (B) potassium concentrations from Experiments 1 and 2. Each point represent a mean of three plants. Error bars were not included on the plots to aid clarity. For reference, standard errors were on average 8.3% and 10.9% of means for shoots and roots respectively. Data are from Experiments 1 and 2 with points at the sampling dates outlined in Section 2.2.1.

other four treatments. The mean average shoot potassium concentration in the highest three potassium treatments across the samples was 102 (99, 106) μmol (g FW)$^{-1}$ and the average root potassium concentration in the same treatments was 56 (54, 59) μmol (g FW)$^{-1}$.

Average uptake rates per gram of root per day for plants sampled after 21, 42, and 63 days are presented in Figure 2.4. A non-linear regression fit of the data resulted in an estimated maximum uptake rate of 63.2 (55.6, 72.7) μmol (g root FW)$^{-1}$ d^{-1} and a half-saturation constant of 63 (42, 103) μM.

Data for the net translocation rate of potassium from roots to shoots and the

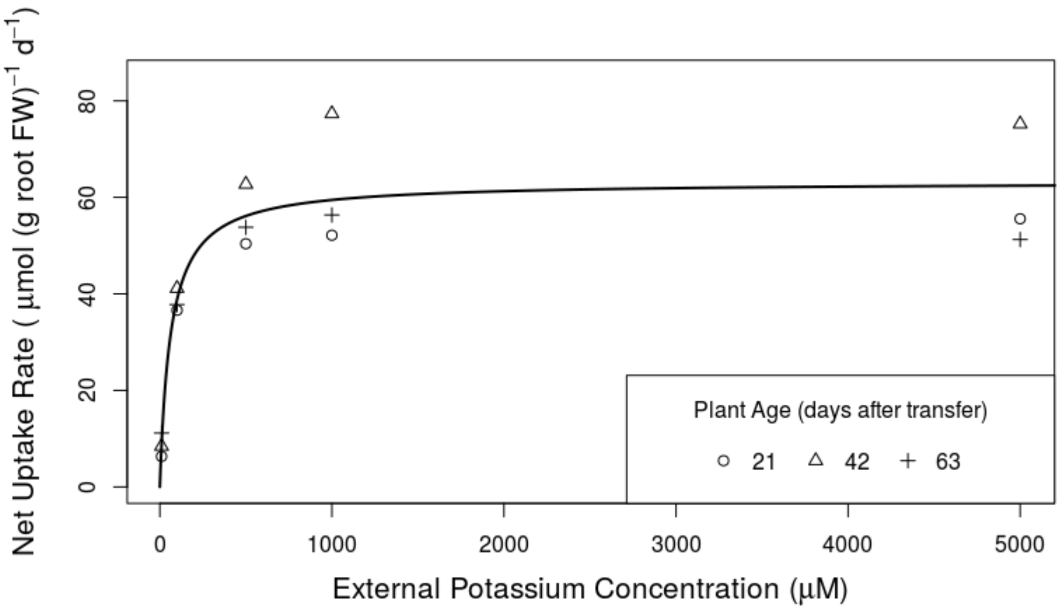


Figure 2.4: Net uptake rates of potassium per gram of root per day relative to the external solution potassium concentration. Points were calculated using the net assimilation rate formula from Williams (1948) using the means of three plants for inputs. The curve represents a non-linear regression fit to these data using a Michaelis-Menten-type function. Data are from Experiments 1 with points at the sampling dates outlined in Section 2.2.1.

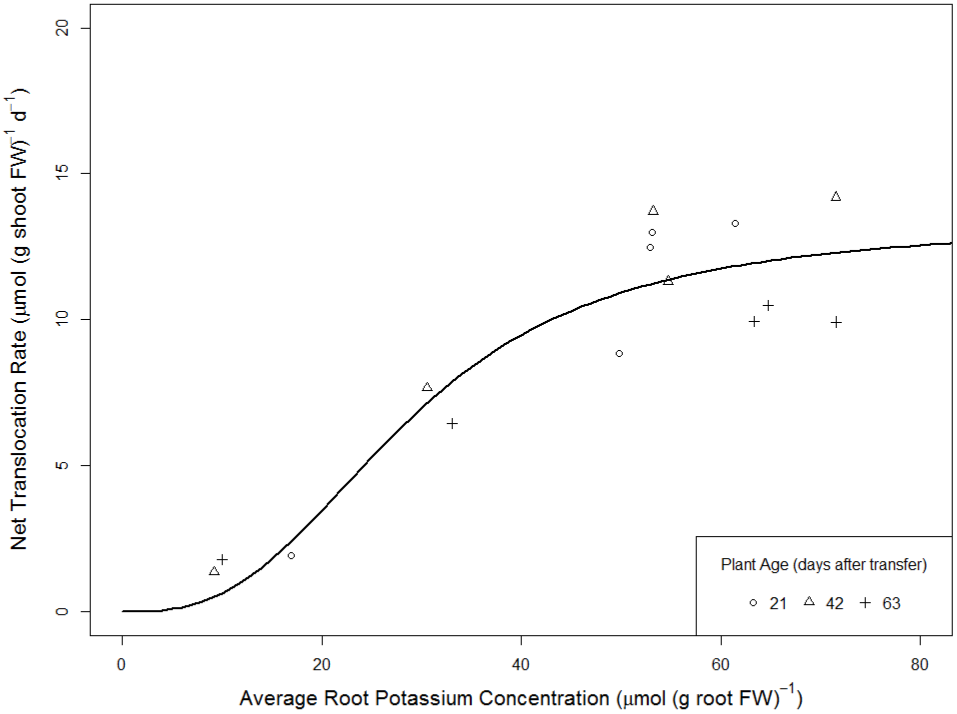


Figure 2.5: Net translocation rates of potassium from roots to shoots relative to the average root potassium concentration between consecutive samples (0, 21, 42, and 63 days post-transfer to hydroponics). Points were calculated using the net assimilation rate formula in Williams (1948) and the means of three plants for inputs. The curve represents a regression fit to these data using a Hill-type function. Data are from Experiments 1 with points at the sampling dates outlined in Section 2.2.1.

results of a non-linear regression fit of a Hill-type function are shown in Figure 2.5. This analysis found a maximum net translocation rate of 13.3 (11.3, 21.0) µmol (g shoot FW)$^{-1}$ d^{-1} and a half-saturation root concentration of 29 (22, 51) µmol (g root FW)$^{-1}$. The Hill coefficient was found to be 2.8 (1.6, 6.9).

For the growth rate of roots, a non-linear regression analysis using a Hill-type function (Figure 2.6) found a maximum relative growth rate of 0.113 (0.085, 0.297) d^{-1}, a half-saturation concentration of 16 (9, 222) µmol (g root FW)$^{-1}$, and the Hill coefficient was found to be 1.0 (0.54, 5.8). For shoots, a maximum relative

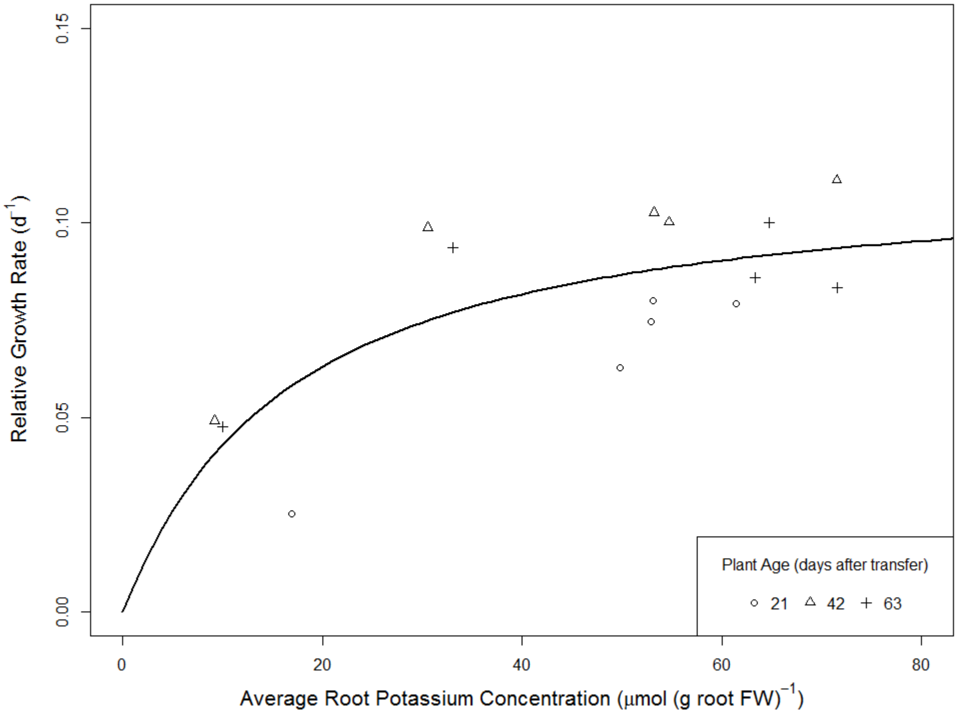


Figure 2.6: Root relative growth as a function of average root potassium concentrations for plants sampled after 0, 21, 42, and 63 days in hydroponics. Each point represents a value calculated using the mean mass of three plants and the curve represents a regression fit to these data using a Hill-type function. Data are from Experiments 1 with points at the sampling dates outlined in Section 2.2.1.

growth rate of 0.103 (0.095, 0.142) d^{-1}, a half-saturation concentration of 40 (37, 61) μmol (g shoot FW)$^{-1}$, and a Hill coefficient of 3.2 (1.4, 6.1) was found (Figure 2.7).

Finally, fitting a Hill-type function to yield data (Figure 2.8) resulted in a maximum yield of 3.7 (2.8, 7.6) g DW and a shoot mass at which the grain mass is half of its maximum of 22.2 (18.2, 45.2) g DW. The Hill coefficient was found to be 12.1 (1.8, 22.4).

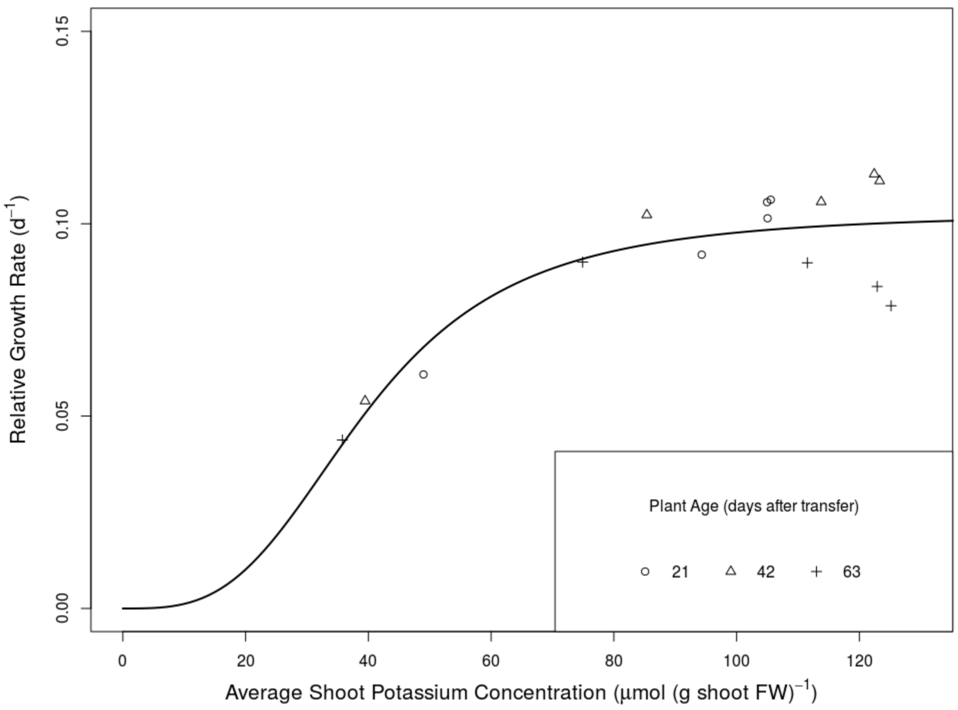


Figure 2.7: Shoot relative growth rates as a function of average shoot potassium concentrations for plants sampled after 0, 21, 42, and 63 days in hydroponics. Each point represents a value calculated using the mean mass of three plants and the curve represents a regression fit to these data using a Hill-type function. Data are from Experiments 1 with points at the sampling dates outlined in Section 2.2.1.

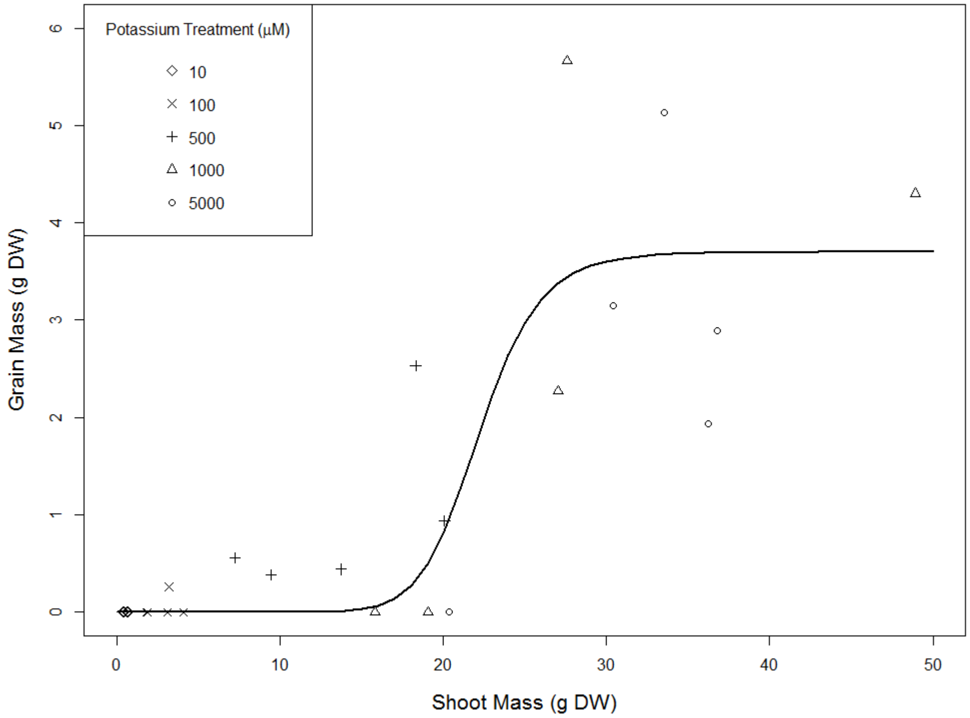


Figure 2.8: Grain mass relative to shoot mass of plants grown at five different external potassium concentrations. Each point represents the grain and shoot mass of one plant. The curve represents a non-linear regression fit to these data using a Hill-type function. Data are from Experiments 2.

2.3.2 Model Parametrisation

Parameter values used in this study are listed in Table 2.2. Maximum likelihood estimates from the non-linear regressions carried out on *IR64* growth data were used for κ, ϕ, θ, β_{root}, σ_{root}, β_{shoot}, σ_{shoot}, ν, and λ (Section 2.3.1). An important finding from the growth experiments is that the root to plant mass ratio is not stable during vegetative growth, with a notable decline relatively early in development (Figure 2.2). This is consistent with reports by White (1993) and Yoshida (1981). At the same time, plants supplied with potassium concentrations of 500 μM or greater maintained stable tissue potassium concentrations (Figure 2.3). Figures 2.5 to 2.7 suggest that net translocation from root to shoot was unaffected by plant age and that the root, but not the shoot, relative growth rate was lower early in growth. This is in keeping with the decline in root to plant mass ratio in Figure 2.2. Taken together, this information suggests that the net uptake rate of potassium per gram of root is temporally variable and Figure 2.4 supports this. Roots were relatively inefficient at taking up potassium early in growth and this is seemingly compensated for by the greater root to plant mass ratio. The uptake rate per gram of root is also lower later in the growth cycle when growth rates decline, as was found by Fageria (1976). Overall, it appears that differences in root to plant mass ratio are compensated by how efficiently each gram of root tissue can absorb potassium from the external solution, allowing tissue potassium concentrations to remain stable under adequate potassium supply.

Using the point estimates of parameters from the regression analyses (Section 2.3.1) resulted in simulated plants which failed to respect the empirical observations of constant root and shoot potassium concentrations in plants exposed to potassium-replete conditions (Figure 2.3). With the above in mind, the most parsimonious explanation for this was the observed temporal variation in potassium uptake and use which was not accounted for in regression models. In order to address this without increasing model complexity, plants were modelled as having a constant root to plant mass ratio adopting the stable value of 0.17 that was reached 21 days after transfer to hydroponics with potassium concentrations above 10 µM. Given the parameter estimates from the regression analyses and assuming stable tissue potassium concentrations of 56 and 102 µmol (g tissue FW)$^{-1}$ (Figure 2.3) as well as a relative growth rate of 0.1 (Section 2.3.1) with 1,000 µM external potassium, the maximum rate constants (μ, χ, α_{root}, and α_{shoot}) that account for the temporal variability present in rice plant development were calculated (Appendix Section 6.1.1). All of these values fall within the relevant 95% confidence intervals from the regression analyses (Section 2.3.1). The resultant set of parameter values (Table 2.2) were used in the subsequent analyses.

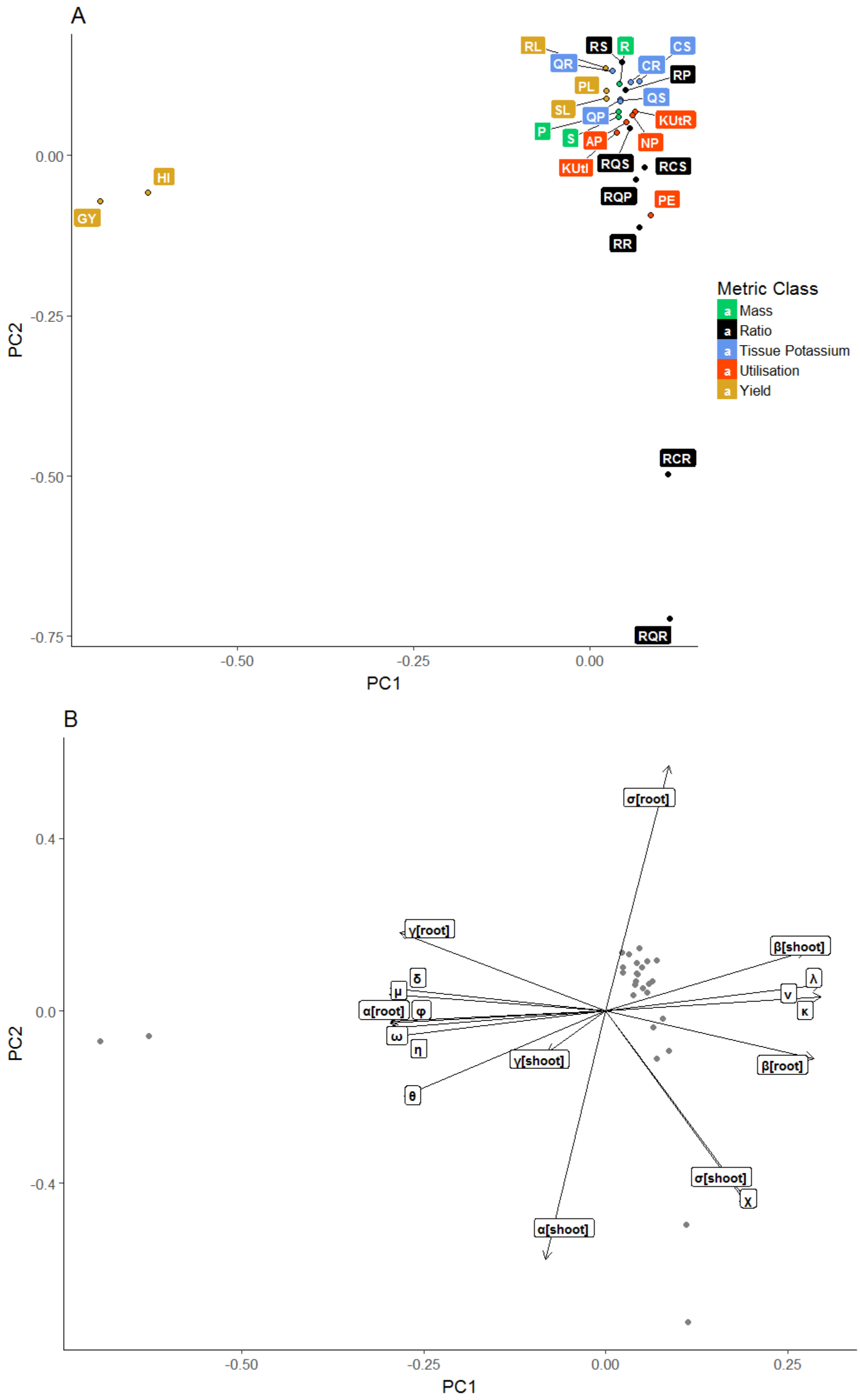
Table 2.2: Summary of model parameters used in this study.

Parameter	**Units**	**Value**
ω	g FW	0.15
δ	-	0.17
γ_{root}	µmol K^+ (g root FW)$^{-1}$	56
γ_{shoot}	µmol K^+ (g shoot FW)$^{-1}$	102
μ	µmol K^+ (g root FW)$^{-1}$ d^{-1}	58.9
κ	µM K^+	63
χ	µmol K^+ (g shoot FW)$^{-1}$ d^{-1}	11.8
ϕ	µmol K^+ (root FW)$^{-1}$	29
θ	-	2.8
α_{root}	d^{-1}	0.129
β_{root}	µmol K^+ (g root FW)$^{-1}$	16
σ_{root}	-	1.0
α_{shoot}	d^{-1}	0.105
β_{shoot}	µmol K^+ (g shoot FW)$^{-1}$	40
σ_{shoot}	-	3.2
ν	g seed DW	4
η	g shoot FW	37
λ	-	12

The regression analysis for seed yield used shoot dry mass at harvest to allow meaningful comparison across potassium treatments. The maximum likelihood mass at which seed yield was half of its highest value was found to be 22.2 g which was 65% of the mean shoot dry weight of flowering rice plants (34.4 g DW). The mean fresh weight of plants grown in the same treatments after 63 days of hydroponics was 57.66 g and so ν was set at 37 g FW.

2.3.3 Sensitivity Analysis

The largest two PCs for metric sensitivities to alterations in model parameters in simulated rice plants grown with 10 and 100 μM fixed external potassium concentrations are displayed in Figure 2.9 and 2.10. In each case, the largest two PCs explained over 85% of the variance in sensitivities of metrics to model parameters. This means that the largest two PCs sufficiently summarise the findings from the sensitivity analyses and that meaningful inferences can be made from plotting metrics PCA scores.



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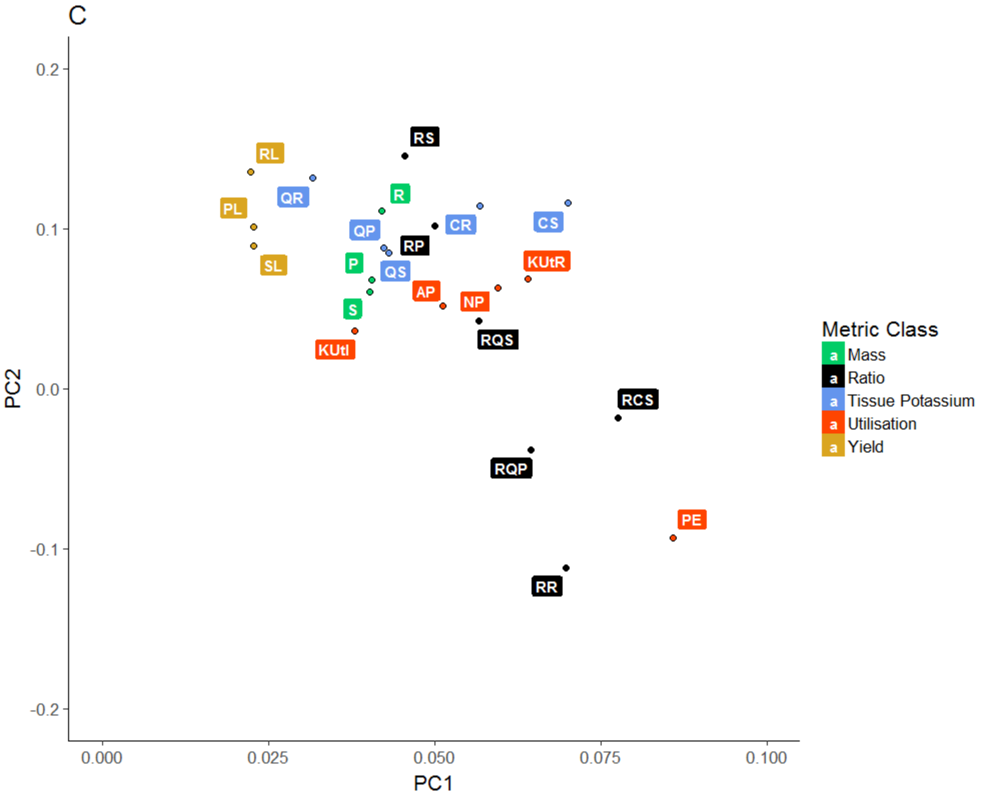
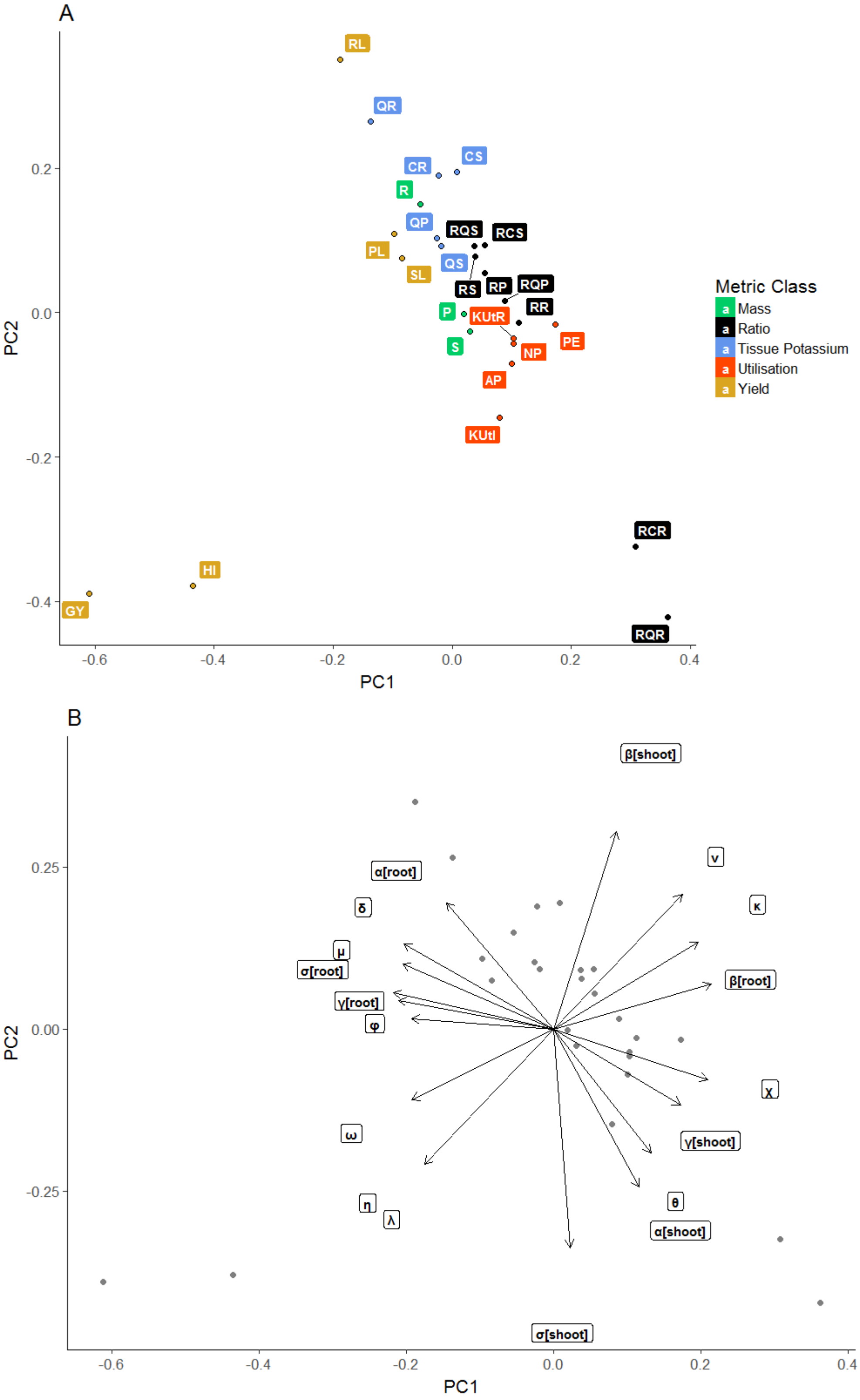


Figure 2.9: Metric scores in the largest two principal components which explain 75% (first) and 17% (second) of the variation respectively with a fixed external potassium treatment if 10 µM. Each dot represents a metric score and labels give the metric identities. Sub-plot A shows the PCA scores of all metrics used in this study for the top two principal components. B shows the loadings of model parameters superimposed on metric scores and C is the same as sub-plot A, but zoomed in on the main cluster of metrics. Metric abbreviations are as defined in Table 2.1.

Yield Metrics

Seed yield-related metrics (HI and GY) are consistently distinct in their sensitivities from the rest of the metrics in the first PC (Figures 2.9 and 2.10). Under 100 (Figure 2.10) and 950 µM (Appendix Figure 6.1) conditions, these two metrics are also different from the major cluster of KUE metrics in the second PC. Inspection of the parameter loadings (Figures 2.9B and 2.10B) reveals that the unique sensitivities of HI and GY to η, ν, and λ are important causes of this distinction. However, other model parameters are also highly relevant. In particular, β_{root}, κ, and ω are important in both the 10 and 100 µM simulations. These point to early growth vigour (ω) and the abilities to maintain root growth and potassium uptake as being key drivers of maintaining seed yield under moderate stress. Under more extreme stress, such as 10 µM external potassium (Figure 2.9B), several other model parameters become important determinants of metric scores on the first PC.

The maximum theoretical shoot RGR (α_{shooot}) has a relatively small loading on the largest principal component for both external potassium concentrations. Furthermore, the theoretical maximum net translocation rate from root to shoot (χ) either has little impact on PC1 (with 10 µM external potassium) or is negatively associated with HI and GY with 100 µM external potassium. Taken together, this suggests that maintenance of the root mass and root to shoot ratio are of primary importance under potassium stress. The parameters in Equation 2.4 are the key determinants of HI and GY being separate from the other metrics under higher potassium treatments (Appendix Figure 6.1). However, this separation only occurs in the second PC, which suggest that when potassium is non-limiting there is greater similarity in response of yield and vegetative metrics to differences in



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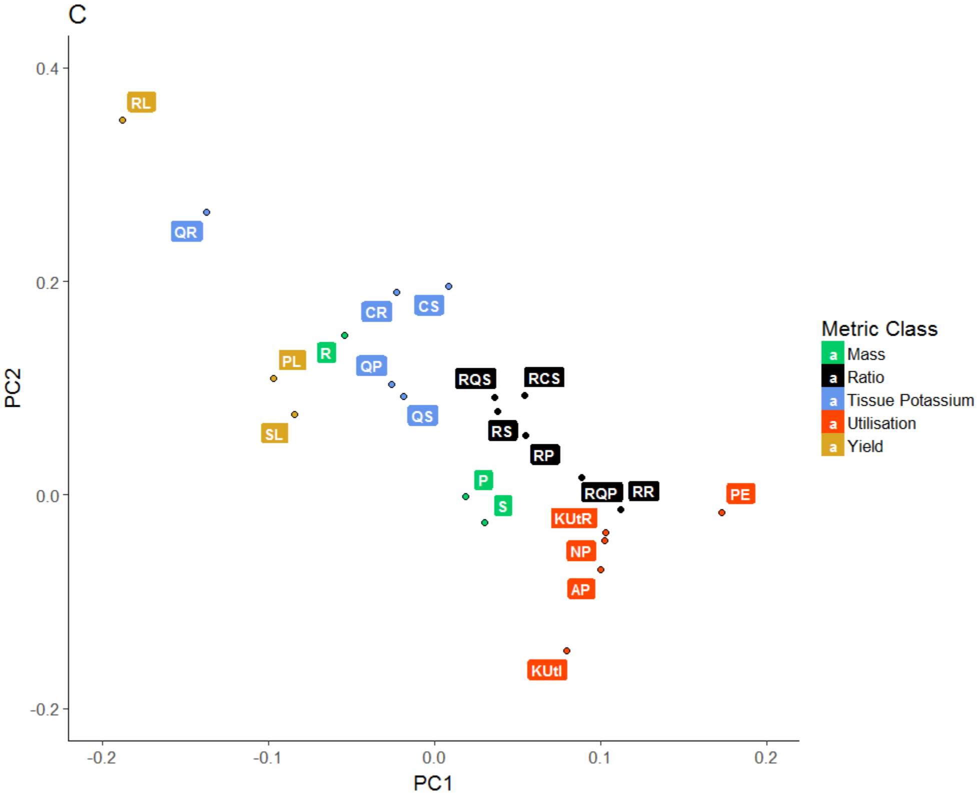


Figure 2.10: Metrics scores in the largest two principal components which explain 63% (first) and 24% (second) of the variation respectively with a fixed external potassium treatment if 100 µM. Each dot represents a metric score and boxes give the metric identities. Sub-plot A shows the PCA scores of all metrics used in this study for the top two principal components. B shows the loadings of model parameters superimposed on metric scores and C is the same as sub-plot A, but zoomed in on the main cluster of metrics. Metric abbreviations are as defined in Table 2.1.

physiological traits.

Long-term plant mass metrics had more in common with the vegetative KUE metrics in this study. In particular, the long-term root (RL), shoot (SL), and plant (PL) mass had relatively similar sensitivities to content measures after four weeks of treatment (QR, QS, and QP). Short term mass metrics (R, S, and P) showed some similarity to their long-term counterparts, especially with 10 μM external potassium. Potassium content metrics include tissue potassium concentrations as well as mass which together is more relevant to the potential future growth of a plant.

Utilisation Metrics

A relationship exists between utilisation efficiency metrics (KUtR, KUtI, AP, NP, and PE; Table 2.1) and the uptake of potassium. All of these are negatively associated with values of parameters related to greater uptake of potassium such as α_{root} and μ (Figure 2.11). This is the case because the metrics relate biomass production to tissue potassium concentration or content and limiting the uptake of potassium makes the denominator smaller in the calculation of these metrics. At the same time, these metrics are also increased through greater α_{shoot} which makes the numerator larger.

Among the utilisation efficiency metrics, PE is the least similar to the other utilisation metrics on the bi-plots (Figures 2.9A and 2.10A). PE responds in the opposite direction to the rest of the utilisation metrics for γ_{root}, γ_{shoot}, and β_{shoot}. This is due to PE comparing mass and potassium content between plants in stressed and replete conditions. These differences in responses favour plants in replete conditions that grow less well and this overrides the relationship between utilisation efficiency under potassium stress. Furthermore, PE is generally more sensitive to

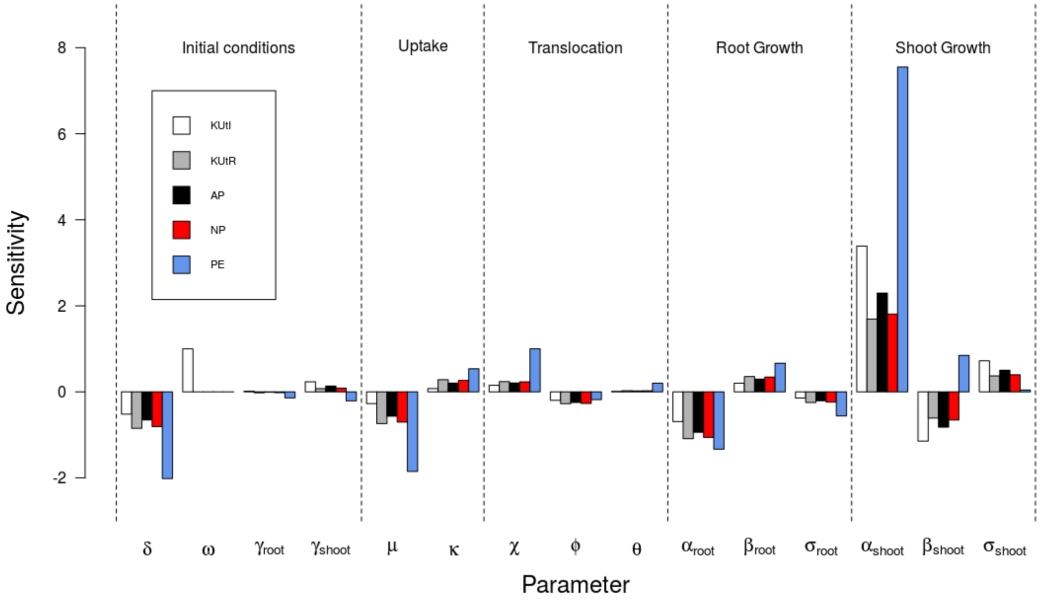


Figure 2.11: Sensitivity profiles of potassium utilisation metrics simulated in a constant 10 μM external potassium solution for 28 days. Metric abbreviations are defined in Table 2.1.

changes in model parameters than the other utilisation metrics (Figure 2.11).

KUtI is also somewhat distinct from KUtR, AP, and NP (Figure 2.11). It has a relatively high sensitivity to the initial plant mass (ω) and is also responds differently to decreasing the uptake capacity of plants. For example, the sensitivity to μ is positive while it is negative for the other utilisation metrics. For α_{root}, the sensitivity is much smaller for KUtI.

Ratio Metrics

The sensitivities of ratio metrics to changes in plant physiology are strongly influenced by the external solution potassium concentration. These metrics have values near unity when concentrations are high and respond very similarly to changes in model parameters (Appendix Figure 6.1). However, once stressed for potassium, the responses of these metrics to differences in physiology were less similar. At 10 and 100 µM external potassium concentrations, RCR and RQR become separate from the main cluster of KUE metrics. This is primarily due to sensitivities that relate to decreasing the root to shoot ratio and the movement of potassium to the shoots (*e.g.* increased α_{shoot} and χ or decreasing α_{root}).

Besides RCR and RQR, the remaining ratio metrics were grouped together at 100 µM external potassium, although RR was closer to utilisation measures than other ratios, particularly KUtR and NP. More extreme stress at 10 µM resulted in the PCA scores of this group splitting up. The shoot and plant mass ratios (RS and RP) were clustered with tissue potassium metrics while the relative shoot and plant potassium metrics (RCS, RQS, RQP) as well as RR respond to changes in plant physiology similarly to utilisation metrics. The cause of this split appears to be the different ways these two groups of ratio metric respond to alterations in the shoot growth rate (Figure 2.9B and 2.12). RCR and RQR remained distinct from the main group of metrics at both 10 and 100 µM external potassium.

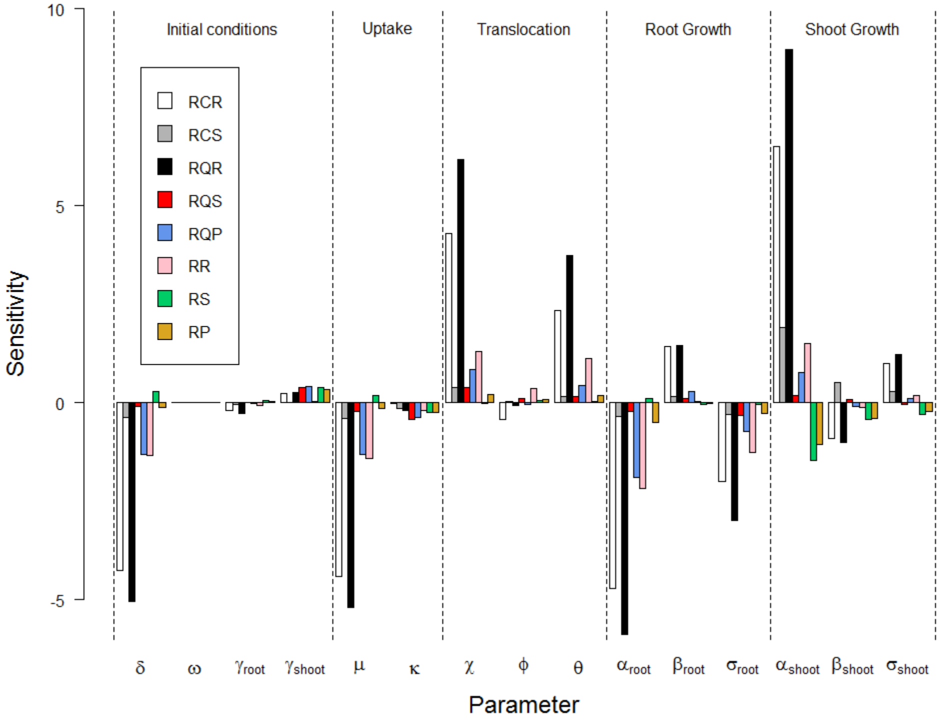


Figure 2.12: Sensitivity profiles of ratio metrics simulated in a constant 10 μM external potassium solution for 28 days.

Ratio metrics also had potentially troublesome sensitivities. Ratios of tissue potassium (RCR, RCS, RQR, RQS, and RQP) were found to have negative associations with the maximal uptake rate (μ) among other relationships that appear counterproductive if the aim is to keep tissue potassium high. Similarly, the mass ratios (RR, RS, and RP) have sensitivities which are negatively associated with strong growth of these tissues (Figure 2.12). The reason for this is similar to the link between better utilisation and worse uptake discussed above. Plants that perform worse under replete conditions lose less under stress and so can attain higher values for ratios with only moderate performance under low-potassium stress.

Tissue Potassium Metrics

The tissue potassium class of metrics mostly groups together across the potassium treatments (Figures 2.9 and 2.10). The exceptions to this are CR and QR which are separate from the major cluster of metrics once the external solution potassium concentration approaches the millimolar range (Appendix Figure 6.1). Sensitivities in this group relate strong uptake but also slower growth in shoots (Figure 2.13). Improved uptake efficiency of the root system (*e.g.* greater μ) or through a larger root mass (*e.g.* greater α_{root}) both aid the ability of a plant to source potassium from the external solution. The strong sensitivity to a larger root to plant mass ratio (δ) suggests that a larger root mass relative to the shoot mass is also beneficial to these metrics. These notions are supported by sensitivities that favour decreasing the net translocation of potassium from roots to shoots (*e.g.* smaller χ and larger ϕ). This also serves to diminish the growth of the shoot mass which can lessen the dilution of tissue potassium concentrations and also means more potassium can be held in the roots under stress rather than needing to be transported to the shoot.

The direction of responses in sensitivities is quite consistent across this class of

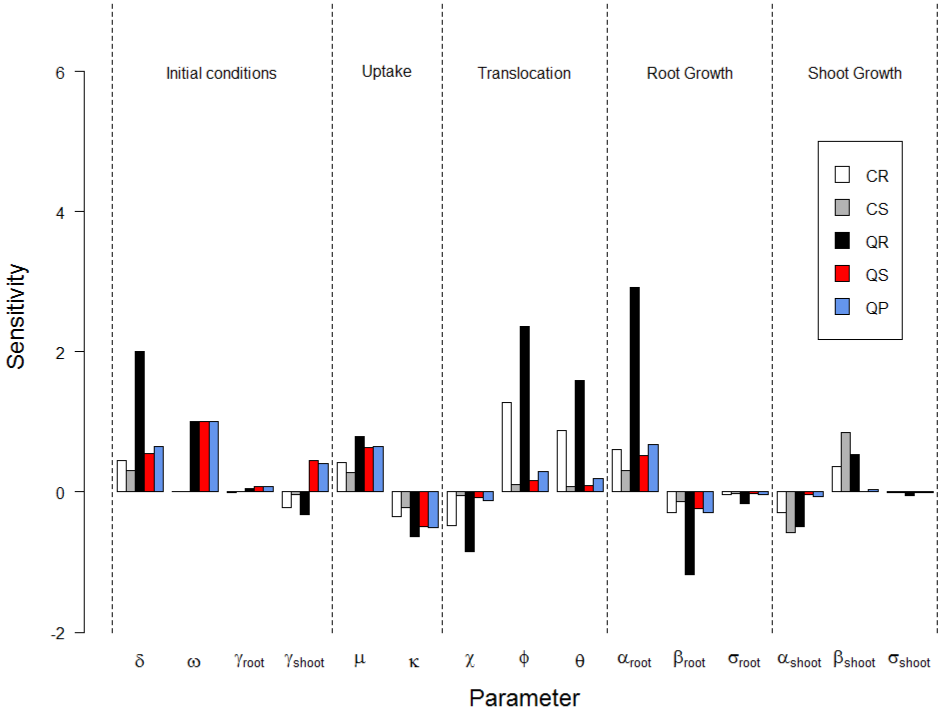


Figure 2.13: Sensitivity profiles of tissue potassium metrics simulated in a constant 10 μM external potassium solution for 28 days. Metric abbreviations are defined in Table 2.1.

metrics, although some small differences are apparent such as in γ_{shoot} and θ (Figure 2.13). There are more pronounced differences in the magnitude of responses to alternations in model constants. For example, there was a split between the concentration (CR and CS) and content (QR, QS, and QP) metrics in their responses to the initial plant mass, ω. The content metrics responded positively to a larger value of ω at which the concentrations were essentially unaffected.

Plant Mass Metrics

Short-term plant mass metrics (R, S, and P) were consistently part of the major cluster of metrics across simulated potassium treatments (Figures 2.9 and 2.10,

Appendix Figure 6.1). S and P are more similar to each other than to R which is due to the plant mass being predominantly derived from the shoot. This split is apparent on bi-plots (Figures 2.9 and 2.10) and sensitivities profiles (Figure 2.14). The root mass is aided by poorer growth of the shoots (*e.g.* lower α_{shoot} or higher β_{shoot}) while the opposite is true for the shoot and plant mass. This is because a greater shoot mass means that more potassium is required by the shoot, leaving less for the root when potassium is limited. The responses to parameters for net translocation (χ, ϕ, and θ) suggests that all three mass metrics are somewhat improved by decreasing the flow of potassium to the shoot. In those circumstances, shoots can gain potassium through having a larger root mass to source potassium and supply them and so prioritising uptake over translocation which is especially beneficial during potassium stress. Evidence in favour of this is that the sensitivities to these translocation parameters approach zero as the external potassium concentration is increased. At an external potassium concentration of 950 µM, the sensitivities of S to χ, ϕ, and θ were -0.014, -0.011, and 0.0034 respectively.

2.4 Discussion

2.4.1 Growth Experiments and Choice of Model

The finding that plant growth rates (Figure 2.1) and tissue potassium concentrations (Figure 2.3) are similar when the external potassium concentration exceeds 100 µM is consistent with several previous studies (*e.g.* Asher and Ozanne, 1967; Fageria, 1976; Spear *et al.*, 1978a). Furthermore, a marked decline in the root to shoot ratio has also been reported before (Fageria, 1976; Yoshida, 1981; White, 1993). The link between seed mass and shoot mass was also in keeping with previous studies (Abbadi *et al.*, 2008; Ma *et al.*, 2013).

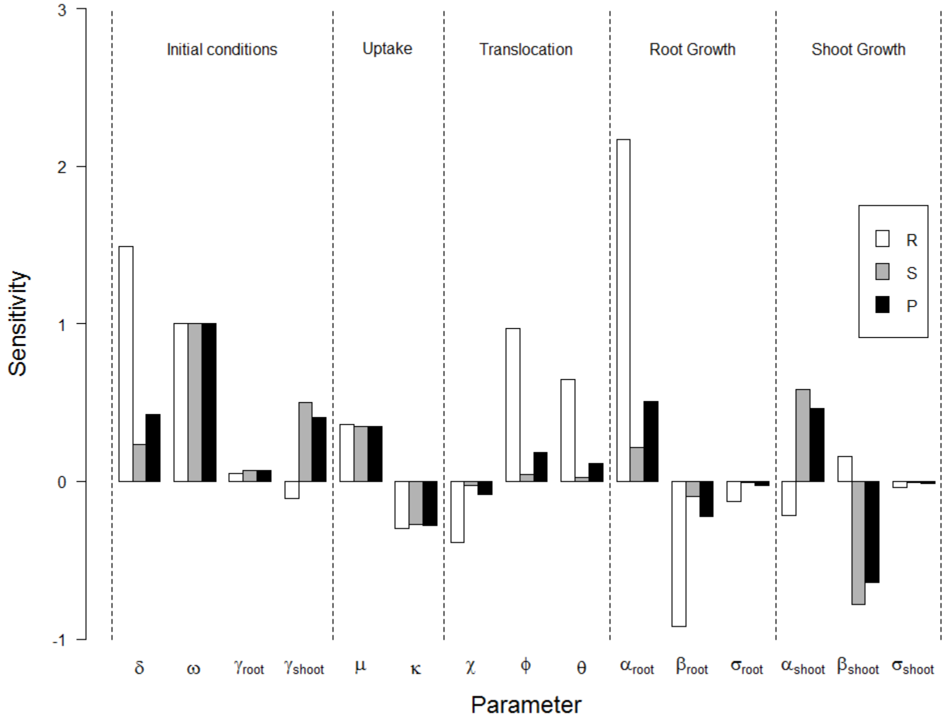


Figure 2.14: Sensitivity profiles of plant mass metrics simulated in a constant 10 μM external potassium solution for 28 days.

The uptake of potassium by plant roots has previously received some attention in modelling studies In these, saturating dynamics are typically described (Claasen *et al.*, 1986; Silberbush *et al.*, 1993; Roshani *et al.*, 2009; Samal *et al.*, 2010). Some of these models consider uptake from the soil which may require more complex models than was necessary in this study which used idealised solutions with fixed potassium concentrations. The impact of such added complexity on metric sensitivities would form an interesting future direction and it would be of great importance to know if KUE metrics respond differently in solution culture and soil. Previous experimental work (Yang *et al.*, 2003; Yang *et al.*, 2004) suggests that initial screening in hydroponics prior to field tests is a useful methodology, but may rely on specific metric selection. In this study, more complex relationships such as a Hill-type function for uptake or including the influence of internal tissue concentrations as proposed by Siddiqi and Glass (1982) did not offer a better model of the data from the growth experiments given the extra parameters required based on the Bayesian Information Criterion (BIC; Schwarz, 1978) which can be used to find parsimonious models. Figure 2.4 shows that a Michaelis-Menten-type formalism adequately described the dynamics for net uptake as a function of the external solution potassium concentration. However, there was some temporal variation apparent when comparing the uptake rate at day 42 against 21 and 63 days post-transfer to hydroponics.

The root to plant mass ratio was not constant across the treatment period (Figure 2.2) but tissue concentrations (Figure 2.3) and growth rates (Figure 2.1) were quite stable across the first 21 days post-transfer to hydroponics. This suggests that the younger plants were relatively inefficient at potassium absorption and have to invest in a relatively large root biomass (Figure 2.4).

The net uptake parameters (μ and κ) were assumed constant along with the root to plant mass ratio in this study. As a result, the apparent temporal variation in uptake (Figure 2.4) and root to plant mass ratio (Figure 2.2) were not modelled explicitly. Future work could examine the impact of these factors on metric sensitivities, but if the variation in net uptake occurs for the reasons suggested above, it is unlikely to have a large impact on results.

The translocation of potassium around a plant is a complex process and involves movement of potassium through xylem and phloem vessels (Section 1.2.3). The transport of nutrients in plants has been the subject of some prior theoretical analysis and models range from relatively simple relationships (*e.g.* Thornley, 1969 and 1972) to more complex mechanistic models (*e.g.* Hölttä *et al.*, 2009). Figure 2.5 shows that the net translocation of potassium from roots to shoots in *IR64* plants closely followed a sigmoidal relationship with the root potassium concentration. This is consistent with declines in shoot concentrations that tend to lag those in root concentrations (Asher and Ozanne, 1967; Spear *et al.*, 1978; White and Karley, 2010) but does not reveal any insights into how potassium translocation is controlled. Xylem (Jeschke *et al.*, 1992; Jeschke *et al.*, 1997; Peuke *et al.*, 2002) and phloem (Marschner *et al.*, 1996; Peuke *et al.*, 2002) fluxes have been found to be influenced by several factors including potassium availability, the presence of competing cations, and the plant status in relation to other nutrients. Nevertheless, the Hill-type function displayed in Figure 2.5 adequately summarises the partitioning of potassium between roots and shoots.

The growth of plant tissues in response to their internal potassium concentration follows a saturating relationship based on the data collected in this study (Figure 2.6 and 2.7) and others (Hommels *et al.*, 1989; White, 1993; Jordan-Meille and Pellerin, 2008). Interestingly, the data from this study point to root and shoot tissues responding differently to their internal potassium concentrations. While the maximal recorded relative growth rates appear quite similar (in the region of 0.1 g g^{-1} d^{-1}), the half-saturation points for these relationships were 16 μmol (g FW)$^{-1}$ for roots and 40 μmol (g FW)$^{-1}$ for shoots. These values are consistent with the data of White (1993) using rye and may reflect a wider phenomenon found in many plant species. Further support for this is the fact that root potassium concentrations are generally reported to be lower than the shoot potassium concentration, regardless of the external potassium treatment (Asher and Ozanne, 1967; Spear *et al.* 1978a; Ma *et al.*, 2013; Ahmad *et al.*, 2016).

Figures 2.6 and 2.7 show relative growth rates as a function of shoot and root potassium concentration respectively. In both cases the experimental data were fitted using a Hill-type function, but while the maximum likelihood Hill coefficients for roots was 1, it was 3.2 for shoots. This difference may point to a physiological difference or it could indicate that root growth rates also follow a sigmoidal relationship with the tissue concentration and that the sample size in this study was not sufficient to resolve this relationship. Figure 2.6 shows that the measured relative growth rates remained high when root potassium concentration was approximately 30 μmol (g root FW)$^{-1}$ or higher but rates rapidly declined in the presence of less than 20 μmol (g root FW)$^{-1}$. However, further data points with extremely low root potassium concentrations would help to further explore this trend.

The correlation between seed and shoot mass was best described by a sigmoidal function (Figure 2.8). This is consistent with several other reports across several species (Brohi *et al.*, 2000; Yang *et al.*, 2004; Abbadi *et al.*, 2008; Fageria *et al.*, 2013; Ma *et al.*, 2013) but a relatively high Hill coefficient was observed in this study compared to the previous literature. It was noted that the 95% confidence interval was quite wide for this parameter. The data are also quite spread out around the line of best fit and the true underlying relationship between shoot and grain mass may well decline more slowly. A further issue is experimental variability. For example, the flowering time was around 69 days in Experiment 1 and 104 days in Experiment 2. Repeating the analyses presented here with a smaller λ did not alter the results from the sensitivity analysis qualitatively (not shown).

Taken together, the data collected from *IR64* rice growth experiments is consistent with the broader literature and the relationships presented adequately describe the key dynamics of this system. It is therefore suitable to use the resultant parameters in a model of potassium use efficiency in crop plants. Furthermore, the broad agreement between experimental results in this study and others across a number of plant species suggests that the model can be applied to crops other than rice.

2.4.2 Sensitivity Analysis of Potassium Use Efficiency Metrics

Sensitivity analysis on the model developed in this study revealed how metrics previously used in the literature to study KUE inter-relate across different external potassium concentrations. In doing so, it was revealed that the classes of metric, as defined by their calculation (Table 2.1), are often meaningful and, within the same class, tend to have similar sensitivities to changes in plant physiology. Therefore, using one metric from most of these classes would be sufficient to fully explore KUE, a notion that agrees well with the previous literature (Baligar *et al.*, 2001; Good *et al.*, 2004; White, 2013). Indeed, the commonly reported utilisation metrics had quite similar sensitivities to altered physiological processes. However, exceptions to this existed. PE was the most distinct from the other utilisation metrics, but these differences are relatively small compared to differences between classes. The ratio group showed strong differences in sensitivities and these were not constant across potassium treatments (Figures 2.9 and 2.10).

The ratio metrics have highly similar sensitivities at relatively high external potassium concentrations (Appendix Figure 6.1) but metrics within this class become increasingly distinct as potassium becomes more limiting (Figure 2.9 and 2.10 and Section 2.3.3: Ratio Metrics). Ratios of root potassium concentration and content were distinct from both the other ratios and from other metrics. The relative shoot and plant mass after 28 days of treatment was somewhat distinct from ratios of shoot potassium concentrations (RCS) as well as the relative root mass (RR) at 10 μM external potassium, but not at 100 μM. As screen studies often use nutrient solutions which have potassium concentrations up to 100 μM for a stress treatment

(Yang *et al.*, 2003; Fan *et al.*, 2013; Ju *et al.*, 2014), the use of three metrics such as RP, RCS, and RCR would cover the diversity of the ratio metrics observed in this study (Figure 2.9A).

This study also highlighted potential weaknesses in metrics. Plants with worse maximal uptake rates per gram of root per day (μ) scored better for most utilisation (Figure 2.11) and ratio metrics (Figure 2.12). This negative association between uptake and utilisation is in agreement with empirical results (White *et al.*, 2010; Chapter 3) and reflects how utilisation and uptake can conflict. More generally, scores for ratio metrics were improved when parameters were changed such that plants performed poorly under potassium replete conditions (*e.g.* lower α_{root} for most of the ratios and α_{shoot} for RS and RP). Such sensitivities have important implications for screening studies since genotypes that are found to be highly efficient do not necessarily show growth and yield traits that are agriculturally desirable.

Undesirable associations between metrics and physiology can potentially be ameliorated. Inclusion of physiologically relevant measures as co-factors in statistical analyses could be used to control for unwanted associations. Some of the parameters in this model are relatively simple to measure experimentally, such as the initial plant mass or root to shoot ratio, while others can be estimated. The amount of potassium a plant contains divided by the root mass under potassium replete conditions could be used as a proxy of the maximal uptake rate, for example. Other parameters would be more challenging to measure, such as the separate determination of relative growth rates of roots and shoots. While a whole plant relative growth rate can be calculated, some metrics respond oppositely to increases in root and shoot relative growth rates (*e.g.* Figures 2.11, 2.12, and 2.14).

It was noted that KUtI responded differently to alterations in potassium uptake parameters (μ and κ) compared to the other utilisation metrics. This could be a reason to use KUtI because it would appear to not select for poor uptake. However, KUtI is sensitive to differences in the initial shoot potassium concentration (γ_{shoot}) and plant mass (ω). Depending on the study, these two parameters could be used as co-variates with KUtI in statistical analyses, but collecting the necessary data may be intractable in large scale screens. Furthermore, of the utilisation metrics, AP, NP and KUtR grouped closely and these represent the most reliable utilisation metrics found by Moriconi and Santa-Marìa (2013).

Previous studies have found conflicting evidence for a link between vegetative metrics used in KUE studies and yield (Woodend and Glass 1993; George *et al.*, 2002; Yang *et al.*, 2003; Damon and Rengel *et al.*, 2007). This study shows that grain yield-related metrics are distinct from vegetative metrics and this is partly due to how the production of seeds responds to potassium stress (*i.e.* parameters in Equation 2.4), much as suggested by Damon and Rengel (2008). However, under more extreme potassium stress (cf. Figures 2.9 and 2.10, B sub-plots), the influence of other physiological processes become relatively more important. This points to a relevance for both strong uptake and high utilisation of potassium. In the context of this study, there was little similarity between vegetative metrics and HI and GY on the first principal component. In the second PC, ratios of shoot potassium concentration and content as well as plant content were most similar in 10 μM conditions (Figure 2.9A). With an external potassium concentration of 100 μM potassium, ratios of root potassium concentration and content were most similar to seed yield-related metrics (Figure 2.10A). This suggests a difference in optimal strategy where, depending on the severity of potassium stress, potassium is directed towards the shoot unless it is extremely limited, at which point preserving root potassium levels is more beneficial.

In future modelling of plant responses to potassium stress and measuring KUE, the relevance of metrics for experimental design could be explored. For example, studies where plants are starved of a nutrient have been recommended to measure utilisation without a distorting effect from uptake (Rose *et al.*, 2011; Santa-Marìa *et al.*, 2015) and this study has shown that metric sensitivities can change in response to potassium supply. The length of time of a study is also potentially important and a cursory analysis of this using the methods outlined above found that little change in metric sensitivities occurred as long as an experiment lasted long enough for tissue potassium concentrations to decline (*i.e.* a few days at least). One notable exception was the relationship between long-term mass (RL, SL, and PL) with their short term counterparts and potassium contents. R, S, and P became increasingly reliable predictors of RL, SL and PL respectively and outperformed QR, QS, and QP as the treatment time approached the flowering time. One interesting avenue for research would be to examine how altering several physiological parameters at once influences sensitivities and plant performance. An exploratory analysis of this using the model presented in this study and a Markov chain Monte Carlo technique found that metrics tended towards imposed extremes in the same directions as found in sensitivity profiles (Figures 2.11 to 2.14) reported here. Furthermore, there is a potential to define new metrics which may be useful in screening KUE. Geometric means of metrics used in this study are one potential option. This study used changes of ± 10% of parameter values to explore the sensitivities of metrics as responses were approximately linear in this range. Large changes can elicit non-linear relationships between metrics and their responses to changes in model parameters which are not adequately captured by Equation 2.5. Exploration of the impacts of larger changes to plant physiology may prove to be a profitable way to further explore how KUE can best be optimised beyond what has been shown in this work.

2.4.3 Conclusions

In summary, this study used a simplified model for the uptake and distribution of potassium in plants parametrised based on growth experiments in IR64 rice. The sensitivities of metrics to changes in the external potassium concentration and a range of physiological processes were determined and in doing so, key physiological influences were revealed. Furthermore, principal components analysis showed how various metrics compared with each other and the links between vegetative metrics and yield were explored and revealed how suitable metrics can change in response to external potassium availability. Based on these outcomes, a more informed selection of metrics can be made which more fully addresses the complexity of KUE in screening studies.

Chapter 3

Characterisation of Potassium Use Efficiency in Rice (*Oryza sativa* L.) and its Underlying Physiology

3.1 Introduction

Rice is a major crop with approximately 500 million tonnes of milled grain produced each year from over 160 million hectares of agricultural land worldwide (FAO, 2015). It is considered a crucial food source for over three billion people (Ali *et al.*, 2011; McCouch *et al.*, 2016) because rice supplies a large proportion of calories consumed in many countries, including over half of those consumed in Bangladesh, Cambodia, and Vietnam (IRRI, 2017). At the same time, agricultural soils around the world are increasingly subject to widespread potassium deficiency. For example, 75% of Chinese rice paddies are believed to suffer potassium exhaustion (Römheld and Kirkby, 2010) and potassium is considered to be the most limiting nutrient in rice production (Yang *et al.*, 2005). A lack of potassium can result in lost yield. For example, 26% of yield was lost with insufficient potassium fertilisation in maize (Dai *et al.*, 2013). Off-take of potassium that was not balanced by intakes has caused soil potassium levels to deplete (Dobermann *et al.*, 1998; Hoa *et al.*, 2006; Pathak *et al.*, 2010).

To meet future food demand in the face of a growing global population, it is imperative that gains in agricultural output are secured. While potash is not a limited resource, and will without doubt help to raise and maintain soil potassium levels, the uneven distribution of its production between the Northern and Southern hemispheres (USGS, 2016) is an issue due to cost and environmental impacts of transport. Furthermore, deep-leached soils are often found in the Southern hemisphere which can limit the effectiveness of potash fertilisers (Leonardos *et al.*, 2000). Another risk factor is price spikes similar to the one observed between 2008 and 2010, when the price of potassium chloride more than quadrupled from under US$ 200 in June 2007 to over US$ 870 in February 2009 (World Bank, 2017). Furthermore, the transport, mining, and application of potash fertilisers can have negative environmental consequences (Ciceri *et al.*, 2015; Sardans and Peñuelas, 2015). Taken together, these issues may limit the effectiveness of increased potassium fertilisation.

A complementary approach is to improve the potassium use efficiency (KUE) of crops so that they require lower potash inputs but still yield adequately (see Chapter 1 for a discussion of KUE definitions). Evidence exists for variation in KUE within and across plant species (*e.g.* Trehan *et al.*, 2005; Damon *et al.*, 2007; Fan *et al.*, 2013; Fageria and Melo, 2014), suggesting that natural variation may be exploited in the production of new elite cultivars. Links between KUE and physiological traits that can improve it have been proposed. For example, improved uptake capacity could be achieved through increasing the root surface area with root hairs (Høgh-Jensen and Pedersen, 2003), greater root length (Samal *et al.*, 2010, or mobilisation of soil potassium using root exudates (Liebersbach *et al.*, 2004; Trehan *et al.*, 2005). Better utilisation of potassium within plant tissues could be achieved through more efficient distribution at the tissue and cellular level, replacement of potassium in biophysical processes with other cations and osmotica, and maintenance of key physiological processes such as water use and photosynthesis during potassium stress (see reviews by Rengel and Damon, 2008; White, 2013; Wang and Wu, 2015).

In rice, evidence exists for considerable variation in KUE among cultivars. Yang *et al.* (2003) found that grain utilisation efficiency (the ratio of grain mass to potassium content, g DW (mg $K^+)^{-1}$) was approximately 40 % lower in inefficient varieties when compared to the most efficient, while seven-fold variation in grain yield efficiency index was described by Fageria *et al.* (2013). Similar levels of variation in potassium uptake rate have been reported by Liu *et al.* (2009). Potassium utilisation by shoots has been found to have 1.5-, and 6-fold variation in *indica* (Liu *et al.*, 2009) and lowland (Yang *et al.*, 2003) rice cultivars respectively. Greater utilisation efficiency at the seedlings stage was found to correlate positively with grain utilisation efficiency and efficient genotypes were found to be better able to maintain their tillering, photosynthetic, and grain filling rates (Yang *et al.*, 2004). Such diversity could be exploited in breeding programmes to improve crop KUE.

In this study, KUE in rice genotypes from all major sub-populations was characterised for the first time for cultivars from the Rice Diversity Panel 1 (RDP1; Zhao *et al.*, 2011; Eizenga *et al.*, 2014). This panel is made up of cultivars and landraces from the five rice subpopulations (*aus*, *indica*, *temperate japonica*, *tropical japonica*, and *aromatic*) as well as admixtures (Eizenga *et al.*, 2014). KUE was assessed using six metrics which capture different aspects of the plant response to low potassium stress based on a mathematical analysis of KUE measurement (Chapter 2). Metrics include plant mass, potassium utilisation, and the amount of potassium a plant contains after four weeks of low-potassium conditions as well as ratios of plant mass and tissue potassium concentrations under low and replete potassium conditions. Evidence for differences in KUE at the genotype and sub-population levels was found. The links between these differences and plant physiological parameters were also explored and revealed key drivers of variation in rice KUE.

3.2 Methods

3.2.1 Plant Growth

Five seeds from each of 324 cultivars (see Supplementary Information file "riceGenotypes.xlsx") were germinated in sand flooded with deionised water. Seedlings were grown for two weeks prior to transfer to hydroponics. The two most visually similar seedlings were weighed before placement in 335 x 210 x 140 mm black plastic boxes filled with 9 litres of an adapted Yoshida nutrient solution (Yoshida *et al.*, 1976) as described in Section 2.2.1. Potassium was added as KCl and the solution had an initial pH of 5.5. One seedling from each cultivar was placed in a solution with either 0.1 mM potassium ('stress treatment') or 1 mM initial potassium concentration. Each box held fifty-four plants arranged according to an alpha lattice design (Patterson and Williams, 1976; Patterson *et al.*, 1978). The hydroponic solution was changed weekly and the plants were grown for four weeks in a glasshouse with 12 hour day and night periods with temperatures of 32 and 28 °C in the day and night respectively and a relative humidity between 50 and 60%. Growth trials were repeated five times.

3.2.2 Trait Measurements

At the end of a four week treatment period, plants were separated into roots and shoots and weighed. Plant tissues were then dried at 80 °C for 72 hours in a fan oven and re-weighed.

Root and shoot potassium and sodium concentrations were measured for each plant by suspending dried tissue sub-samples in 20 mM $CaCl_2$ for twenty-four hours. The relevant cation concentrations of the resultant solutions were measured using a flame photometer (Sherwood Scientific, Cambridge, UK).

3.2.3 Trait Calculations

Six KUE metrics were used in this study and are outlined in Table 3.1. These were all calculated using data from the growth experiments outlined above. Physiological traits for use in regression analyses were also calculated from experimental measurements and are outlined in Table 3.2.

3.2.4 Statistical Analyses

Genotypes with fewer than three measurements for a given trait were excluded from analyses. Associations between the cultivar and traits were tested by using one-way multivariate analysis of variance (MANOVA) and analysis of variance (ANOVA) using the *Anova* function from the **car** R package (Fox and Weisburg, 2011). When the data failed to meet the assumptions of the tests, appropriate Box-Cox power transformations were calculated using the *boxcox()* function from the **MASS** R package (Venables and Ripley, 2002). Where values for traits are presented, the values have been back-transformed to the original scale. Post-hoc testing was carried out using *t*-tests to identify statistically significant differences between the individual cultivar means and the grand mean of all genotypes. Multiple testing was accounted for using a 5% false discovery rate (Benjamini and Hochberg, 1995).

Associations between KUE metrics and physiological traits were identified using best subsets regression with the *regsubsets* command in the **leaps** R package (Lumley, 2017). The physiological traits are listed in Table 3.2. The most parsimonious

Table 3.1: Summary of potassium use efficiency metrics used in this study.

Metric	**Abbreviation**	**Calculation**	**Units**
Plant dry mass	P	W_{plant}	g DW
Potassium utilisation ratio	KUtR	$1 / \mathrm{KS}_{LT}$	g shoot DW (µmol shoot $\mathrm{K}^{+})^{-1}$
Plant potassium content	QP	$(\mathrm{KR}_{LT}\ \mathrm{W}_{root,LT}) + (\mathrm{KS}_{LT}\ \mathrm{W}_{shoot,LT})$	µmol plant K^{+}
Relative plant mass	RP	$\mathrm{W}_{LT} / \mathrm{W}_{HT}$	-
Relative root potassium concentration	RCR	$\mathrm{KR}_{LT} / \mathrm{KR}_{HT}$	-
Relative shoot potassium concentration	RCS	$\mathrm{KS}_{LT} / \mathrm{KS}_{HT}$	-

DW stands for dry weight, *KS* and *KR* are the shoot and root potassium concentrations respectively, *LT* stands for the low potassium treatment (0.1 mM), *HT* is the high potassium treatment (1 mM), *W* is the mass.

model was selected using the Bayesian information criterion (BIC; Schwarz, 1978). Where necessary, values used to calculate the KUE metric were removed from each analysis to avoid redundant correlations. For example, KUtR (Table 3.1) was calculated using the shoot potassium concentration obtained in the potassium stress treatment and therefore this parameter would not be included as a potential predictor in the multiple regression analysis of KUtR. The relative importance of each predictor in the multiple regression model with the lowest BIC was assessed by decomposing the model coefficient of determination (R^2) using the *booteval.relimp()* function from the **relaimpo** R package (Grömping, 2006). Relative importance of predictors is presented as a percentage of the model R^2 (Tables 3.3 to 3.8). Adjusted R^2 values are also reported. When these two values are similar, it suggests that there is little redundancy in a model (*i.e.* explanatory power would be lost by removing any of the predictors from the model).

All calculations and analyses were carried out using R 3.3.3 (R Core Team, 2017).

Table 3.2: Summary of physiological traits used in multiple regression analyses.

Trait	**Units**	**Calculation**
Initial plant fresh weight, W_0	g plant FW	-
Low treatment root potassium concentration, KR_{LT}	µmol K^+ (g root DW)$^{-1}$	-
High treatment root potassium concentration, KR_{HT}	µmol K^+ (g root DW)$^{-1}$	-
Low treatment shoot potassium concentration, KS_{LT}	µmol K^+ (g shoot DW)$^{-1}$	-

High treatment shoot potassium concentration, KS$_{HT}$	µmol K^{+} (g shoot DW)$^{-1}$	-
Low treatment root sodium concentration, NaR$_{LT}$	µmol Na^{+} (g root DW)$^{-1}$	-
High treatment root sodium concentration, NaR$_{HT}$	µmol Na^{+} (g root DW)$^{-1}$	-
Low treatment shoot sodium concentration, NaS$_{LT}$	µmol Na^{+} (g shoot DW)$^{-1}$	-
High treatment shoot sodium concentration, NaS$_{HT}$	µmol Na^{+} (g shoot DW)$^{-1}$	-
Low treatment potassium uptake per gram of root, U$_{LT}$	µmol K^{+} (g root DW)$^{-1}$	Plant potassium content / DW
High treatment potassium uptake per gram of root, U$_{HT}$	µmol K^{+} (g root DW)$^{-1}$	Plant potassium content / root DW
Low treatment potassium distribution index, KDI$_{LT}$	-	shoot potassium content / root potassium content
High treatment potassium distribution index, KDI$_{HT}$	-	shoot potassium content / root potassium content
Low treatment root to shoot ratio, RSR$_{LT}$	-	Root DW / shoot DW

High treatment root to shoot ratio, RSR_{HT}	-	Root DW / shoot DW
Low treatment relative growth rate, RGR_{LT}	d^{-1}	$(\ln(\mathrm{W}_{final})-\ln(\mathrm{W}_0))/28$
High treatment relative growth rate, RGR_{HT}	d^{-1}	$(\ln(\mathrm{W}_{final})-\ln(\mathrm{W}_0))/28$
Low treatment fresh weight to dry weight ratio, FWDW_{LT}	-	plant FW / plant DW
High treatment fresh weight to dry weight ratio, FWDW_{HT}	-	plant FW / plant DW

3.3 Results

3.3.1 Growth and Cation Concentrations

The solution potassium concentration had a large impact on the growth of rice plants as well as on the tissue potassium and sodium concentrations. With respect to growth, the initial plant fresh weight on transfer to hydroponics did not have a statistically significant difference between treatments (Paired *t*-test, t_{633} = -0.33, P = 0.74). However, the final mass of plants in the 0.1 mM potassium treatment was on average 60% of the mass of plants in the 1 mM treatment (Paired *t*-test, t_{517} = -17.73, $P < 2.2 \times 10^{-16}$; Figure 3.1). The root to shoot mass ratio after four weeks in treatment was statistically significantly higher in the 0.1 mM potassium treatment (Paired *t*-test, t_{631} = 3.16, P = 0.0017), although this difference was

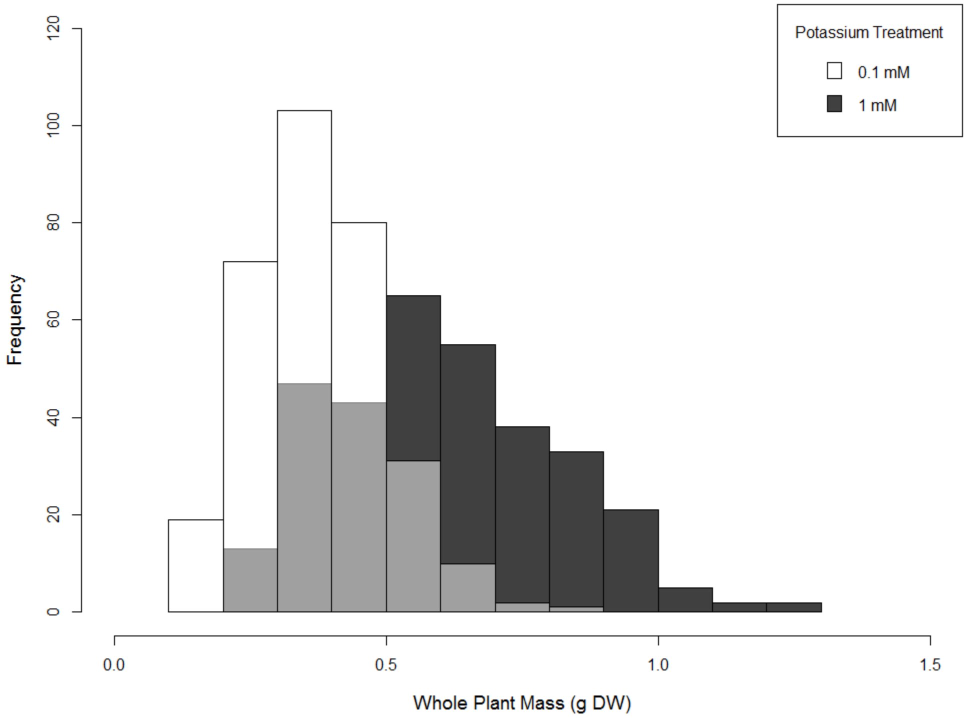


Figure 3.1: Least squares means for whole plant dry mas of 318 rice genotypes grown in nutrient solutions with initial potassium concentrations of 0.1 or 1 mM.

small with 0.1 mM potassium plants having an average root to shoot ratio of 0.20 compared 0.19 for those in the 1 mM treatment.

Tissue potassium concentrations were lower when plants were grown in a 0.1 mM external potassium concentration compared to 1 mM potassium nutrient solutions. Root concentrations were 70% lower on average in the 0.1 mM potassium treatment and this was statistically significantly different (Paired t-test, t_{347} = -35.83, P =<2.2 x 10^{-16}; Figure 3.2A). Shoots exhibited a greater decline in potassium concentration across the two treatments, with plants in the lower treatment found to have only 23% of the potassium concentration found in high treatment plants (Paired t-test, t_{360} = -68.75, P =<2.2 x 10^{-16}; Figure 3.2B).

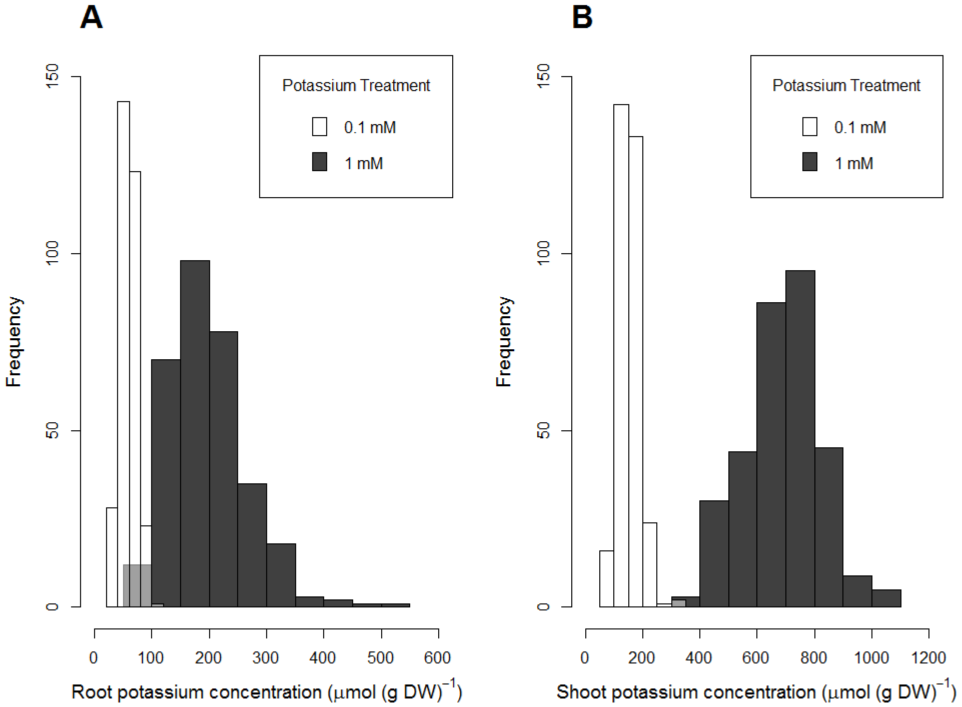


Figure 3.2: Least squares means for root (A) and shoot (B) potassium concentrations of 318 rice genotypes grown in nutrient solutions with initial potassium concentrations of 0.1 or 1 mM.

Conversely, tissue sodium concentrations were increased when plants were cultivated in the lower external solution potassium concentration. Root sodium concentrations were on average 1.9 times greater in 0.1 mM potassium solutions compared to plants given 1 mM potassium (Paired t-test, t_{479} = 22.26, P =<2.2 x 10^{-16}; Figure 3.3A). This effect was considerably larger in shoots where a 7.1 times greater average sodium concentration was recorded in the low potassium treatment compared to the 1 mM external potassium treatment (Paired t-test, t_{328} = 33.62, P =<2.2 x 10^{-16}; Figure 3.3B). However, the change in tissue sodium was not uniform across genotypes. Dividing the root sodium concentrations under low and high potassium supplies for each genotype revealed that 16 genotypes actually had a lower root sodium concentration in the 0.1 mM potassium treatment compared to the 1 mM treatment.

3.3.2 Genotypic Variation in Rice Potassium Use Efficiency

Genotype was statistically significantly associated with KUE (MANOVA, $\Lambda_{316,1896}$ = 0.06, P <2.2 x 10^{-16}) and the distributions of least squares means for each metric are displayed in Figure 3.4. P, KUtR, and QP showed 6.6-, 4.6-, and 14-fold variation respectively between the largest and smallest values. For the three ratio metrics, 3.4-, 19.3-, and 7.6-fold variation was observed for RP, RCR, and RCS respectively. Statistically significant correlations were found in pairwise comparisons between several metrics (Figure 3.5). Most of these correlations were relatively weak (r <0.3), but a strong positive correlation was observed between P and QP. Moderately strong negative correlations existed between KUtR and RCS as well as KUtR and QP.

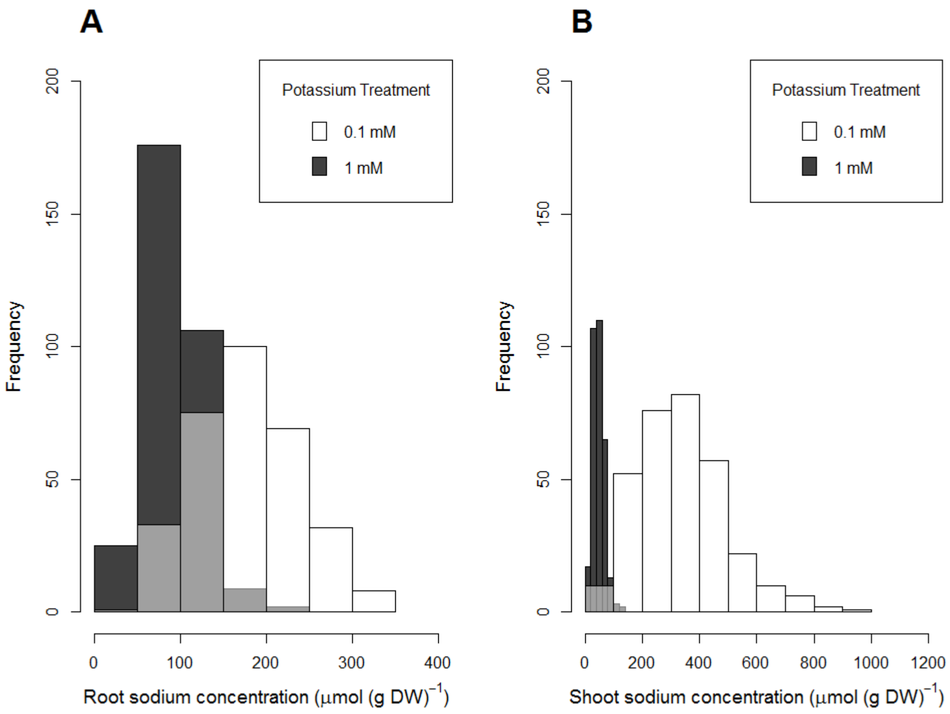


Figure 3.3: Least squares means for root (A) and shoot (B) sodium concentrations of 318 rice genotypes grown in nutrient solutions with initial potassium concentrations of 0.1 or 1 mM.

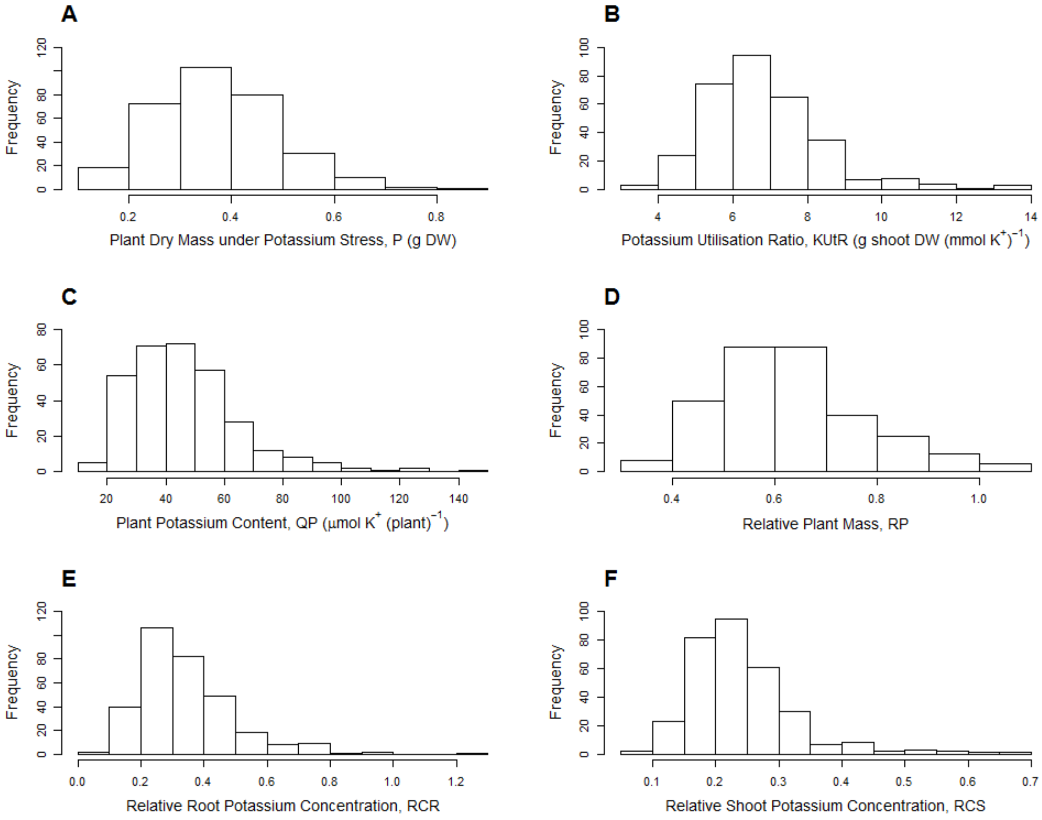
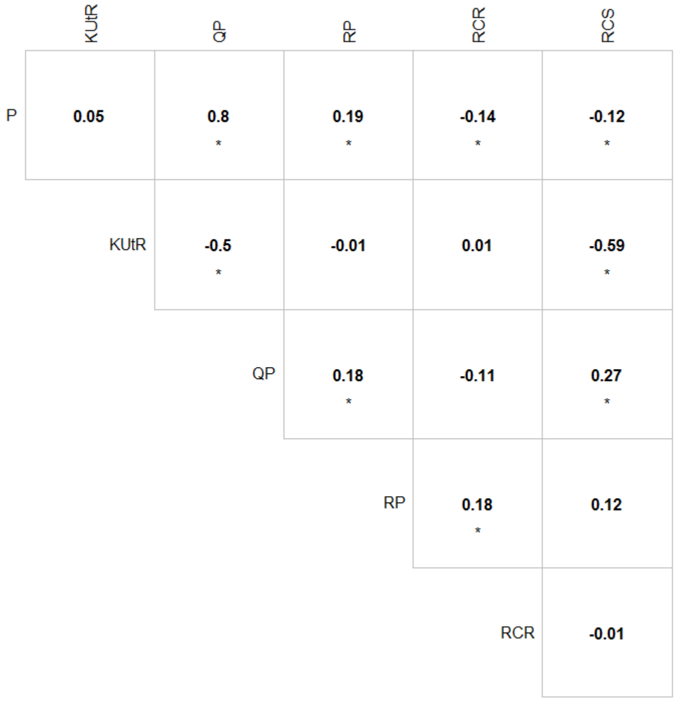


Figure 3.4: Summary of least squares means for each of 318 rice genotypes for six measures of potassium use efficiency. Genotype was a statistically significant predictor of: (A) the plant dry weight under potassium stress, P (ANOVA, $F_{317,1198} = 5.1$, $P < 2.2 \times 10^{-16}$); (B) the potassium utilisation ratio, KUtR (ANOVA, $F_{317,1197} = 1.5$, $P = 7.8 \times 10^{-6}$); (C) the plant potassium content, QP (ANOVA, $F_{317,1196} = 2.8$, $P < 2.2 \times 10^{-16}$); (D) the relative plant dry mass, RP (ANOVA, $F_{317,1170} = 1.5$, $P = 1.0 \times 10^{-6}$); (E) the relative root potassium concentration, RCR (ANOVA, $F_{317,1155} = 1.4$, $P = 2.4 \times 10^{-5}$); (F) the relative shoot potassium concentration, RCS (ANOVA, $F_{316,1153} = 1.3$, $P = 7.1 \times 10^{-4}$). All six tests had a false discovery rate <5%.



	KUtR	QP	RP	RCR	RCS
P	**0.05**	**0.8** *	**0.19** *	**-0.14** *	**-0.12** *
KUtR		**-0.5** *	**-0.01**	**0.01**	**-0.59** *
QP			**0.18** *	**-0.11**	**0.27** *
RP				**0.18** *	**0.12**
RCR					**-0.01**

Figure 3.5: Pairwise Pearson's correlation coefficients (r) between potassium use efficiency metrics. Asterisks denote statistically significant correlations with a false discovery rate <5%.

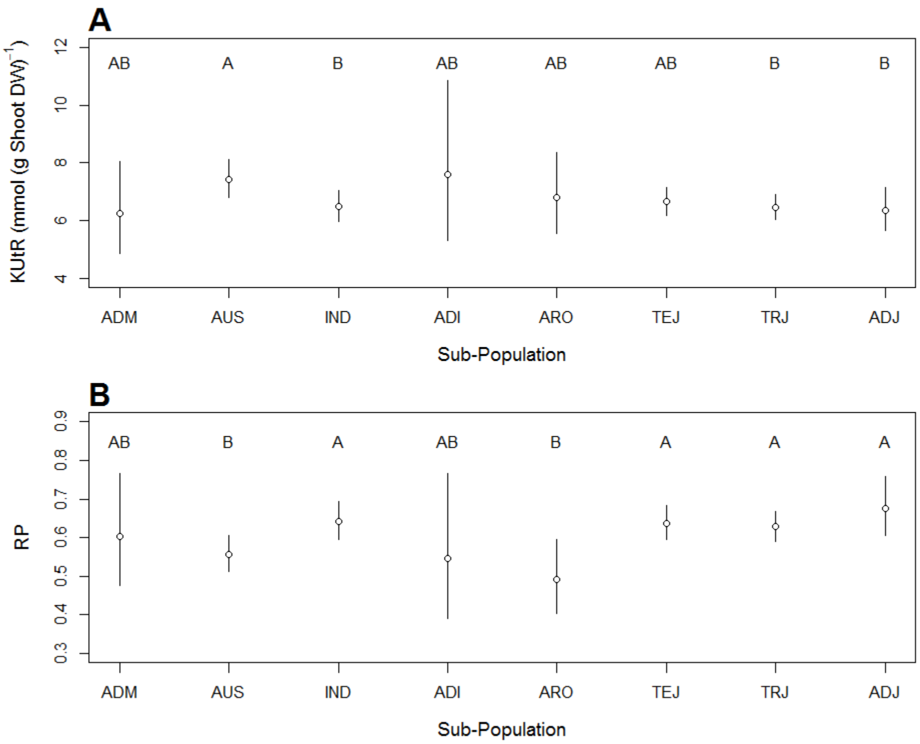


Figure 3.6: Least squares means for the potassium utilisation ratio (KUtR, A) and the relative plant dry mass (RP, B) for each rice sub-population. Letters denote statistically significant differences between sub-populations at a 5% false discovery rate. Sub-population abbreviations are as follows: ADM for *admixed*, AUS for *aus*, IND for *indica*, ADI for *admixed indica*, ARO for *aromatic*, TEJ for *temperate japonica*, TRJ for *tropical japonica*, and ADJ for *admixed japonica*. Error bars are the 95% confidence interval of the mean.

The sub-population membership was also statistically significantly associated with KUE metrics (MANOVA, $\Lambda_{7,1368} = 0.74$, $P = 2.91 \text{ x } 10^{-5}$). However, this was not the case for all KUE metrics and sub-population was a statistically significant factor for KUtR (ANOVA, $F_{7,297} = 2.4$, $P = 0.02$) and RP (ANOVA, $F_{7,297} = 2.02$, $P = 7.0 \text{ x } 10^{-5}$) only (Figure 3.6).

Table 3.3: Summary of the lowest BIC model from a best subsets regression analysis for the whole plant dry mass (P) for plants grown in nutrient solutions with a 0.1 mM initial potassium concentration. The bootstrapped 95% confidence intervals for the percentage of variation in P explained by each predictor are listed in parentheses. The model R^2 and R^2 adjusted were 0.73.

Predictor	**Coefficient**	P	**% Variance Explained**
High treatment relative growth rate, RGR_{HT}	6.5	$<2.2 \times 10^{-16}$	39.6 (33.6, 44.9)
Initial plant mass, W_0	2.1	$<2.2 \times 10^{-16}$	28.2 (23.4, 33.2)
Low treatment shoot sodium concentration, NaS_{LT}	0.00016	1.4×10^{-10}	3.4 (1.4, 6.2)
Low treatment fresh weight to dry weight ratio, $FWDW_{LT}$	-0.034	5.8×10^{-6}	2.1 (1.3, 3.4)

3.3.3 Links Between Potassium Use Efficiency and Physiology

Regression models which most parsimoniously described associations between the six metrics used in this study and plant physiology based on BIC are summarised in Tables 3.3 to 3.8. For P, the high treatment relative growth rate (RGR_{HT}) was the most important predictor (Table 3.3) while the initial plant mass (W_0) explained approximately 30% of the variation in P. Smaller proportions of the model R^2 were attributed to the shoot sodium concentration (NaS_{LT}) and the fresh weight to dry weight ratio ($FWDW_{LT}$) in the 0.1 mM potassium concentration nutrient solutions. All associations were positive except for $FWDW_{LT}$. The low potassium treatment relative growth rate (RGR_{LT}) was not included in this analysis as P was used in its calculation and a strong positive correlation exists between RGR_{LT} and P (Appendix Figure 6.2).

The most important correlation of the potassium utilisation ratio (KUtR) was with the potassium uptake per gram of root in the low potassium treatment (U_{LT}) which

Table 3.4: Summary of the lowest BIC model from a best subsets regression analysis for the potassium utilisation ratio (KUtR) for plants grown in nutrient solutions with a 0.1 mM initial potassium concentration. The bootstrapped 95% confidence intervals for the percentage of variation in KUtR explained by each predictor are listed in parentheses. The model R^2 and adjusted R^2 were 0.84.

Predictor	**Coefficient**	***P***	**% Variance Explained**
Low treatment potassium uptake per gram of root, U_{LT}	-7.3 x 10^{-6}	<2.2 x 10^{-16}	55.0 (50.4, 59.1)
Low treatment potassium distribution index, KDI_{LT}	-3.0 x 10^{-5}	0.0005	14.2 (10.6, 17.8)
Low treatment root to shoot ratio, RSR_{LT}	-0.033	<2.2 x 10^{-16}	13.7 (10.8, 17.1)
Initial plant mass, W_0	0.021	<2.2 x 10^{-16}	1.4 (0.4, 3.1)

explained 55% of the variation in KUtR (Table 3.4). The potassium distribution index (KDI_{LT}) and root to shoot ratio (RSR_{LT}) were both in the lowest BIC model, but explained only a relatively small proportion of the variation in KUtR than U_{LT}. The initial plant mass was also included in the model but explained only a small amount of the variation in KUtR. The low treatment shoot concentration (KS_{LT}) was not included in this analysis as it was used to calculate KUtR.

The plant potassium content in 0.1 mM initial potassium concentration solutions (QP) was associated with several physiological traits. The most important correlates of QP were with W_0 and RGR_{LT} (Table 3.5). KDI_{LT} was also positively associated with QP and explained just over 10% of the variation in this metric. Small portions of the variance were explained by positive correlations with the low potassium treatment shoot and root sodium concentrations (NaS_{LT} and NaR_{LT} respectively), and root to shoot ratio (RSR_{LT}) as well as potassium uptake per gram of root in the 1 mM potassium treatment (U_{HT}). On the other hand, $FWDW_{LT}$ was negatively associated with QP. KR_{LT}, KS_{LT}, and U_{LT} were not included in

Table 3.5: Summary of the lowest BIC model from a best subsets regression analysis for the potassium content (QP) for plants grown in nutrient solutions with a 0.1 mM initial potassium concentration. The bootstrapped 95% confidence intervals for the percentage of variation in QP explained by each predictor are listed in parentheses. The model R^2 and adjusted R^2 were 0.77 and 0.76 respectively.

Predictor	**Coefficient**	***P***	**% Variance Explained**
Low treatment relative growth rate, RGR_{LT}	1236	$<2.2 \times 10^{-16}$	30.4 (24.5, 35.5)
Initial plant mass, W_0	333.7	$<2.2 \times 10^{-16}$	23.5 (17.9, 29.0)
Low treatment potassium distribution index, KDI_{LT}	1.6	$<2.2 \times 10^{-16}$	12.1 (7.3, 18.0)
Low treatment shoot sodium concentration, NaS_{LT}	0.015	0.0003	4.2 (1.5, 7.8)
Low treatment fresh weight to dry weight ratio, $FWDW_{LT}$	-9.1	1.1×10^{-13}	2.3 (1.7, 3.3)
High treatment potassium uptake per gram of root, U_{HT}	0.0015	0.008	2.2 (0.7, 5.2)
Low treatment root sodium concentration, NaR_{LT}	0.058	1.45×10^{-6}	1.3 (0.9, 2.5)
Low treatment root to shoot ratio, RSR_{LT}	96.7	1.9×10^{-6}	0.7 (0.5, 1.5)

this analysis due to overlaps in their calculations with QP.

The relative plant mass (RP) was positively associated with the shoot sodium concentration in both the 0.1 and 1 mM potassium treatments (Table 3.6). A negative association with $FWDW_{LT}$ was also found. Relative growth rates in both potassium treatments were not included in this analysis as the plant mass was used to calculate them. However, weak correlations were observed between these traits and RP. RGR_{LT} was positively associated while RGR_{HT} was negatively correlated with RP (Appendix Figure 6.2).

Table 3.6: Summary of the lowest BIC model from a best subsets regression analysis for the relative plant mass (RP) between 0.1 and 1 mM potassium treatments. The bootstrapped 95% confidence intervals for the percentage of variation in RP explained by each predictor are listed in parentheses. The model R^2 and adjusted R^2 were 0.20 and 0.19 respectively.

Predictor	**Coefficient**	***P***	**% Variance Explained**
Low treatment shoot sodium concentration, NaS_{LT}	3.3×10^{-4}	3.02×10^{-10}	9.3 (5.1, 13.6)
Low treatment fresh weight to dry weight ratio, $FWDW_{LT}$	-0.088	7.0×10^{-10}	7.5 (3.9, 11.7)
High treatment shoot sodium concentration, NaS_{HT}	0.0012	0.002	3.2 (0.6, 7.1)

The relative root potassium concentration (RCR) was found to be correlated with the distribution of potassium between root and shoots (KDI_{HT} and KDI_{LT}) and the uptake of potassium per gram of root in both potassium treatments (U_{HT} and U_{LT}; Table 3.7). However, these correlations differed between treatments. For example, with 1 mM potassium, U_{HT} was negatively linked with RCR while KDI_{HT} associated positively. Other traits included in the best regression model were the high treatment root sodium concentration (NaR_{HT}) and the initial plant mass (W_0). Of all these traits, KDI_{HT} explained the most variation in RCR. The root potassium concentrations in both treatments were not included in this analysis as they were used to calculate RCR, but a strong negative correlation between KR_{HT} and RCR was noted (Appendix Figure 6.2).

The relative shoot potassium concentration between the two treatments (RCS) shared many of the same associations as RCR, although differences were apparent. For RCS, the uptake traits U_{LT} and U_{HT} were found to be the most important

Table 3.7: Summary of the lowest BIC model from a best subsets regression analysis for the relative root potassium concentration (RCR) between 0.1 and 1 mM potassium treatments. The bootstrapped 95% confidence intervals for the percentage of variation in RCR explained by each predictor are listed in parentheses. The model R^2 and adjusted R^2 were 0.84.

Predictor	**Coefficient**	***P***	**% Variance Explained**
High treatment potassium distribution index, KDI_{HT}	0.014	$<2.2 \times 10^{-16}$	44.9 (38.4, 50.7)
Low treatment potassium distribution index, KDI_{LT}	-0.017	$<2.2 \times 10^{-16}$	13.8 (10.2, 18.0
High treatment uptake per gram of root, U_{HT}	-8.5×10^{-5}	$<2.2 \times 10^{-16}$	10.3 (7.8, 13.9))
High treatment root sodium concentration, NaR_{HT}	2.5×10^{-4}	0.0004	9.1 (5.5, 13.4)
Low treatment uptake per gram of root, U_{LT}	3.2×10^{-4}	$<2.2 \times 10^{-16}$	5.5 (4.2, 7.6)
Initial plant mass, W_0	-0.029	0.015	0.7 (0.3., 2.2)

drivers (Table 3.8) as opposed to the potassium distribution traits which explained most of the variation in RCR. Furthermore, RCS responded positively to KDI_{LT}. Further positive associations of RCS were found with RSR_{LT}, NaS_{LT}, and KR_{HT} while negative links were observed with RSR_{HT} and NaR_{HT} also existed.

3.4 Discussion

In this study, over 300 rice genotypes were grown under potassium stressed and replete conditions. A thorough exploration of the complex KUE trait was facilitated by using six metrics which were previously (Chapter 2) found to reflect different physiological aspects of growth and development at different external potassium concentrations. This served to quantify the scope of variation in rice KUE as

Table 3.8: Summary of the lowest BIC model from a best subsets regression analysis for the relative shoot potassium concentration (RCS) between 0.1 and 1 mM potassium treatments. The bootstrapped 95% confidence intervals for the percentage of variation in RCS explained by each predictor are listed in parentheses. The model R^2 and adjusted R^2 were 0.85.

Predictor	**Coefficient**	***P***	**% Variance Explained**
Low treatment uptake per gram of root, U_{LT}	$2.7 \text{ x } 10^{-4}$	$<2.2 \text{ x } 10^{-16}$	29.4 (22.9, 34.7)
High treatment uptake per gram of root, U_{HT}	$-6.6 \text{ x } 10^{-5}$	$<2.2 \text{ x } 10^{-16}$	26.3 (18.8, 34.1))
Low treatment potassium distribution index, KDI_{LT}	0.0014	$<2.2 \text{ x } 10^{-16}$	10.5 (6.3, 15.9
High treatment root to shoot ratio, RSR_{HT}	-1.43	$<2.2 \text{ x } 10^{-16}$	8.8 (6.5, 11.5)
Low treatment root to shoot ratio, RSR_{LT}	1.17	$<2.2 \text{ x } 10^{-16}$	5.3 (3.9, 7.2)
Low treatment shoot sodium concentration, NaS_{LT}	$4.9 \text{ x } 10^{-5}$	0.0002	2.1 (0.5, 4.7)
High treatment root potassium concentration, KR_{HT}	$1.5 \text{ x } 10^{-4}$	$2.1 \text{ x } 10^{-6}$	1.4 (0.2, 4.8)
High treatment root sodium concentration, NaR_{HT}	$-2.4 \text{ x } 10^{-4}$	0.0008	1.4 (0.44, 3.0)

well as examine the links between physiological characteristics of rice plants and KUE.

The mostly weak associations found between these six metrics (Figure 3.5) suggests that there was little redundancy in the metric selection and even when the correlation between metrics was strong, as was the case for P and QP, they were not found to have the same key physiological drivers (Tables 3.3 and 3.5). As would be expected, low potassium treatment plants had lower growth rates and tissue potassium concentrations in comparison to those in the 1 mM treatment (Figures 3.1 and 3.2). Furthermore, tissue sodium concentrations were generally higher under potassium stress (Figure 3.3) and this is consistent with sodium being used as a partial replacement for potassium, as has been reported (Subbarao *et al.*, 1999; Horie *et al.*, 2007; Gattward *et al.*, 2012). Positive association between tissue sodium concentration and P, QP, RP, and RCS provide further evidence for a beneficial impact of sodium in potassium stressed plants.

Large scale variation was observed across rice genotypes for all six metrics. Variation in metrics across genotypes ranged from just over three-fold for RP to nearly twenty-fold differences for RCR. Variation in KUE values reported in the literature are comparable: around two-fold differences between highest and lowest values have been reported for biomass (Woodend and Glass, 1993; Liu *et al.*, 2009), utilisation efficiency (Liu *et al.*, 2009), and plant potassium content (Guoping *et al.*, 1999; Yang *et al.*, 2003; Damon *et al.*, 2007; Liu *et al.*, 2009). The relatively high levels of variation described for rice in this study are likely to be at least partially due to the large and diverse dataset used. Notably higher levels of variation were found in KUtR by Yang *et al.* (2003) using 134 rice genotypes and by White *et al.* (2010) who found over 35-fold variation in potassium content across *Brassica* species. Ratio metrics (RP, RCR, and RCS) reported here also had similar values to those of other studies (*e.g.* Damon *et al.*, 2007; Fan *et al.*, 2013) with treatment-dependent growth reductions between zero and over 60% (Figure 3.4).

Statistically significant differences for all metrics were observed at the genotype level but only for KUtR and RP at the sub-population level (Section 3.3.2). Greater variety within than between sub-populations has been found for a wide variety of agricultural traits in rice (*e.g.* supplementary information of Zhao *et al.*, 2011; Crowell *et al.*, 2016) and variation at the genotype level is typically reported in crop species (*e.g.* Guoping *et al.*, 1999; George *et al.*, 2002; Damon *et al.*, 2007; Fageria and Melo, 2014; Ju *et al.*, 2014). Taken together, this abundant variation and its relationship to population structure points to there being substantial scope to improve KUE in rice, but that differences in average performance is small between sub-populations (Figure 3.6).

It is important to understand which physiological processes are key determinants of KUE, both to better understand this complex trait and for breeding purposes. Several potentially important physiological traits can been proposed such as replacement with other cations and osmotica, more efficient (re-)distribution of potassium, and enhanced root architecture (*e.g.* White, 2013; Shin, 2014; Wang and Wu, 2015). Furthermore, the relevance of some traits has been identified experimentally, such as greater photosynthetic and tillering rates being linked with better KUtR in rice (Yang *et al.*, 2004). Traits such as sub-cellular distribution of potassium between the vacuole and cytoplasm (Memon *et al.*, 1985) and substitution with sodium (Subbarao *et al.*, 1999) have also been linked with improved potassium utilisation. Low-potassium stress can also be tackled with greater root hair length (Høgh-Jensen and Pedersen, 2003), root mass (George *et al.*, 2002), and high-affinity uptake (Trehan and Claassen, 1998) for example. Multiple regression, as used in this study, provides a method to simultaneously assess the relevance of several physiological traits to KUE, although this approach is not without potential pitfalls.

A key problem that can confound multiple regression analyses is co-linearity between predictors. Correlation coefficients between predictor variables in this study were generally weak, although some moderate to strong relationships existed, such as between potassium uptake (U_{LT}) and distribution (KDI_{LT}) in the 0.1 mM potassium treatment (Appendix Figure 6.2). Removal of highly co-linear variables based on their variance inflation factor, (VIF) is a commonly applied method to deal with this problem (Zuur *et al.*, 2010). However, this approach is not guaranteed to provide an optimal analysis (O'Brien, 2007) and in the context of this study, would result in the removal of physiologically distinct traits. It was noted that the same drivers of differences in KUE were identified using least absolute shrinkage and selection operator (LASSO) regression (Tibshirani, 1996; Friedman *et al.*, 2010), a similar technique which does not require VIF-based removal of correlated predictors. This suggests that the results of the analysis as presented here are robust to methodological choice. Nevertheless, the model selection procedure described in this study revealed several models with similar but slightly larger BIC values. These models identified the same key drivers but showed weaker associations with the metrics. As a result, traits which explain only small percentages of the variability in metrics (such as <5%) should be regarded with caution. However, the aim of this analysis was to identify key drivers and the outcomes were consistent in this respect regardless of methodology.

As may be expected, several traits impacted on multiple KUE metrics. The initial plant mass (W_0), shoot sodium concentration under potassium stress (NaS_{LT}), and potassium distribution index in the 0.1 mM treatment (KDI_{LT}) were all associated with four metrics, the highest number observed. KDI_{LT} explained more than 10% of the variation in its associations with KUtR, QP, RCR, and RCS. W_0 explained over 20% of the variation in P and QP, while NaS_{LT} was the predictor which explained the most variation in RP (9.3%). However, best regression model for RP explained the least variation in the six metrics with a model R^2 of 0.26 compared to 0.73 to 0.85 in the other five lowest BIC regression models. This relatively low R^2 for RP may be as a result of the true physiological drivers of this metric not being measured in this study.

The key drivers of the metrics identified in this work suggest that some metrics respond most strongly to traits that are not explicitly linked to potassium stress. For example, P responds positively to W_0 and the relative growth rate in the potassium replete (1 mM) treatment (RGR_{HT}). Therefore, short-term studies based on vegetative plants mass may select for plants which have innately strong growth rather than KUE-specific traits. Furthermore, the key traits that would improve some metrics may not be compatible with agricultural demands. For example, high KUtR was primarily linked with plants that have a poor potassium uptake (Table 3.4) in this screen. An example is the genotype *Arias* which had the lowest potassium content (QP) but was in the top ten best genotypes for KUtR (Appendix Table 6.2). Similarly, the *aus* genotype *Sathi* had the third highest KUtR and the lowest U_{LT} of all the genotypes tested (Appendix Table 6.2). Such trade-offs could be deleterious in plants grown to maturity as they may struggle to absorb sufficient potassium to maintain growth and ultimately yield. However, it should be noted that this metric has been previously found to be a predictor of grain yield under potassium stress in rice (Yang *et al.*, 2003).

Furthermore, the ratio metrics (RP, RCR, and RCS) favour plants that perform poorly under replete conditions because they are correlated negatively with traits measured under replete conditions (RGR_{HT}, KR_{HT}, or KS_{HT}). At the same time, they are also positively correlated with strong performance under low-potassium stress. For example, RCR is positively linked with KDI_{HT} and negatively with U_{HT}, meaning that there is a relatively low potassium concentration in the roots and consequently less is lost under stress. U_{HT} is also negatively linked with RCS which again serves to minimise the amount of tissue potassium that is lost under stress. Although a low tissue potassium requirement could be considered a positive trait in the context of KUE, a root system that is inefficient at using its mass for potassium absorption would seem an unlikely candidate for optimal KUE. Thus, an important conclusion can be drawn: genotypes that are deemed to be efficient according to some KUE metrics do not necessarily select for agriculturally beneficial traits. This is exemplified by the KUE metric RP. For example, the highest RP genotype (*Okshitmayin*) had an average plant mass of 0.42 g DW under low-potassium stress (P) that was approximately half of the mass of the highest P genotype (0.83 g DW for *Zerawchanica Karatalski*). More broadly, none of the top ten highest RP genotypes were among the top ten genotypes for P (Appendix Tables 6.1 and 6.4).

Identification of physiological traits that are most important to metrics can help to reveal useful targets for crop improvement. The aforementioned positive associations of KUE metrics with the shoot sodium concentration not only points to this as a potential focus for high KUE crops, but also suggests that moderate fertilisation with sodium salts could generate savings in production costs. Crop plants can use sodium as a replacement cation under low-potassium stress (Leigh *et al.*, 1986; Subbarao *et al.*, 1999; Gattward *et al.*, 2012) and sodium fertilisers offer a cheap alternative to potash (Benito *et al.*, 2014). However, plant stress resulting from excess sodium in soils is a growing global problem (Yamaguchi and Blumwald, 2005; Shabala and Cuin, 2007) and the proportion of variation in KUE metrics explained by the shoot sodium concentration was always less than 10% (Tables 3.3, 3.5, 3.6, and 3.8). Therefore, the risks associated with the use of sodium fertilisers need to be considered alongside the potential benefits of replacing potassium with sodium in crops.

While RGR_{LT} and RGR_{HT} have a strong positive correlation (Appendix Figure 6.2), it is the low treatment relative growth rate that is most important to QP and this metric, as well as RCS, is positively associated with KDI_{LT}. This indicates an importance of maintaining an adequate supply of potassium to the shoot when under stress. The fresh weight to dry weight ratio in the 0.1 mM potassium treatment was negatively associated with P, QP, and RP, although it never explained more than 10% of the variation in these metrics. This may indicate a small advantage for plants which decrease their water content under potassium stress and this could help to maintain potassium concentrations on a fresh weight basis.

Future work could explore whether the key associations identified here are consistent across species. It may be the case that crop species differ in their key physiological drivers of coping with low-potassium stress. A related issue would be to determine the relative importance of traits not measured in this study. For example, Yang *et al.* (2004) described how the re-distribution of potassium from older to younger leaves could result in difference in KUE between rice genotypes, while the ability to mobilise non-exchangeable potassium from soil has been implicated in potato KUE (Trehan *et al.*, 2005). For the traits that were measured, the exact underlying physiology is often unknown. For example, the uptake per gram of root is likely to be influenced by root architecture and transporter activity. Furthermore, though partitioning of potassium between roots and shoots was examined, details of distribution between and in other organs and tissues remains to be explored, as is intracellular allocation of potassium. The importance of traits identified at the vegetative stage for grain yield in rice also warrants further investigation and would further inform breeding efforts. The genetic underpinnings of metrics and their component physiological traits is also highly relevant to crop improvement and can be explored using the results of this work in conjunction with genome-wide association studies as exemplified in Chapter 4.

In conclusion, this study has characterised the response of diverse rice genotypes to low potassium stress and revealed much of the physiology that underlies differences in performance. From this it is apparent that rice germplasm holds considerable variation in KUE which could be exploited in crop development. Furthermore, analysis of the component traits that define KUE revealed that some metrics have greater agronomic relevance than others.

Chapter 4

Genetic Architecture of the Response to Potassium Deficiency in Rice *Oryza sativa* L.

4.1 Introduction

Potassium use efficiency (KUE) is an important trait for crop improvement (see Section 1.1) and so knowledge of its genetic underpinnings is important. While some target genes can been proposed, particularly those involved in potassium transport and its regulation (Shin, 2014; Wang and Wu; 2015), a more complete understanding of the genetics of KUE is required. Studies conducted with a range of species have identified quantitative trait loci (QTLs) associated with plant responses to potassium deficiency (*e.g.* Wu *et al.*, 1998; Prinzenberg *et al.*, 2010; Kong *et al.*, 2013; Zhao *et al.*, 2014) and the transcriptome has also been examined (Maathuis *et al.*, 2003; Armengaud *et al.*, 2004; Wang *et al.*, 2012; Zeng *et al.*, 2014).

Transcriptomics studies using rice roots have found many genes to be differentially expressed when plants experience low-potassium stress. Genes for membrane proteins as well as those involved in transport and transcriptional regulation are commonly reported (Ma *et al.*, 2012; Shankar *et al.*, 2013; Takehisa *et al.*, 2013; Zhang *et al.*, 2017), although the genes identified in these studies come from a wide variety of functional groups and are quite variable between studies. Despite this, some genes are typically found to be differentially expressed with potassium deficiency. For example, the high-affinity potassium transporter *OsHAK1* was found to be up-regulated under potassium stress (Miyamoto *et al.*, 2012; Shankar *et al.*, 2013) and also down-regulated on potassium re-supply (Shankar *et al.*, 2013). Surprisingly, Zhang *et al.* (2017) found this gene to be down-regulated in response to potassium deficiency. The *Arabidopsis* homologue of *OsHAK1*, *AtHAK5*, has also been found to be up-regulated with low-potassium stress and down-regulated with potassium re-supply (Ahn *et al.*, 2004; Armengaud *et al.*, 2004) providing evidence for the importance of these homologues in the response of plants to low-potassium conditions.

Genes which code for HKT sodium transporters are also often found to be differentially expressed during low-potassium stress in rice. *OsHKT2;4* was found to be down-regulated in both the absence of potassium and its re-supply (Shankar *et al.*, 2013) while *OsHKT2;1* has been found to be up-regulated in response to low-potassium stress (Ma *et al.*, 2012; Takehisa *et al.*, 2013). Furthermore, *OsHKT2;1* has been implicated in helping rice plants cope with low-potassium stress through replacement of potassium with sodium (Horie *et al.*, 2007; Miyamoto *et al.*, 2015).

While transcriptomics studies help to explore the response of gene expression to low-potassium stress, they have seldom been used to analyse the mechanistic basis of KUE, let alone to show what drives differences in KUE between genotypes of a species. For this, studies which associate genomic regions with a particular phenotype have been used. Hybrid populations, derived from parental genotypes known to differ for a phenotype and which have been genotyped for genetic markers spread across the genome provide one method for identifying quantitative trait loci (QTLs; *e.g. e.g.* Wu *et al.*, 1998; Harada and Leigh, 2006; Prinzenberg *et al.*, 2010; Miyamoto *et al.*, 2012; Fang *et al.*, 2015). In rice, QTLs for several traits, including potassium uptake and tissue potassium concentration and content in salt- and non-stressed plants, have been found (Koyama *et al.*, 2001; Lin *et al.*, 2004; Garcia-Oliveira *et al.*, 2009). Furthermore, QTLs in the context of potassium deficiency have also been reported (Wu *et al.*, 1998; Miyamoto *et al.*, 2012; Fang *et al.*, 2015), although little overlap between studies in the identified genomic regions was apparent. However, both Miyamoto *et al.* (2012) and Fang *et al.* (2015) described associations in a region approximately 24 to 31 Mb along chromosome 6, although these associations were found for different phenotypes: Miyamoto *et al.* (2012) found this region to be linked with the shoot sodium concentration while Fang *et al.* (2015) found it to be associated with the shoot potassium and calcium concentrations. While this region is large, evidence that it may contain an important gene (or genes) relevant to low-potassium stress is strengthened by being identified in separate studies.

A complementary technique for identifying genetic associations with a phenotype is the genome-wide association study (GWAS). First used to implicate complement factor H in human age-related macular degeneration (Haines *et al.*, 2005; Klein *et al.*, 2005), this technique compares the phenotypes of individuals to their genotypes for markers spread across the genome. The genetic markers used are typically single nucleotide polymorphisms (SNPs). A statistical test is used to determine whether a specific marker has a statistically significant association with the phenotype. This results in a P-value for each marker that can be used to identify genomic regions and potentially genes that gave rise to the observed phenotypic differences between individuals.

Diversity panels exist for crop species, such as the Rice Diversity Panel 1 (RDP1; Zhao *et al.*, 2011; Eizenga *et al.*, 2014) which has over 300 diverse rice genotypes genotyped for 700,000 SNPs (McCouch *et al.*, 2016). Studies using the RDP1 have examined abiotic stresses such as aluminium (Famoso *et al.*, 2011) and salt (Kumar *et al.*, 2015; Campbell *et al.*, 2017; Patishtan *et al.*, 2017) and were able to detect novel loci as well as *a priori* candidates. For example, GWAS was used in conjunction with characterisation studies to identify *OsHKT1;1* as important in greater sodium accumulation in *Indica* genotype rice roots compared to *Japonica* genotypes under salt stress (Campbell *et al.*, 2017). However, the response of rice to potassium deficiency has yet to be examined using GWAS.

In this study, GWAS was therefore used to explore the genetic architecture of low-potassium stress in rice for the first time using the RDP1. In doing so, novel QTLs were detected as well as genomic regions which co-localised with those in the previous literature. Furthermore, genes within QTLs were also found which have been reported to be differentially expressed under potassium deficiency. This, as well as the known functions of genes within QTLs from this study and co-localisation of genes with SNPs associated with phenotypes, made it possible to propose candidate genes for improvement of KUE.

4.2 Methods

4.2.1 Trait Measurement

Least squares means of 318 diverse rice genotypes for the metrics and traits (henceforth phenotypes) used in the regression analyses in Chapter 3 (Tables 3.1 and 3.2) were used in this study. The rice genotypes had been previously genotyped for 700,000 SNPs distributed across the rice genome (McCouch *et al.*, 2016). Growth experiments and the calculation of least squares means were described in detail in Section 3.2. Briefly, each rice genotype was grown in potassium deficient (0.1

mM) and replete (1 mM) nutrient solutions (adapted from Yoshida *et al.*, 1976) for four weeks (see Section 3.2.1). Root and shoot mass as well as potassium and sodium concentrations were measured (see Section 3.2.2). Phenotype values were calculated for each genotype from these data (see Section 3.2.3). These values were then used in GWAS analyses outlined below.

4.2.2 Genome-Wide Association Studies

GWAS was carried out using the **GenABEL** package (Aulchenko *et al.*, 2007) in R 3.3.3. Linear mixed models (LMMs) were used to test the statistical significance of SNP genotype to a phenotype as they have previously been found to effectively control for the population structure present in rice (Zhao *et al.*, 2011). LMMs were of the form:

$$\mathbf{y} = \mu + X\mathbf{B}_X + \mathbf{g}B_g + \mathbf{u} + \mathbf{e} \tag{4.1}$$

where $\mathbf{y}$ is a (nx1, where n is the number of genotypes) vector of phenotype values, μ is the intercept, X is the (nxk, where k is the number of covariates) matrix of covariates (principal components, PCs, calculated from kinship matrices for each grouping of genotypes), $\mathbf{B}_X$ is a (kx1) vector of covariate (PC) effects, $\mathbf{g}$ is a (nx1) vector of SNP genotypes, B_g is the fixed effect of the SNP genotype, $\mathbf{u}$ is a (nx1) vector of random polygenic effects, and $\mathbf{e}$ is a (nx1) vector of random residuals (Svishcheva *et al.*, 2012).

Sub-species- or sub-population-specific associations have been found for many phenotypes in previous studies (*e.g.* Zhao *et al.*, 2011; Crowell *et al.*, 2016). In addition to analyses across all genotypes, separate GWAS analyses were therefore carried out for different sub-species (*i.e. Indica* or *Japonica*) or sub-populations (*aus*, *indica*, *temperate japonica*, or *tropical japonica*). The *aromatic* sub-population was not analysed on its own due to having only 9 members in the dataset. For each analysed group of genotypes, SNPs with a minor allele frequency (MAF) <0.05 as well as a call rate <0.9 were removed from analyses to minimise the risk of spurious associations.

PCs were not included for analyses at the sub-population level, but were used for sub-species and analyses which used all of the genotypes as sub-population and sub-species clusters exist within these groupings (Zhao *et al.*, 2011; McCouch *et al.*, 2016). However, PCs were not used when their inclusion resulted in deflation of test statistics measured using the genomic inflation factor (λ; Devlin and Roeder, 1999). The model with λ nearest to 1 was used for each analysis.

Associations between SNPs and phenotypes were declared significant if their *P*-value was $<1 \times 10^{-5}$ (a threshold used by Crowell *et al.*, 2016) and their false discovery rate (Benjamini and Hochberg, 1995) was <10%.

4.2.3 Identification of Quantitative Trait Loci and Candidate Genes

Quantitative trait loci (QTLs) were defined as genomic regions ± 100 kbp either side of a significantly associated SNP. This window size was used because linkage disequilibrium in rice declines rapidly (on average) over the first 100 kbp (Zhao *et al.*, 2011; McCouch *et al.*, 2016). If two QTLs from the same association (*i.e.* one that used the same phenotype and group of genotypes) overlapped, the QTls were grouped into a single QTL.

Genes within QTLs were found from the Rice Genome Annotation Project website (http://rice.plantbiology.msu.edu/pub/data/Eukaryotic_Projects/o_sativa/annotation_dbs/pseudomolecules/version_7.0/). Candidate genes were identified based on containing a non-synonymous SNP statistically significantly associated with a trait (identified using the Rice Diversity Allele Finder: http://rs-bt-mccouch4.biotech.cornell.edu/AF/), known and putative functions of genes related to low-potassium stress, or evidence that genes are differentially expressed under low-potassium stress in rice (based on studies by Ma *et al.*, 2012; Shankar *et al.*, 2013; Takehisa *et al.*, 2013; Zhang *et al.*, 2017).

4.3 Results

4.3.1 Quantitative Trait Loci Associated with Potassium Deficiency in Rice

GWAS analyses identified 208 SNPs as statistically significantly associated with phenotypes. Manhattan plots (*e.g.* Figure 4.2 and 4.3A) which summarise the results of each GWAS analysis are provided in the Supplementary Information file "gwasPlots.pdf". These statistically significantly associated SNPs resulted in 98 QTLs (Table 4.1), of which twenty were for analyses using all genotypes and the remaining QTLs were for sub-population and sub-species level analyses. Co-localisation between the 98 QTLs found in this work and previously identified QTLs was apparent (Figure 4.1).

Thirteen genomic regions were found for which two or more QTLs from the GWAS analyses carried out in this study overlapped. These were spread across all chromosomes except 3, 5, 11, and 12 (Table 4.1) and several were for the same phenotype but at different levels of the population hierarchy. An example of this was for the potassium content (QP) in the *Japonica* sub-species and the *temperate japonica* sub-population where the QTLs QP.JAPONICA-7.2 and QP.tej-7.3 spanned the same region on chromosome 7 (Table 4.1). Some of these were also in the same regions as QTLs from other potassium deficiency studies using rice, such as on chromosome 6 (QTLs NaR.lt-6.1 and NaS.lt-6.1), as well as KUtR.ALL-9.1, KUtR.INDICA-9.1, and KR.ht.ALL-9.1 on chromosomes 9 (Figure 4.1). Novel associations were found for several phenotypes in other parts of the genome, for example at approximately 13 Mbp along chromosome 3 and approximately 20 Mbp along chromosome 8 (Figure 4.1).

Table 4.1: Summary of quantitative trait loci (QTLs) identified in this study using genome-wide association studies of rice under potassium deficiency. QTL names begin with the phenotype the QTL was associated with, followed by the group of genotypes it relates to (*i.e.* the sub-species, sub-population, or all of the genotypes). The chromosome and a count to distinguish between QTLs on the same chromosome for the same analysis are then given. The abbreviations are as follows: LT for low treatment (0.1 mM solution potassium concentration), HT for high treatment (1 mM solution potassium concentration); P for whole plant mass, KUtR for the potassium utilisation ratio, QP for plant potassium content, RP for relative plant mass, RCR for relative root potassium concentration, RCS for relative shoot potassium concentration, KR for root potassium concentration, KS for the shoot potassium concentration, NaR for root sodium concentration, NaS for shoot sodium concentration, U for potassium uptake per gram of root, KDI for potassium distribution index, RSR for root to shoot ratio, FWDW for fresh weight to dry weight ratio, RGR for relative growth rate. Further information on the metrics and traits is available in Tables 3.1 and 3.2. For genotype groupings, ALL means the genome-wide association study used data from every rice genotype in this study, INDICA and JAPONICA refer to associations using the respective sub-species, and aus, ind, tej, and trj symbolise associations which used the *aus*, *indica*, *temperate japonica*, and *tropical japonica* sub-populations respectively.

Quantitative Trait Locus	**Chromosome**	**Start (bp)**	**End (bp)**	**Notes**
KDI.ht.JAPONICA-1.1	1	3,054,419	3,254,419	-
RCR.tej-1.1	1	13,257,732	13,457,732	-
RCR.tej-1.2	1	14,514,661	14,714,661	-
P.JAPONICA-1.1	1	18,867,813	19,067,813	-
RGR.lt.INDICA-1.1	1	22,259,080	22,484,204	>1 SNP
RGR.lt.ALL-1.1	1	22,260,180	22,463,799	>1 SNP
QP.JAPONICA-1.1	1	22,594,881	22,794,881	-
RSR.ht.ALL-1.1	1	32,476,423	32,676,423	-
KR.lt.trj-1.1	1	33,210,677	33,410,677	-
KUtR.ALL-1.1	1	34,363,159	34,563,159	-
U.lt.INDICA-1.1	1	38,400,811	38,600,811	-
RCR.ALL-1.1	1	42,940,025	43,140,025	-

KUtR.INDICA-2.1	2	2,586,991	2,786,991	-
RCS.trj-2.1	2	10,641,216	10,841,216	-
QP.tej-2.1	2	23,908,600	24,267,552	>1 SNP
RCR.JAPONICA-2.1	2	24,814,028	25,014,028	-
QP.tej-2.2	2	25,866,610	26,239,961	>1 SNP
KDI.lt.trj-2.1	2	27,324,500	27,524,500	-
KR.ht.ALL-2.1	2	27,364,358	27,564,358	-
QP.tej-2.3	2	27,843,200	28,043,200	-
KDI.lt.trj-3.1	3	3,580,300	3,780,300	-
RCS.JAPONICA-3.1	3	4,041,814	4,241,814	-
RP.JAPONICA-3.1	3	12,707,870	12,907,870	-
RCS.JAPONICA-3.2	3	12,837,754	13,148,789	>1 SNP
RCS.trj-3.1	3	12,837,754	13,037,754	-
KR.ht.ALL-3.1	3	13,079,729	13,279,729	-
NaS.ht.trj-3.1	3	13,639,419	13,839,419	-
RCS.JAPONICA-3.3	3	19,568,515	19,768,515	-
RCS.trj-3.2	3	20,096,338	20,296,338	-
QP.trj-3.1	3	27,317,271	27,517,271	-
KR.lt.trj-1.3	3	32,320,151	32,520,151	-
KUtR.INDICA-3.1	3	35,067,724	35,267,724	-
RCS.JAPONICA-4.1	4	890,270	1,090,270	-
RCS.trj-4.1	4	890,270	1,090,270	-
RCS.JAPONICA-4.2	4	2,502,716	2,702,716	-
KR.ht.ALL-4.1	4	12,255,015	12,455,015	-
FWDW.lt.JAPONICA-4.1	4	18,840,302	19,040,302	-
RCR.tej-4.1	4	31,645,530	31,845,530	-
KR.ht.ALL-4.2	4	33,156,379	33,356,379	-

RGR.ht.tej-4.1	4	33,193,193	33,393,193	-
KUtR.INDICA-4.1	4	34,278,649	34,478,649	-
KR.lt.JAPONICA-5.1	5	10,983,374	11,183,374	-
KS.lt.ind-5.1	5	13,526,636	13,726,636	-
RCS.JAPONICA-5.1	5	17,080,084	17,280,084	-
KDI.ht.JAPONICA-5.1	5	17,540,601	17,74,0601	-
U.lt.ALL-5.1	5	18,375,248	18,575,248	-
KDI.ht.JAPONICA-5.2	5	27,268,520	27,468,520	-
RCS.trj-6.1	6	0	126,034	-
FWDW.lt.JAPONICA-6.1	6	8,391,304	8,591,304	-
QP.tej-6.1	6	12,112,734	12,312,734	-
QP.JAPONICA-6.1	6	21,591,579	21,791,579	-
P.ALL-6.1	6	21,754,339	21,954,339	-
RCR.tej-6.1	6	22,216,793	22,416,793	-
RCR.tej-6.1	6	22,216,793	22,416,793	-
KR.lt.JAPONICA-6.1	6	24,887,468	25,087,468	-
QP.JAPONICA-6.2	6	26,841,219	27,041,219	-
NaR.lt.ALL-6.1	6	29,440,164	29,640,591	>1 SNP
NaS.lt.ALL-6.1	6	29,440,164	29,640,591	>1 SNP
KDI.ht.JAPONICA-7.1	7	1,820,065	2,020,065	-
QP.tej-7.1	7	5,219,318	5,462,586	>1 SNP
QP.JAPONICA-7.1	7	9,387,632	9,587,632	-
QP.tej-7.2	7	12,692,645	12,892,645	-
QP.JAPONICA-7.2	7	20,494,818	20,694,818	-
QP.tej-7.3	7	20,494,818	20,694,818	-
RCS.JAPONICA-7.1	7	22,716,173	22,916,173	-
KR.ht.ALL-7.1	7	22,949,108	23,149,108	-

RCS.JAPONICA-7.2	7	23,278,661	23,478,661	-
RCS.trj-7.1	7	23,278,661	23,478,661	-
QP.tej-7.4	7	24,628,157	25,052,100	>1 SNP
QP.JAPONICA-7.3	7	24,655,435	24,962,827	>1 SNP
KR.lt.trj-7.1	7	26,562,735	26,762,735	-
QP.tej-7.5	7	28,842,868	29,165,544	>1 SNP
RCS.JAPONICA-8.1	8	5,378,001	5,578,001	-
RCS.JAPONICA-8.2	8	7,416,036	7,616,036	-
RCS.JAPONICA-8.3	8	13,397,546	13,597,546	-
RCS.JAPONICA-8.4	8	14,032,648	14,232,648	-
RCS.JAPONICA-8.5	8	19,801,229	20,385,187	>1 SNP
RCS.trj-8.1	8	20,180,990	20,942,059	>1 SNP
RCR.JAPONICA-8.1	8	20,484,968	20,684,968	-
RCS.trj-8.2	8	22,823,792	23,175,801	>1 SNP
KUtR.ALL-9.1	9	3,721,410	3,921,410	-
KUtR.INDICA-9.1	9	3,721,410	3,921,410	-
KR.ht.ALL-9.1	9	3,894,822	4,094,822	-
QP.tej-9.1	9	7,775,589	7,975,589	-
KR.ht.ALL-9.2	9	13,367,729	13,567,729	-
RCS.trj-9.1	9	13,958,209	15,088,238	>1 SNP
KS.lt.ind-10.1	10	5,539,043	5,739,043	-
RCS.ind-10.1	10	5,539,043	5,739,043	-
KS.lt.INDICA-10.1	10	7,238,220	7,438,220	-
RCS.trj-10.1	10	13,658,426	13,858,426	-
P.ALL-10.1	10	16,425,036	16,625,036	-
FWDW.ht.JAPONICA-11.1	11	2,341,192	2,541,192	-
QP.trj-11.1	11	16,933,927	17,133,927	-

NaR.lt.ALL-11.1	11	22,044,534	22,244,534	-
RCS.trj-12.1	12	8,988,538	9,188,538	-
KUtR.ALL-12.1	12	10,586,529	10,786,529	-
KR.ht.ALL-12.1	12	10,793,826	10,993,826	-
RCS.trj-12.2	12	17,981,301	18,830,926	>1 SNP

Most of the QTLs identified in these analyses have only 1 SNP that satisfied the significance criteria. However, some contained several SNPs such as RCS.trj-9.1 (Table 4.1) which spans a 1.1 Mb window that includes 53 statistically significantly associated SNPs (Figure 4.2). The relative shoot potassium concentration (RCS; shoot potassium concentration in the 0.1 mM potassium solution divided by the shoot potassium concentration in the 1 mM potassium solution) had the most QTLs with 26. These were primarily found for the *Japonica* sub-species (13) and the *temperate japonica* sub-population (12) while the *indica* sub-population had one QTL (Table 4.1). Overlapping QTLs between sub-species and their sub-populations were often found (*e.g.* between the *Japonica* and *tropicical japonica* for RCS on chromosomes 3, 4, 7, and 8), although several sub-population specific QTLs were also observed (Table 4.1). The *Japonica* sub-species had the most QTLs across phenotypes with 32 compared to 21 for GWAS analyses using all genotypes. Conversely, associations involving the *Indica* sub-species and the sub-populations within it returned few QTLs, with seven for the sub-species and three QTLs for the *indica* sub-population. No QTLs were detected for the *aus* sub-population. Furthermore, no statistically significant association was found for the phenotypes initial plant mass (W_0), shoot potassium concentration (KS_{HT}), root sodium concentration (NaR_{HT}), and uptake per gram of root (U_{HT}) in the 1 mM potassium treatment as well as the root to shoot ratio in the 0.1 mM potassium

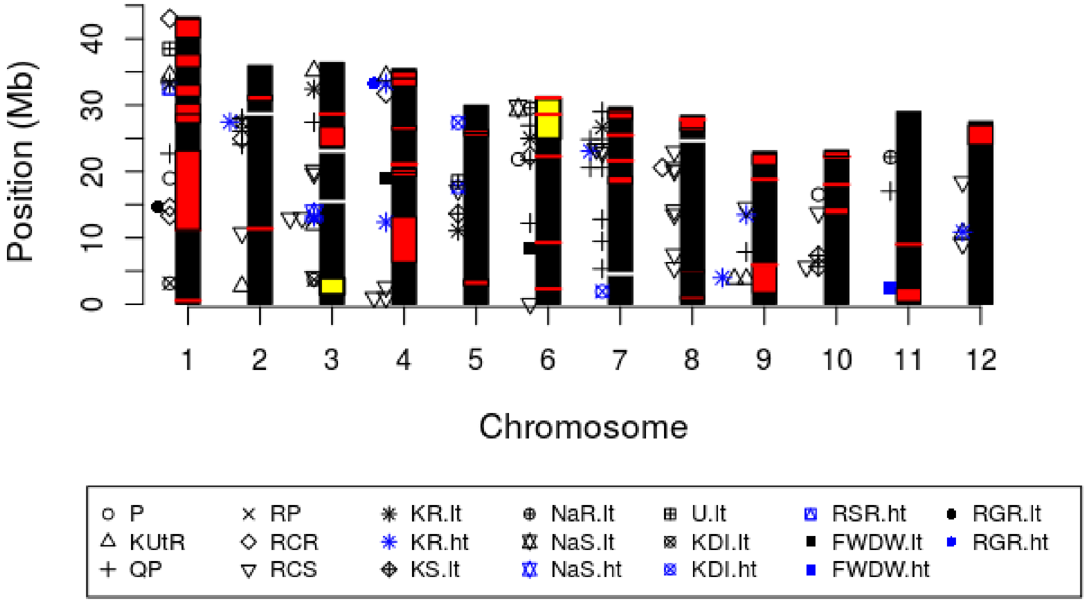


Figure 4.1: Summary of quantitative trait loci (QTLs) identified in this study. QTL locations are shown using points to the left of black bars which represent rice chromosomes. Each point represents the phenotypes associated with the QTL and these are listed in the legend. The abbreviations are as follows: LT for low treatment (0.1 mM solution potassium concentration), HT for high treatment (1 mM solution potassium concentration); P for whole plant mass, KUtR for the potassium utilisation ratio, QP for plant potassium content, RP for relative plant mass, RCR for relative root potassium concentration, RCS for relative shoot potassium concentration, KR for root potassium concentration, KS for the shoot potassium concentration, NaR for root sodium concentration, NaS for shoot sodium concentration, U for potassium uptake per gram of root, KDI for potassium distribution index, RSR for root to shoot ratio, FWDW for fresh weight to dry weight ratio, RGR for relative growth rate. Further information on the metrics and traits is available in Tables 3.1 and 3.2. Coloured rectangles represent QTLs and markers identified in previous studies in rice under potassium deficiency (Wu *et al.* 1998 in white; Miyamoto *et al.*, 2012 in yellow; Fang *et al.*, 2015 in red). Marker positions were found at http://archive.gramene.org/db/markers.

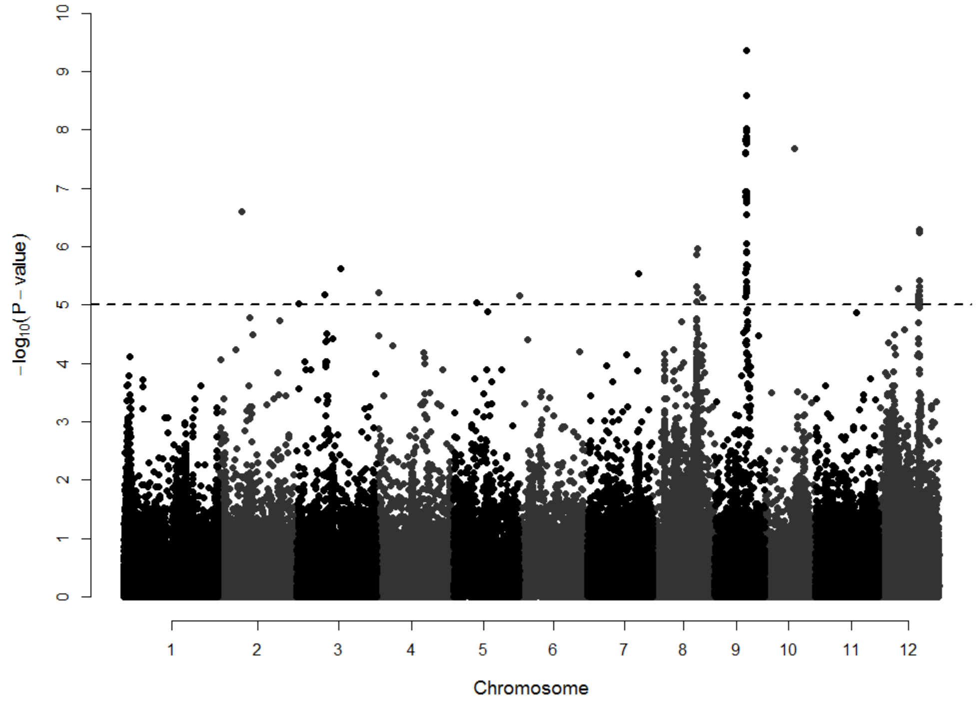


Figure 4.2: Genome-wide association study for relative shoot potassium concentration (RCS) in 86 *tropical japonica* rice genotypes. Each point represents the $-log_{10}(P)$ for an association between a SNP with RCS. There were 121,085 SNPs used in this analysis (minor allele frequency $>$ 0.05 and call rate $>$0.9). The dashed line represents a significance threshold above which all SNPs have a P-value $<$1 x 10^{-5} and a false discovery rate $<$10%. Peaks, (*e.g.* on chromosome 9, represent regions of the rice genome for which several physically close SNPs had strong statistical associations with the phenotype.

treatment (RSR$_{LT}$) across all groups of genotypes.

4.3.2 Genes in Quantitative Trait Loci

The 98 QTLs identified in this study contained 3,034 unique genes (listed in Supplementary Information file "genesList.xlsx"). Of these genes, 1,251 of which were not annotated as '(retro)transposons', 'hypothetical proteins', or 'expressed proteins' without further annotation on the Rice Genome Annotation Project Website.

Twenty-eight genes were found among the full list of genes which were involved in cation transport. These included three genes with gene ontology (GO) terms for potassium transport. The potassium channel KAT2 (LOC_Os04g02720) was associated with the relative shoot potassium concentration in *Japonica* rice (QTLs RCS.JAPONICA-4.1 and RCS.trj-4.1) and is primarily expressed in guard cells and has been suggested to be the rice functional orthologue of *AtKAT1* (Hwang *et al.*, 2013). A putative potassium transporter (LOC_Os08g36340) was also related to the same trait, but only in the *tropical japonica*. The latter gene was found to be expressed relatively highly in the stem and panicle compared to other tissues in *cv.* Minghui 63 rice (Wang *et al.*, 2010; http://rice.plantbiology.msu.edu/cgi-bin/ORF_infopage.cgi) Furthermore, B4-BTB1 (LOC_Os02g39910) was found to have a GO term related to potassium transport. This gene has been described as coding for a potassium channel tetramerisation domain-containing protein (Hirose *et al.*, 2007).

However, GO singular enrichment analysis of this collection of genes revealed no GO terms that were statistically significantly (false discovery rate <5%) enriched relative to their background abundance in the rice genome (using Fisher's exact test with a Yekutieli adjustment for multiple testing using the AgriGO gene ontology analysis tool kit: http://bioinfo.cau.edu.cn/agriGO/analysis.php). Therefore, identifying genes as candidates based on their GO terms is problematic as they may be chance associations.

4.3.3 Single Nucleotide Polymorphisms Associated with Traits and Within Genes

Of the 208 unique statistically significantly associated SNPs across all associations, 85 were within 67 different genes. Twenty SNPs were found to result in in non-synonymous substitutions in 16 different genes and are listed in Table 4.2, with one resulting in a nonsense mutation in a hypothetical protein (LOC_Os09g24280).

Eleven of the sixteen non-synonymous SNPs fell within the 1.1 Mb wide QTL on chromosome 9 for the relative shoot potassium concentration in the *tropical japonica* noted above (RCS.trj-9.1; Table 4.2).

Table 4.2: Non-synonymous single nucleotide polymorphisms (SNPs) identified using genome-wide association studies (GWAS). Chromosome is abbreviated to Chrom.

Quantitative Trait Locus	**Gene**	**Chrom.**	**SNP Position (bp)**	**Amino Acid Change**
RCR.tej-1.2	LOC_Os01g25810: prenyltransferase	1	18,967,813	Gly to Asp
RGR.lt.INDICA-1.1; RGR.lt.ALL-1.1	LOC_Os01g39640: retrotransposon protein	1	22,360,981	Pro to Leu
KR.lt.trj-1.3	LOC_Os03g56920: expressed protein	1	32,420,151	Ile to Val
QP.tej-6.1	LOC_Os06g21140: glycine-rich cell wall structural protein precursor	6	12,212,734	Gly to Arg
RCR.tej-6.1	LOC_Os06g37670: S-locus-like receptor protein kinase	6	22,316,793	Thr to Ser
KR.lt.trj-7.1	LOC_Os07g44680: expressed protein	7	26,662,735	Asp to His
RCS.trj-9.1	LOC_Os09g23660: ZIM motif family protein	9	14,058,209	Val to Ala
RCS.trj-9.1	LOC_Os09g24190: expressed protein	9	14,359,383	Ala to Glu
RCS.trj-9.1	LOC_Os09g24240: pentatricopeptide	9	14,391,529	Asp to Val

RCS.trj-9.1	LOC_Os09g24240: pentatricopeptide	9	14,392,003	Asp to Val
RCS.trj-9.1	LOC_Os09g24240: pentatricopeptide	9	14,392,186	Met to Thr
RCS.trj-9.1	LOC_Os09g24280: hypothetical protein	9	14,426,745	Met to Val
RCS.trj-9.1	LOC_Os09g24280: hypothetical protein	9	14,426,787	Gln to Stop
RCS.trj-9.1	LOC_Os09g24290: agenet domain-containing protein	9	14,440,057	Val to Ile
RCS.trj-9.1	LOC_Os09g24310: expressed protein	9	14,449,786	Ser to Phe
RCS.trj-9.1	LOC_Os09g24310: expressed protein	9	14,451,402	Arg to His
RCS.trj-9.1	LOC_Os09g24440: expressed protein	9	14,536,300	Asn to Ser
QP.trj-11.1	LOC_Os11g29360: pentatricopeptide	11	17,033,927	Ile to Thr
KR.ht.ALL-12.1	LOC_Os12g18810: expressed protein	12	10, 893,826	Asp to Gly
RCS.trj-12.2	LOC_Os12g30630: transposon protein	12	18,396,339	Arg to Lys

4.3.4 Genes Identified in Previous Transcriptomics Studies

A total of 42 genes was identified in this study which had been previously found to be differentially expressed in rice under potassium deficiency (Table 4.3). These genes had a statistically significantly high proportion of regulatory and transcriptional GO terms (Fisher's exact test, false discovery rate <0.05; http://bioinfo.cau.edu.cn/agriGO/analysis.php).

Table 4.3: Summary of 42 genes within quantitative trait loci (QTLs) identified from genome-wide association studies which have been previously found to be transcriptionally regulated by potassium.

Quantitative Trait Locus	**Gene**	**Reference**
KDI.ht.JAPONICA-1.1	LOC_Os01g06640: putative basic helix-loop-helix	Zhang *et al.*, 2017
QP.JAPONICA-1.1	LOC_Os01g40070: expressed protein	Ma *et al.*, 2012
QP.JAPONICA-1.1	LOC_Os01g40094: putative protein phosphatase 2C	Ma *et al.*, 2012
KUtR.ALL-1.1	LOC_Os01g59530: OsCML1 - Calmodulin-related calcium sensor protein	Shankar *et al.*, 2013
RCR.ALL-1.1	LOC_Os01g74410: putative MYB family transcription factor	Takehisa *et al.*, 2013
QP.tej-2.1	LOC_Os02g39910: B4-BTB1 - Bric-a-Brac, Tramtrack, Broad Complex BTB domain with B4 subfamily conserved sequence	Ma *et al.*, 2012
RCR.JAPONICA-2.1	LOC_Os02g41510: putative MYB family transcription factor	Takehisa *et al.*, 2013

RCR.JAPONICA-2.1	LOC_Os02g41510: putative MYB family transcription factor	Takehisa *et al.*, 2013
RCR.JAPONICA-2.1	LOC_Os02g41670: putative phenylalanine ammonia-lyase	Takehisa *et al.*, 2013
RCR.JAPONICA-2.1	LOC_Os02g41680: putative phenylalanine ammonia-lyase	Takehisa *et al.*, 2013
QP.tej-2.3	LOC_Os02g45770: OsMADS6 - MADS-box family gene with MIKCc type-box	Takehisa *et al.*, 2013
QP.tej-2.3	LOC_Os02g46030: putative MYB family transcription factor	Shankar *et al.*, 2013
KDI.lt.trj-3.1	LOC_Os03g07226: putative thioredoxin	Ma *et al.*, 2012
RCS.JAPONICA-3.1	LOC_Os03g07940: AP2 domain containing protein	Shankar *et al.*, 2013
RCS.JAPONICA-3.1	LOC_Os03g08320: putative ZIM domain containing protein	Takehisa *et al.*, 2013
RCS.JAPONICA-3.2; RCS.trj-3.1	LOC_Os03g22590: putative nodulin MtN3 family protein	Takehisa *et al.*, 2013
NaS.ht.trj-3.1	LOC_Os03g24220: putative villin protein	Ma *et al.*, 2012

RGR.ht.tej-4.1	LOC_Os04g56030: putative glycine-rich cell wall structural protein precursor	Takehisa *et al.*, 2013
RCS.JAPONICA-5.1	LOC_Os05g29750: putative cytochrome P450 71E1	Takehisa *et al.*, 2013
QP.tej-6.1	LOC_Os06g21210: putative glycine rich protein family protein	Ma *et al.*, 2012
NaR.lt.ALL-6.1; NaS.lt.ALL-6.1	LOC_Os06g48800: OsHKT2;4 - Na+ transporter	Shankar *et al.*, 2013
NaR.lt.ALL-6.1; NaS.lt.ALL-6.1	LOC_Os06g48810: OsHKT2;1 - Na+ transporter	Takehisa *et al.*, 2013
QP.tej-7.2	LOC_Os07g22680: putative SKP1-like protein 1B	Takehisa *et al.*, 2013
QP.tej-7.2	LOC_Os07g22730: AP2 domain containing protein	Takehisa *et al.*, 2013
QP.JAPONICA-7.2; QP.tej-7.3	LOC_Os07g34400: putative lipase class 3 family protein	Takehisa *et al.*, 2013

RCS.JAPONICA-7.1	LOC_Os07g37990: putative glycosyltransferase sugar-binding region containing DXD motif	Ma *et al.*, 2012
RCS.JAPONICA-7.2; RCS.trj-7.1	LOC_Os07g38810: putative lectin receptor-type protein kinase	Takehisa *et al.*, 2013
QP.tej-7.3; QP.JAPONICA-7.4	LOC_Os07g41240: putative cytochrome P450	Takehisa *et al.*, 2013
QP.tej-7.4	LOC_Os07g41694: putative acidic leucine-rich nuclear phosphoprotein 32-related protein 1	Ma *et al.*, 2012
QP.tej-7.5	LOC_Os07g48340: OsCML24 - Calmodulin-related calcium sensor protein	Takehisa *et al.*, 2013
QP.tej-7.5	LOC_Os07g48490: putative stress responsive protein	Takehisa *et al.*, 2013
QP.tej-7.5	LOC_Os07g48680: zinc finger, C3HC4 type domain containing protein	Takehisa *et al.*, 2013

RCS.JAPONICA-8.1	LOC_Os08g09640: OsFBL43 - F-box domain and LRR containing protein	Takehisa *et al.*, 2013
RCS.JAPONICA-8.5	LOC_Os08g32080: CPuORF27 - conserved peptide uORF-containing transcript	Zhang *et al.*, 2017
RCS.JAPONICA-8.5	LOC_Os08g32520: expressed protein	Ma *et al.*, 2012
RCS.JAPONICA-8.5; RCS.trj-8.1	LOC_Os08g32750: putative bifunctional monodehydroascorbate reductase and carbonic anhydrasenectarin-3 precursor	Takehisa *et al.*, 2013
RCS.trj-9.1	LOC_Os09g24370: expressed protein	Ma *et al.*, 2012
RCS.trj-9.1	LOC_Os09g24710: putative alpha/beta hydrolase fold	Ma *et al.*, 2012
RCS.trj-9.1	LOC_Os09g25060: WRKY76	Takehisa *et al.*, 2013
RCS.trj-12.1	LOC_Os12g16010: putative sex determination protein tasselseed-2	Ma *et al.*, 2012
RCS.trj-12.2	LOC_Os12g31160: putative MLA10	Takehisa *et al.*, 2013

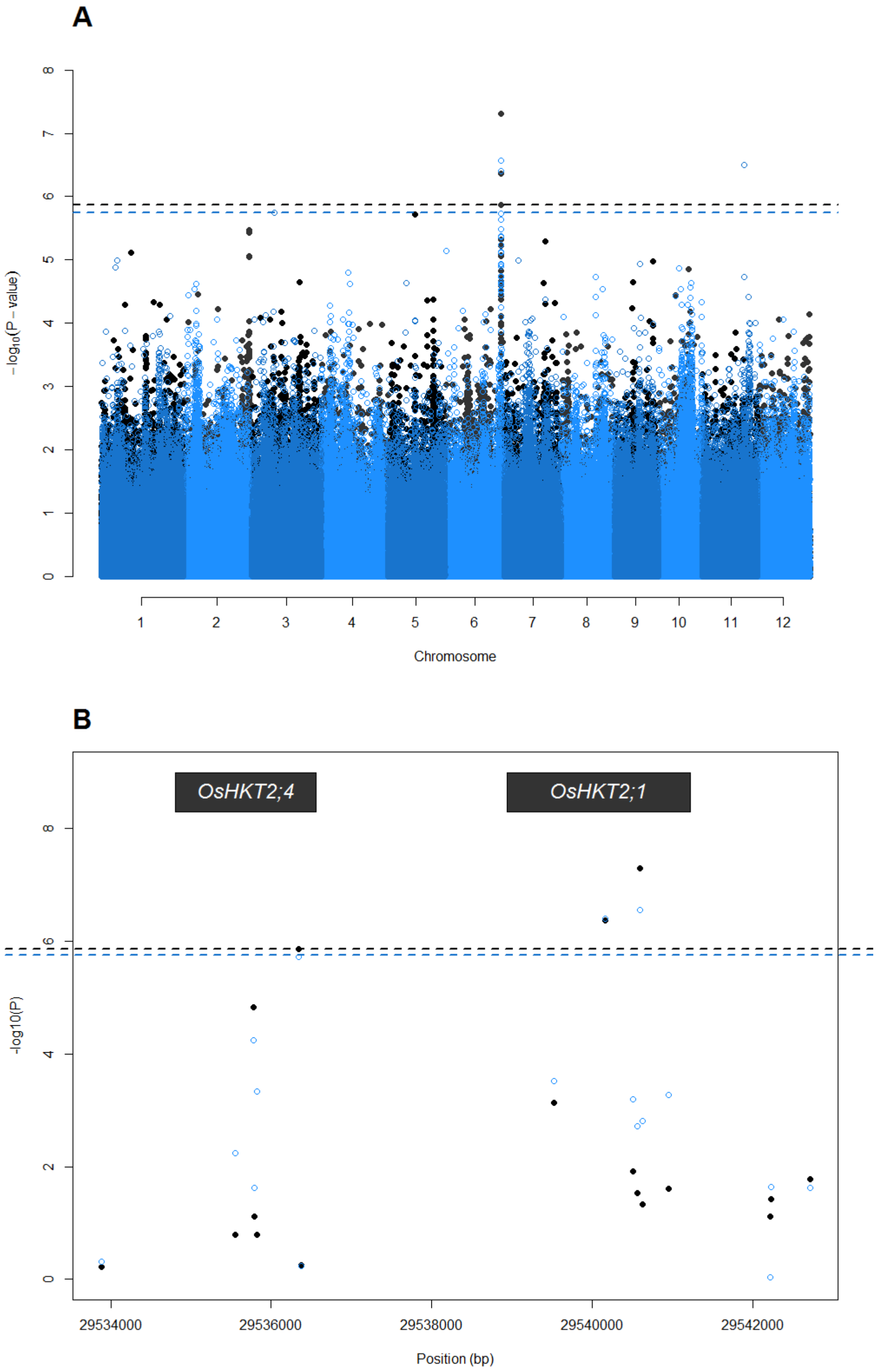
Two of the genes listed in Table 4.3 also contained a SNP that was statistically significantly associated with a phenotype. One of these was a putative bifunctional monodehydroascorbate reductase and carbonic anhydrasenectarin-3 precursor (LOC_Os08g32750) which was in two QTLs for the relative shoot potassium concentration for the *Japonica* sub-species and *tropical japonica* sub-population (RCS.JAPONICA-8.5 and RCS.trj-8.1). The associated SNP was in the 3' untranslated region of the gene.

The gene for the sodium transporter HKT2;1 contains two synonymous SNPs that were statistically significantly associated with the root and shoot sodium concentrations for all genotypes (NaR.lt.ALL-6.1; NaS.lt.ALL-6.1; Figure 4.3). *OsHKT2;1* is positioned close to *OsHKT2;4*, another sodium transporter, on chromosome 6 (Figure 4.3B). However, no statistically significantly associated SNPs were found within *OsHKT2;4* although several SNPs in this region were in relatively high linkage disequilibrium with each other (Figure 4.3C).

4.4 Discussion

In this study, the genetic architecture of rice in response to potassium treatment was explored using GWAS for the first time. A total of 98 QTLs was identified across traits and groupings of genotypes. Most of these were associated with phenotypes of plants in potassium stressed conditions or ratios between stressed and replete conditions, suggesting that a lot of genetic variation exists for potassium stress, but less was apparent when plants were not stressed. Furthermore, rice has extensive population structure (Garris *et al.*, 2005; Zhao *et al.*, 2010) and many QTLs were found that were sub-population and/or sub-species specific. Several of these showed overlaps between sub-species and their relevant sub-populations. This sub-population specificity could have important repercussions in an agronomic context. For example, strategies for improving KUE may not be the same for all sub-populations. Furthermore, it may also mean that crosses between groups of rice genotypes can benefit crop improvement.

It should be noted that QTLs were found much more readily in the *Japonica* than *Indica* rice. This may be as a result of statistical power as the dataset contained 195 *Japonica* genotypes compared to only 110 *Indica* genotypes that passed the quality control criteria. On the other hand, previous GWAS using the RDP1 appear to have been able to find many associations in the *aus* and *indica* subspecies for several different phenotypes (Famoso *et al.*, 2011; Zhao *et al.*, 2011). A recent dataset which combines the RDP1 and Rice Diversity Panel 2 (RDP2) greatly increases the number of available genotypes for such analyses (McCouch *et al.*, 2016) and this larger dataset could be used to re-assess the genetic architecture of KUE in the *Indica*.



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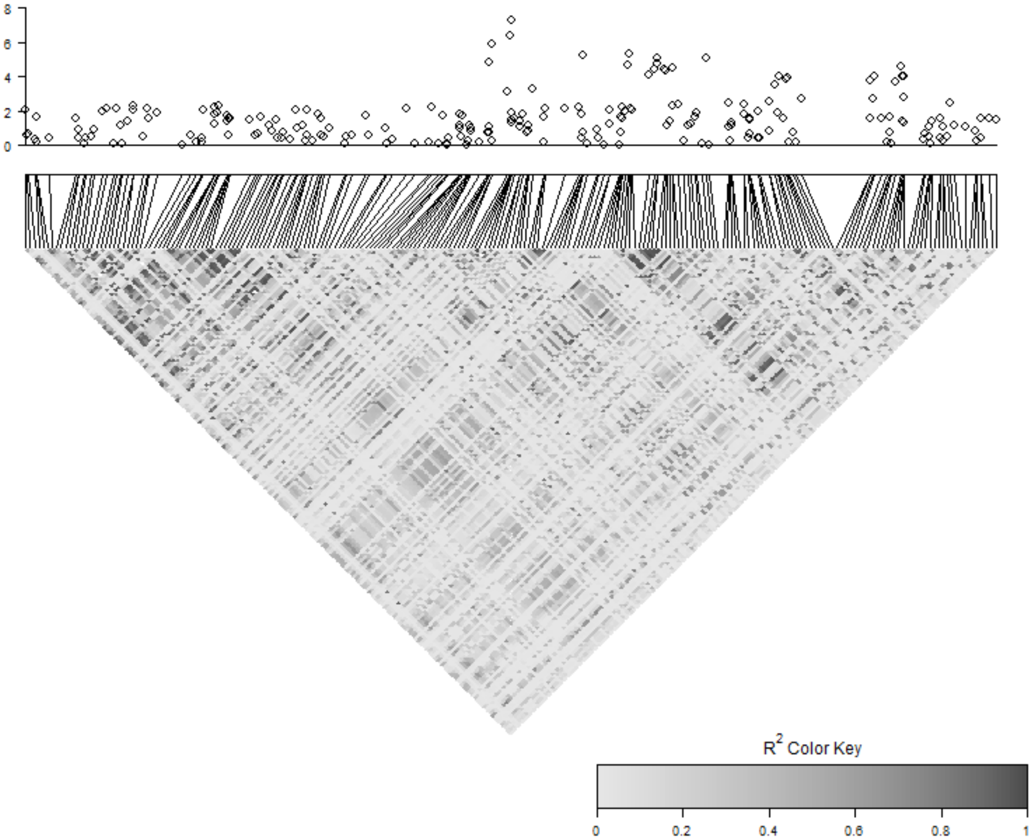


Figure 4.3: Summaries of genome-wide association studies (GWAS) for root (blue) and shoot (black) sodium concentrations in 318 rice genotypes. Manhattan plots for both phenotypes are shown in sub-plot A where the dashed lines indicate the 10% false discovery rate threshold in for roots and shoots in their respective colours. Sub-plot B shows an enlargement for the top of the peaks on chromosome 6 in A in relation to the positions of *OsHKT2;1* and *OsHKT2;4*. The linkage disequilibrium (LD) across the quantitative trait loci NaR.lt.ALL-6.1 and NaS.lt.ALL-6.1 identified from the GWAS results is depicted in sub-plot C. The GWAS results for the shoot sodium concentration are shown on the Manhattan plot above a triangle depicting the pairwise linkage disequilibrium between single nucleotide polymorphisms (measured using R^2) where darker shading represents stronger LD.

The presence of several different QTLs across many phenotypes in the context of low potassium supply is in keeping with previous QTL studies which used crosses between small numbers of rice genotypes (Figure 4.1). In particular, Fang *et al.* (2015) reported QTLs which together spanned a large expanse of the rice genome, although the QTLs described in the present study were generally much narrower regions of the rice genome. Nevertheless, some QTLs from the GWAS analyses could still be in the range of one million base pairs wide when several closely situated SNPs were found to be statistically significantly associated with a phenotype (*e.g.* RCS.trj-9.1, see Table 4.1).

Co-localisation of QTLs identified in different studies has been relatively rare for potassium deficiency in rice (Figure 4.1). One reason for this is that the identification of QTLs can be sensitive to differences in environmental conditions, as was the case for comparisons between glasshouse and field conditions in *Brassica oleracea* (White *et al.*, 2010). Nevertheless, seventeen QTLs found in this study overlapped with previously described rice potassium deficiency QTLs (Figure 4.1). Additionally, QTLs have also been described for potassium concentration in salt stressed rice plants and the QTL KDI.ht.JAPONICA-7.1 co-localises with a QTL for root potassium concentration and content found by Lin *et al.* (2004) located on chromosome 7. QTLs that are repeatedly found across different studies may represent more promising candidates for crop improvement.

Of great potential importance to crop improvement is knowledge of which genes within QTLs are the drivers of differences in KUE. It should be noted that there were no statistically significantly over-represented GO terms relative to the background in the rice genome for all of the genes found within QTLs in this study and so candidate genes should be treated with appropriate caution. For example, while the discovery of three genes with GO terms relating to potassium transport may appear highly relevant, the number of genes coding for proteins involved in cation transport is no more than would be expected by chance.

Identification of genes found within QTLs in this study and those known to be differentially expressed in potassium stressed rice roots could provide an approach for identifying more robust candidates (Table 4.3). However, comparison with previous transcriptomics studies is not without limitations. As with QTLs, a lot of variation exists in the genes identified as differentially expressed between studies. Despite this, genes which co-occur in this and previous transcriptomics studies may prove to be good targets for crop improvement. Interestingly, the genes which have been found to be differentially expressed and which occur within QTLs (Table 4.3) tend to be involved in regulatory roles, pointing to a potential importance of proteins such as transcription factors (for example) rather than transporters for improving KUE. Furthermore, genes within QTLs which co-localised with the previous literature and that were also found to be differentially regulated under potassium stress were present. The QTL QP.tej-7.5 was in the same genomic region as a QTL for shoot dry weight, plant dry weight, and height found by Fang *et al.* (2015). This QTL encompassed the genes LOC_Os07g48340 (OsCML24 - Calmodulin-related calcium sensor protein, expressed), LOC_Os07g48680 (zinc finger, C3HC4 type domain containing protein, expressed), and LOC_Os07g48490 (stress responsive protein, putative, expressed) which were found to have a transcription level response to potassium deficiency (Takehisa *et al.*, 2013). Furthermore, a putative expressed thioredoxin (LOC_Os03g07226) was present within the QTL KDI.lt.trj-3.1 and it was also found to be down-regulated under potassium (Ma *et al.*, 2012) as well as nitrogen and phosphorus (Cai *et al.*, 2013) deficiency in rice.

The statistically significantly associated SNPs themselves may also point to candidates for crop improvement. While GWAS does not guarantee that a causative SNP will be identified (Korte and Farlow, 2013), the presence of SNPs associated with traits that are within genes should not be ignored as they may influence KUE and linkage disequilibrium is (on average) strongest over short distances. Sixty-seven genes contained statistically significantly associated SNPs from the

GWAS analyses. Sixteen of these (Table 4.2) contained non-synonymous SNPs that could be causative of differences in phenotypes (*e.g.* Chao *et al.*, 2014; Hartley and Maathuis, 2016). Seven of these exist within a single QTL (RCS.trj-9.1) and a single gene within this QTL (LOC_Os09g24240: pentatricopeptide) contains three non-synonymous SNPs. Interestingly, not only can there be several non-synonymous SNPs within a QTL, several genes reported to be differentially expressed can also be found within single QTLs. This may be indicative of QTLs containing several genes relevant to KUE. However, linkage disequilibrium in rice complicates fine-mapping of QTLs from GWAS studies and this may be a particular problem in the *Japonica* sub-species, where linkage disequilibrium can remain relatively high over several hundred kbp (Zhao *et al.*, 2011; McCouch *et al.*, 2016).

Two synonymous SNPs exist within the gene for the root sodium-selective transporter *OsHKT2;1* (Figure 4.3). This gene was also identified in a QTL found by Miyamoto *et al.* (2012) and it is expressed more strongly in roots than shoots (Miayomoto *et al.*, 2015), with particularly strong expression in peripheral root tissues (Jabnoune *et al.*, 2009). Its expression is increased under potassium deficiency (Ma *et al.*, 2012) and positively associated with sodium accumulation in rice, although over-expressing lines only accumulated sodium in shoots under potassium deficient conditions (Miyamoto *et al.*, 2015). Another class II HKT gene, *OsHKT2;4*, is adjacent to *OsHKT2;1* in the rice genome. This gene is expressed more strongly in shoots than roots (Miyamoto *et al.*, 2015) and its expression is not up-regulated by low-potassium stress (Shankar *et al.*, 2013). This would point to *OsHKT2;1* being the more promising candidate for improving rice tolerance of potassium stress through partial replacement with sodium.

In summary, GWAS was used for the first time to explore the genetic architecture of KUE in a crop. Several QTLs were identified, many of which co-localised with previously identified QTLs for potassium deficiency in rice. Furthermore, many novel QTLs were described and some of these were specific to sub-species and sub-populations in rice. Candidate genes relevant to KUE could be proposed from within QTLs based on containing SNPs identified in GWAS analyses as well as previously identified transcription-level response to potassium deficiency in rice. Among these, the gene encoding a sodium-selective OsHKT2;1 transporter provides a compelling target for improving tolerance of rice to KUE based on the findings in this study and previous characterisation in the literature.

Chapter 5

General Discussion

5.1 Potassium Use Efficiency in Crops and Targets for Improvement

Potassium use efficiency (KUE) is an important agricultural trait that needs to be optimised in order to ensure adequate yield for future food demand (Section 1.1; Rengel and Damon, 2008; Römheld and Kirkby, 2010; White, 2013; Wang and Wu, 2015). Large areas of agricultural land around the world are currently deficient for potassium (Römheld and Kirkby, 2010) and potash fertilisers need to be applied to soils to balance agricultural off-take and compensate historical under-fertilisation (Dobermann, *et al.*, 1998; Pathak *et al.*, 2010; Timsina *et al.*, 2013). While potash reserves are plentiful (USGS, 2017), the geographical distribution of their production (Ciceri *et al.*, 2015), potential for price spikes (World Bank, 2017), and environmental consequences of potash production, distribution, and use (Ciceri *et al.*, 2015; Sarands and Peñuelas, 2015) mean that increasing crop KUE would be highly beneficial (see Section 1.1).

This work set out to address important issues in improving crop KUE. Firstly, details of how KUE is best measured were addressed (Chapter 2), before KUE itself was measured and linked to physiological traits in rice (Chapter 3). The genetic underpinnings of these differences in KUE were then explored (Chapter 4). The results and implications of the work presented in this thesis are discussed below.

5.1.1 The Measurement of Potassium Use Efficiency

The way in which KUE is measured is of great importance when attempting to identify efficient crop genotypes. To this end, many potential metrics have been proposed (*e.g.* Baligar *et al.*, 2001; Good *et al.*, 2004; Moriconi and Santa-María, 2013) and papers routinely report several different metrics. Given the multitude of processes involved in potassium acquisition, distribution, and use in plants (see Section 1.2), the use of a selection of metrics that explore different facets of KUE can be useful. However, it is still the case that different metrics can measure apparently similar processes and so optimal metric selection in screening studies is therefore not a trivial issue.

In Chapter 2, a model of potassium in plants was constructed based on observations from growth experiments using *cv.* IR64 rice exposed to a range of external solution potassium concentrations (10 to 5,000 μM). Sensitivity analysis on this model was then used to reveal which plant physiological parameters were theoretically the most important in causing genotype-specific differences in metrics. Summarising this information using principal component analysis provided a way to explore how similarly different metrics responded to changes in plant physiology.

The model presented in Chapter 2 provided a powerful tool with which to explore the implications of differences in plant physiological parameters on KUE measurement without experimental constraints or potentially limited variation within a selection of rice genotypes. At the same time, in summarising key aspects of the uptake, distribution, and use of potassium by plants using relatively simple mathematical functions, some potentially important nuances of potassium in plants were not modelled explicitly. For example, the re-distribution of potassium from older to younger leaves has been found to increase utilisation efficiency in rice (Yang *et al.*, 2004), but the mathematical model used here considered the shoot as a uniform compartment. Despite this, the impact of efficient distribution of potassium in the shoot could be observed through the parameters β_{shoot} and σ_{shoot}, which governed how the shoot growth rate responded to its internal potassium concentrations. However, parameters such as redistribution in the shoot would also apply for the impacts on growth of replacement of potassium by sodium, for example, and so a full mechanistic explanation of KUE was not present in the model. Nevertheless, relatively simple models of potassium in plants have been used successfully before to explore potassium utilisation efficiency (Moriconi and Santa-María, 2013) and the model presented in Chapter 2 both captured key features of potassium in plants (Figures 2.4 to 2.8) and contained enough complexity to distinguish the importance of several relevant physiological parameters to KUE metrics (Figures 2.11 to 2.14).

This work showed that the classes of metrics that were defined based on their calculation (*e.g.* utilisation or ratio metrics) are generally good indicators of how a metric responds to physiology. However, exceptions existed. Examples of this were ratio metrics for root potassium concentration or content (RCR and RQR) which showed diverging responses compared to other ratio metrics at lower external potassium concentrations. The latter shows that metrics can respond to experimental conditions as well as physiology and this is an important consideration since external potassium concentrations can vary greatly over the length of an experiment.

Another important question is how well KUE metrics measured at the vegetative stage of plant growth related to economic outputs such as grain yield. Screening often takes place on young plants due to lower costs and time considerations, but the evidence has been mixed as to how useful this may be for predicting yield under potassium stress (*e.g.* Woodend and Glass, 1993; Yang *et al.*, 2003; Damon *et al.*, 2007). Results from this work suggest that vegetative metrics cannot be relied upon to predict grain yield if the harvest index varies in response to stress. This is in agreement with Rengel and Damon (2008) who suggested that the response of the harvest index to potassium stress may be a dominant determinant of KUE in terms of economic output, although the importance of this may vary between species. Previous evidence in rice suggests that vegetative measures of utilisation efficiency were linked with yield (Yang *et al.*, 2003), suggesting that while the model identifies large theoretical influence of harvest index response to potassium stress, this may not manifest itself in cultivars.

The key drivers that were identified by the sensitivity analyses as underlying difference in KUE metrics warrant some attention. While it is tempting to define any plant that scores well for a given metric as a good genotype to use under potassium stress, evidence from this work shows how such a notion can be potentially misleading. For example, the ratio metrics were highly responsive to poor performance under potassium replete conditions, allowing moderately performing plants to appear highly efficient in response to low-potassium stress. This was observed in the screen (see Section 3.4), where, for example, the highest performing genotypes for the relative plant mass (RP) were not among the genotypes with the largest average plant mass under potassium stress. Similarly, utilisation efficiency metrics have a risk of selecting for plants that take up relatively small amounts of potassium rather than having optimised use of potassium in tissues. Indeed, the apparent trade-off between uptake and utilisation found in experiments is not unique to potassium, with evidence for a similar relationship for nitrogen (Chardon *et al.*, 2010) and phosphorus (Rose *et al.*, 2011) use efficiency.

Both the mathematical and empirical analyses of KUE presented in Chapters 2 and 3 highlight how great care should be taken in interpreting KUE metrics. Different experimental design has been suggested as a way to alleviate the problem of a trade-off between uptake and utilisation, such as growing plants in separate nutrient solutions with very low amounts of a nutrient available (Rose *et al.*, 2011). While this may have some impact for utilisation metrics, other issues, such as the potential for ratio metrics to select for unremarkable performance under stress coupled with poor performance under replete conditions would not be solved through alterations in experimental procedures. A solution to such problems could be statistical correction by including agronomically deleterious correlates as co-factors when calculating use efficiency metrics. In this way, agronomically unappealing aspects of metrics may be controlled as long as the correlates could be measured experimentally.

Ultimately, work presented in Chapter 2 was able to explore how different ways of measuring KUE inter-relate and respond to difference in plant physiology. From this, it was possible to classify metrics and suggest a selection of metrics that represents each of these groups. Using such a selection of metrics enables studies to explore KUE fully while minimising the risk of redundancy in metric choice. At the same time, potential drawbacks of KUE metrics were found that could be alleviated through experimental or analytical techniques. Taken together, the study described in Chapter 2 was able to advance understanding of KUE measurement and inform metric choice.

5.1.2 Quantification of Potassium Use Efficiency in Rice

The identification of potassium efficient crop genotypes is a crucial step in the development of new elite lines for use in agriculture. Rice is a good crop to use for such work as it is both highly important to agriculture and relatively tractable experimentally and genetically (Section 1.4). Some previous work has explored KUE in rice (Yang *et al.*, 2003; Liu *et al.*, 2009), but studies with a comparable diversity and number of genotypes as available in the Rice Diversity Panel 1 (RDP1) have not previously been carried out. The aim of Chapter 3 was to utilise the RDP1 resource and experimentally explore the underlying physiology of differences in rice KUE.

Over 300 rice cultivars were analysed in this study using a selection of KUE metrics based on the results of Chapter 2. Large scale variation at the genotype level was found for these metrics, suggesting that there is plentiful genetic variation in rice for future crop improvement. The scale of the variation was quite high across the traits compared to the previous literature and this may result from the large scale and diversity of the panel used in this study.

The high levels of variation in KUE observed in the RDP1 lends support to the belief that germplasm diversity can be mined for future crop improvement (Rengel and Damon, 2008; Hawkesford, 2012; Wang and Wu, 2015). Furthermore, variation in KUE is generally high between genotypes of other crops besides rice (*e.g.* Woodend and Glass, 1993; Fageria *et al.*, 2001; Trehan *et al.*, 2005; Damon *et al.*, 2007; White *et al.*, 2010). To effectively exploit germplasm diversity, the causes of the variation need to be known so that the traits which give rise to enhanced KUE can be bred for. While the importance of traits may vary between species, many key drivers of differences in the six KUE metrics used in Chapter 3 were identified.

Multiple regression models which associated the KUE metrics with physiological parameters generally explained high proportions of the variation in these traits ($R^2 > 0.7$), except for the relative plant mass between low- and replete-potassium conditions (RP; $R^2 = 0.19$). For this metric, the shoot sodium concentration was the most important predictor and was positively correlated with RP. Indeed, the shoot sodium concentration was found to be positively associated with four of the six metrics analysed. This suggests that replacement of potassium by sodium may offer a broadly beneficial target for KUE improvement in rice and there is evidence to suggest that other crop species also benefit from moderate sodium uptake in the absence of potassium (Leigh *et al.*, 1986; Subbarao *et al.*, 1999; Gattward *et* *al.*, 2012).

The key physiological drivers of variation in KUE between rice genotypes differed between the various metrics but generally agreed with those identified by sensitivity analyses as presented in Chapter 2. For example, the importance of a high translocation rate of potassium from root to shoot for a high relative root potassium concentration (RCR) was found in rice (Table 3.7) and in the model presented in Chapter 2 (Figure 2.12). The link between a high potassium utilisation ratio (KUtR) and low rates of potassium uptake, as described in Chapter 2, was found to be a key driver of differences in rice KUtR at the genotypic level (Table 3.4), lending support for the validity of the findings of Chapter 2. Given the potential for metrics to select for agronomically deleterious traits as observed in Chapters 2 and 3, it may be the case that future work could have greater benefit to agriculture by focussing on physiological traits that can benefit KUE rather than metrics for KUE.

Many of the traits identified as key drivers of differences in rice KUE agree with plant characteristics previously proposed as candidates for crop improvement, such as early growth vigour and efficient uptake of potassium (Rengel and Damon, 2008; White, 2013). Several of these are likely to be beneficial for use efficiencies of other elements too (Hawkesford, 2012; White 2013). For example, root systems that can exploit large areas can aid nitrogen and phosphorus uptake as well as potassium. It may be that greater overall benefit to agriculture is gained through targeting crop traits that are generic to the use efficiencies of several nutrients, but this does not mean that nutrient-specific traits should be ignored. Replacement of potassium by sodium is an example of a trait that is more KUE-specific. However, sodium could also act as a counter ion to nitrate, aiding the delivery of nitrogen to the shoot. Indeed, the importance of potassium to the use and distribution of other nutrients and water (Clarkson and Hanson, 1980; Zörb *et al.*, 2014) means that even apparently potassium-specific crop improvements could be more broadly beneficial to plants.

Taken together, data from Chapter 3 not only quantified the large scale variation in KUE present across the rice diversity panel, but also empirically identified key physiological traits that give rise to this variation. Many of these traits could form targets for crop improvement.

5.1.3 The Genetic Architecture of Potassium Use Efficiency in Rice

Knowledge of the genetic underpinnings of KUE is an important resource for crop improvement. However, the identification of reliable and robust markers can be challenging. Previous work to identify potassium related quantitative trait loci (QTLs) in rice has yielded little overlap in genomic regions and the use of transcriptomics studies has shown that differential expression of genes in response to low-potassium stress is also highly variable. Genome-wide association studies (GWAS) provide a complementary technique which can identify QTLs that are typically narrower than those previously described, usually resulting in genomic regions with tens of genes rather than hundreds. This greater resolution facilitates subsequent analysis of specific markers for their relevance to KUE. Furthermore, the use of large populations allows identification of QTLs that are specific to sub-species or sub-populations.

The application of GWAS to KUE in the RDP1 yielded 98 QTLs across all phenotypes and population sub-structure level combinations (Chapter 4). The presence of QTLs specific to only parts of rice population sub-structure may seem contradictory with the results in Chapter 3 where differences in KUE between sub-populations were small or non-existent. However, it may be the case that different sub-populations achieve similar levels of KUE through different means at the genetic level. Potassium deficiency can be found across Asia (Tanaka and Yoshida, 1965) and it is likely that ancestral rice plants from both the *Japonica* and *Indica* sub-species were required to cope with low-potassium conditions and still yield adequately. This may provide an explanation for why similar KUE levels across the RDP1 genotypes are associated with different QTLs.

Some of the QTLs identified in this study either co-localised with previously identified QTLs and/or with genes found to be differentially expressed when potassium is in limited supply. Among these was the sodium transporter OsHKT2;1. The function of HKT2;1 (Horie *et al.*, 2007; Miyamoto *et al.*, 2015) in combination with GWAS and transcriptomics data (Takehisa *et al.*, 2013) make it a compelling candidate for crop improvement. The QTL containing the gene that encodes this protein was associated with root and shoot sodium concentration, while the shoot sodium concentration was positively associated with four of the six KUE metrics used in Chapter 3. Sodium can replace potassium in many of its functions within plants (Subbarao *et al.*, 1999; Gattward *et al.*, 2012; Benito *et al.*, 2014) and was found to accumulate to greater concentrations in the low-potassium treatment compared to the potassium replete treatment (Figure 3.3). Furthermore, characterisation studies on this gene confirm its relevance as a method for sodium entry into plants (Horie *et al.*, 2007; Miyamoto *et al.*, 2015), facilitating the replacement of potassium. Interestingly, Oomen *et al.* (2012) identified a new root-expressed HKT isoform, No-*OsHKT2;2/1*, in a study which utilised natural polymorphisms. This isoform was implicated in salt tolerance as it maintains high potassium permeability, even when external sodium concentrations are high, unlike OsHKT2;1.

Apart from HKT2;1, comparison of the genes identified in this study with those that have been found to be differentially expressed under low-potassium stress revealed few transport proteins. In fact, only three genes encoding proteins with annotations relating to potassium transport were found within QTLs from this study, a number no higher than would be expected by chance. However, genes encoding proteins with regulatory roles were abundant among those identified in this study and previous transcriptome analyses. This may point to regulatory processes being a more promising target for crop improvement than transport proteins.

An important issue in seeking genetic improvement in crop characteristics is the heritability of important characteristics (*i.e.* how much of the observed variation is a result of genetic as opposed to environmental causes). The environment can have an important influence on genomic regions identified as related to a trait. For example, the flowering time of rice was found to have different regions of the genome associated depending on where the rice plants were grown (see supplementary information of Zhao *et al.*, 2011). The narrow-sense heritability varied greatly among the six KUE metrics. The potassium utilisation ratio (KUtR) had a low heritability of 0.06 (on scale of 0 to 1, estimated using the *polygenic()* function in **GenABEL**; Aulchenko, 2007). The relative shoot potassium concentrations (RCS) also had a very low heritability of 0.006. The other ratio metrics for root potassium concentration (RCR) and plant dry mass (RP) had a greater heritability, with values of 0.31 and 0.36 respectively. The whole plant potassium content (QP; 0.40) and plant mass (P; 0.56) had the highest heritability values. This suggests that RCS and KUtR may be poor candidates for genetic improvement of rice KUE, while P and QP are more promising. The lack of especially high heritability values for KUE may be a reason for limited progress in improving crop KUE. Heritability for phosphorus use efficiency has also been described as low (van der Wiel *et al.*, 2016), and so a lack of genetic basis to variation observed in screens may pose a problem to attempts to improve nutrient use efficiency in general.

However, KUE can still be considered a heritable property, even if environmental variation can have an important influence on study results. As a result, the identification of QTLs and candidate genes remains a valid approach for improving crop KUE. To this end, the study presented in Chapter 4 used a novel approach to contribute to the collection of KUE studies at the genetic level (*e.g.* Wu *et al.*, 1998; Miyamoto *et al.*, 2012; Fang *et al.*, 2015). Along with several novel loci, the relevance of sub-population structure to the genetics of KUE was explored for the first time. Evidence has been reported for sub-population-specific associations for many traits in rice (Famoso *et al.*, 2011; Zhao *et al.*, 2011; Crowell *et* *al.*, 2016) and several were found for KUE (Table 4.1). Re-discovery of QTLs and genes previously identified as relevant to low-potassium stress adds confidence that these associations could be causative as opposed to spurious links that arose from environmental factors or physical linkage.

In summary, having applied GWAS for the first time to study KUE in rice, several novel QTLs were identified including many which co-localised with those described in the literature. Many QTLs were found to be sub-population or sub-species specific, suggesting that the genetic diversity in rice could be mined for future crop improvement. Furthermore, candidate genes could be proposed from this work that may prove to be credible targets for improvement of crop KUE.

5.2 Concluding Remarks

The improvement of KUE in crops is required to ensure agricultural output that meets food demand while also being economic and minimising environmental impacts. Therefore, understanding the mechanisms by which KUE differs at a physiological and genetic level is of great importance to the development of new elite cultivars. The work presented in this thesis aimed to address key issues in this process. Firstly, the way in which KUE is measured in experiments was explored and metric selection was based upon the results of Chapter 2. Next, KUE was measured in rice and the scope of variation in this trait was quantified along with empirical identification of drivers of differences in KUE between genotypes (Chapter 3). Finally, the genetic underpinnings giving rise to these differences was explored and revealed a rich genetic architecture as well as potential targets for improvement of crop KUE. Exploitation of the results of this work could both aid further exploration of this important trait and may eventually lead to crops with improved KUE.

Chapter 6

Appendices

6.1 Appendix for Chapter 2

6.1.1 Calculation of Model Parameters

Maximal Theoretical Uptake Rate

Assuming a constant root to plant mass ratio, δ, with a replete external solution potassium concentration of 1,000 µM, the root and shoot compartments must grow with the same realised relative growth rate, R. Furthermore, the root, γ_{root}, and shoot, γ_{shoot}, tissue potassium concentrations are assumed constant under these conditions. As a result, the whole plant demand for potassium per day ($\left(\frac{dQ_{plant}}{dt}\right)^*$), which is satisfied by root uptake, can be expressed as:

$$\left(\frac{dQ_{plant}}{dt}\right)^* = R\gamma_{root}W_{root} + R\gamma_{shoot}W_{shoot} \tag{6.1}$$

where all parameters are as defined above and in Chapter 2.

Equation 6.1 can be substituted into Equation 2.1 to give:

$$R\gamma_{root}W_{root} + R\gamma_{shoot}W_{shoot} = \frac{\mu S}{\kappa + S}W_{root} \tag{6.2}$$

which can be re-arranged, expressing the mass of root and shoot as a proportion of the whole plant mass, to give:

$$\frac{R\gamma_{root}\delta W_{plant} + R\gamma_{shoot}(1-\delta)W_{plant}}{\delta W_{plant}} = \frac{\mu S}{\kappa + S} \tag{6.3}$$

and this can be simplified to give:

$$\frac{R(\gamma_{shoot} + \delta(\gamma_{root} - \gamma_{shoot}))}{\delta} = \frac{\mu S}{\kappa + S} \tag{6.4}$$

and re-arranged to make μ the subject as follows:

$$\mu = \frac{\left(\frac{R(\gamma_{shoot}+\delta(\gamma_{root}-\gamma_{shoot}))}{\delta}\right)(\kappa + S)}{S} \tag{6.5}$$

from which the maximal uptake rate, μ, can be calculated. Using values from Table 2.2 for δ, κ, γ_{root}, and γ_{shoot}, as well as 0.1 for R (Section 2.3.1) and 1,000 µM for S results in a value of μ of 58.9 (to 3 significant figures) µmol (g root FW)$^{-1}$ d^{-1}.

Maximal Theoretical Net Translocation Rate of Potassium to the Shoot

A value for maximal rate of net potassium supply to the shoot per gram of shoot per day that is consistent with the above assumptions can be calculated in a similar fashion to the maximal net uptake rate described above. The shoot demand for potassium under the outlined conditions $\left(\frac{dQ_{shoot}}{dt}\right)^*$ can be expressed as:

$$\left(\frac{dQ_{shoot}}{dt}\right)^* = R\gamma_{shoot}W_{shoot} \tag{6.6}$$

This can be substituted into Equation 2.2, with the root potassium concentrations expressed as γ_{root}, as follows:

$$R\gamma_{shoot}W_{shoot} = \frac{\chi\gamma_{root}^{\theta}}{\phi^{\theta} + \gamma_{root}^{\theta}}W_{shoot} \tag{6.7}$$

This can be re-arranged and simplified to give:

$$\chi = \frac{(R\gamma_{shoot})(\phi^{\theta} + \gamma_{root}^{\theta})}{\gamma_{root}^{\theta}} \tag{6.8}$$

From this, a value of 11.8 (to 3 significant figures) µmol (g shoot FW)$^{-1}$ d^{-1} for χ could be calculated using the values for ϕ, θ, γ_{root}, and γ_{shoot} from Table 2.2 and 0.1 for R.

Maximal Theoretical Relative Growth Rates

Maximum theoretical relative growth rates (α_{root} and α_{shoot}) were calculated as outlined below given the conditions and assumptions outlined above. For the i^{th} tissue (*i.e.* root or shoot), the growth rate in under the conditions outlined above ($\left(\frac{dW_i}{dt}\right)^*$) is:

$$\left(\frac{dW_i}{dt}\right)^* = RW_i \tag{6.9}$$

and this can be substituted into Equation 2.3, with the tissue potassium tissues represented with γ_i, as follows:

$$RW_i = \frac{\alpha_i \gamma_i^{\sigma_i}}{\beta_i^{\sigma_i} + \gamma_i^{\sigma_i}} W_i \tag{6.10}$$

which can be re-arranged and simplified to give:

$$\alpha_i = \frac{R(\beta_i^{\sigma_i} + \gamma_i^{\sigma_i})}{\gamma_i^{\sigma_i}} \tag{6.11}$$

and using the values in Table 2.2 for β_{root}, σ_{root}, γ_{root} and using 0.1 for R resulted in a values of α_{root} of 0.129 (to 3 significant figures) d^{-1}. For α_{shoot}, a value of 0.105 (to 3 significant figures) d^{-1} using values from Table 2.2 for β_{shoot}, σ_{shoot}, γ_{shoot} and using 0.1 for R.

6.1.2 Potassium Use Efficiency Metrics with Potassium Replete Conditions

The results of applying sensitivity analysis to the model (described in Chapter 2) with a fixed external potassium concentration of 950 µM are summarised in Figure 6.1.

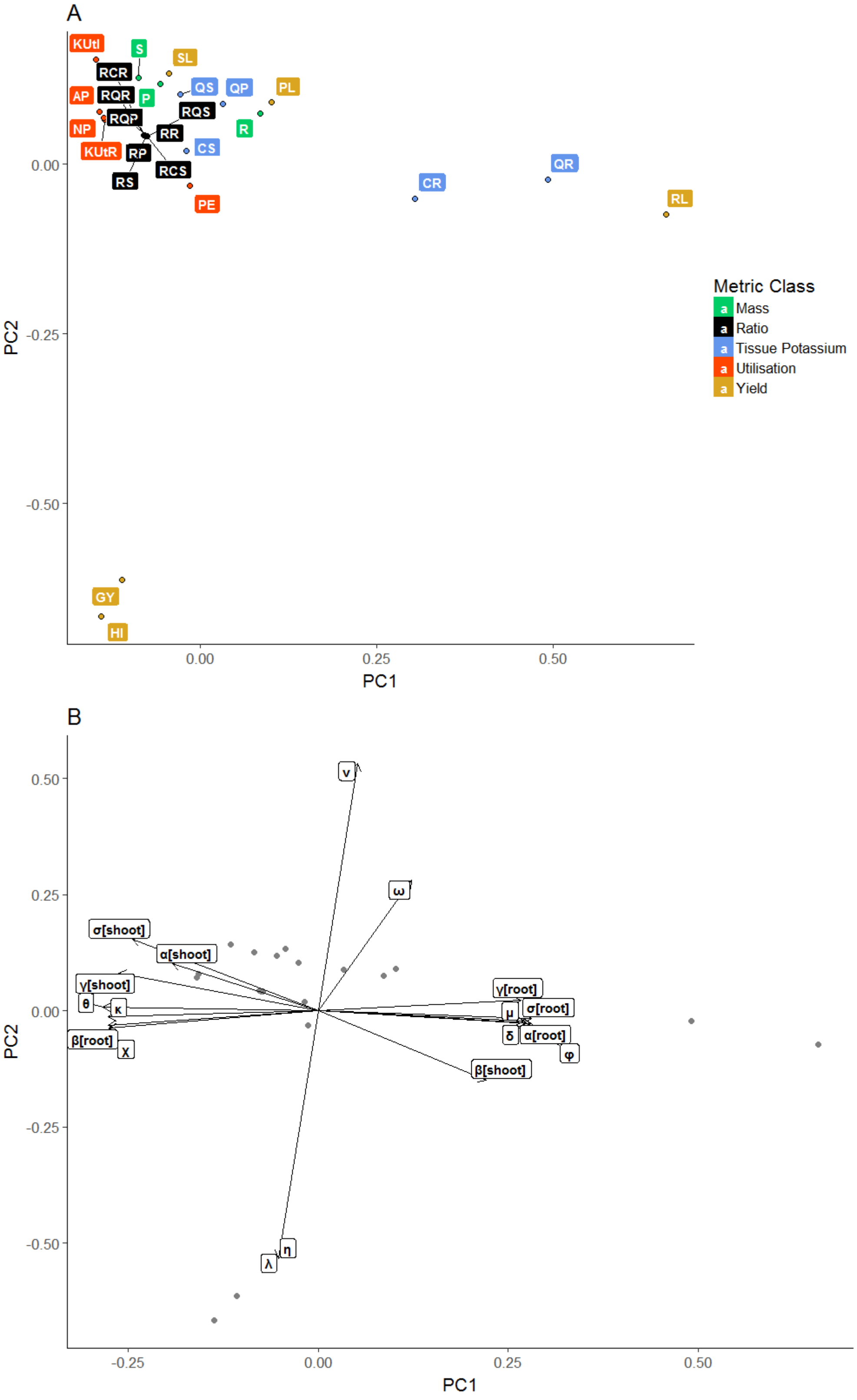


Figure continued on next page.

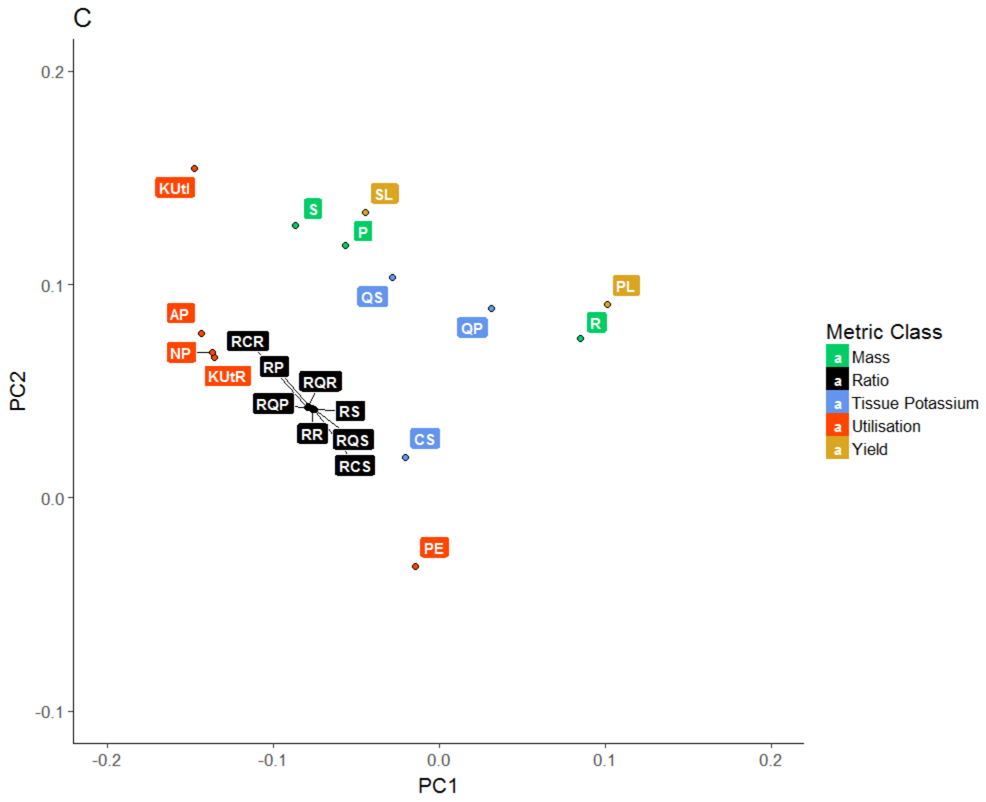
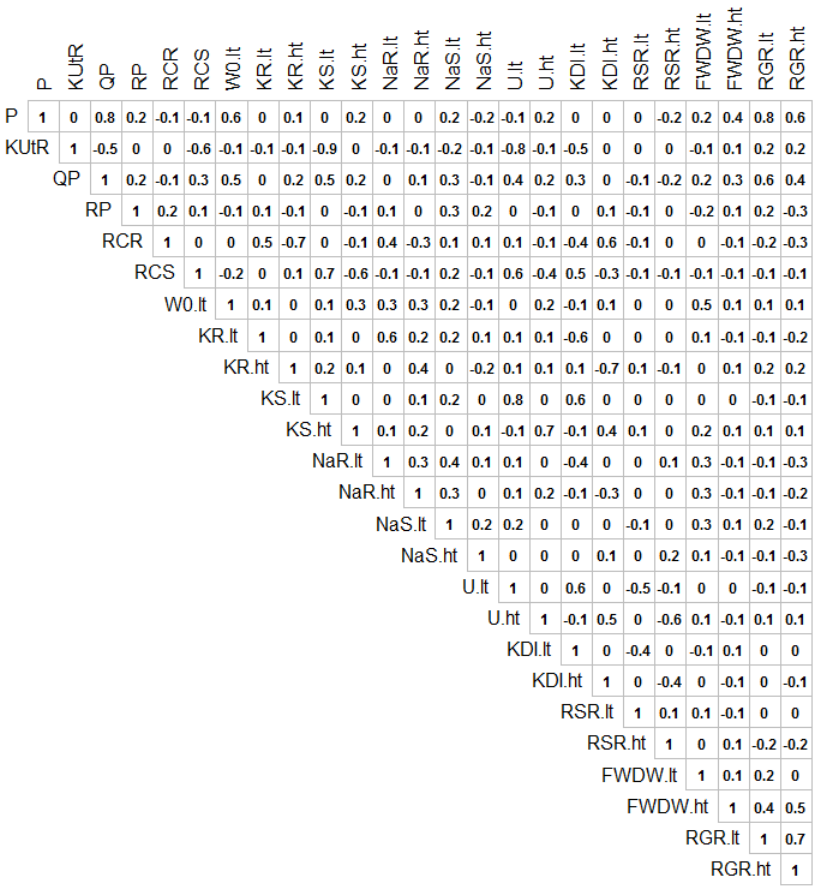


Figure 6.1: Metrics scores in the largest two principal components which explain 69% (first) and 18% (second) of the variation respectively with a fixed external potassium treatment if 950 µM. Each dot represents a metric score which and labels give the metric identities. Sub-plot A shows the PCA scores of all KUE metrics used in this study for the top two principal components. B shows the loadings of model parameters and C is the same as sub-plot A, but zoomed in on the main cluster of metrics.

6.2 Appendix for Chapter 3



	P	KUtR	QP	RP	RCR	RCS	W0.lt	KR.lt	KR.ht	KS.lt	KS.ht	NaR.lt	NaR.ht	NaS.lt	NaS.ht	U.lt	U.ht	KDI.lt	KDI.ht	RSR.lt	RSR.ht	FWDW.lt	FWDW.ht	RGR.lt	RGR.ht
P	1	0	0.8	0.2	-0.1	-0.1	0.6	0	0.1	0	0.2	0	0	0.2	-0.2	-0.1	0.2	0	0	0	-0.2	0.2	0.4	0.8	0.6
KUtR		1	-0.5	0	0	-0.6	-0.1	-0.1	-0.1	-0.9	0	-0.1	-0.1	-0.2	-0.1	-0.8	-0.1	-0.5	0	0	0	-0.1	0.1	0.2	0.2
QP			1	0.2	-0.1	0.3	0.5	0	0.2	0.5	0.2	0	0.1	0.3	-0.1	0.4	0.2	0.3	0	-0.1	-0.2	0.2	0.3	0.6	0.4
RP				1	0.2	0.1	-0.1	0.1	-0.1	0	-0.1	0.1	0	0.3	0.2	0	-0.1	0	0.1	-0.1	0	-0.2	0.1	0.2	-0.3
RCR					1	0	0	0.5	-0.7	0	-0.1	0.4	-0.3	0.1	0.1	0.1	-0.1	-0.4	0.6	-0.1	0	0	-0.1	-0.2	-0.3
RCS						1	-0.2	0	0.1	0.7	-0.6	-0.1	-0.1	0.2	-0.1	0.6	-0.4	0.5	-0.3	-0.1	-0.1	-0.1	-0.1	-0.1	-0.1
W0.lt							1	0.1	0	0.1	0.3	0.3	0.3	0.2	-0.1	0	0.2	-0.1	0.1	0	0	0.5	0.1	0.1	0.1
KR.lt								1	0	0.1	0	0.6	0.2	0.2	0.1	0.1	0.1	-0.6	0	0	0	0.1	-0.1	-0.1	-0.2
KR.ht									1	0.2	0.1	0	0.4	0	-0.2	0.1	0.1	0.1	-0.7	0.1	-0.1	0	0.1	0.2	0.2
KS.lt										1	0	0	0.1	0.2	0	0.8	0	0.6	0	0	0	0	0	-0.1	-0.1
KS.ht											1	0.1	0.2	0	0.1	-0.1	0.7	-0.1	0.4	0.1	0	0.2	0.1	0.1	0.1
NaR.lt												1	0.3	0.4	0.1	0.1	0	-0.4	0	0	0.1	0.3	-0.1	-0.1	-0.3
NaR.ht													1	0.3	0	0.1	0.2	-0.1	-0.3	0	0	0.3	-0.1	-0.1	-0.2
NaS.lt														1	0.2	0.2	0	0	0	-0.1	0	0.3	0.1	0.2	-0.1
NaS.ht															1	0	0	0	0.1	0	0.2	0.1	-0.1	-0.1	-0.3
U.lt																1	0	0.6	0	-0.5	-0.1	0	0	-0.1	-0.1
U.ht																	1	-0.1	0.5	0	-0.6	0.1	-0.1	0.1	0.1
KDI.lt																		1	0	-0.4	0	-0.1	0.1	0	0
KDI.ht																			1	0	-0.4	0	-0.1	0	-0.1
RSR.lt																				1	0.1	0.1	-0.1	0	0
RSR.ht																					1	0	0.1	-0.2	-0.2
FWDW.lt																						1	0.1	0.2	0
FWDW.ht																							1	0.4	0.5
RGR.lt																								1	0.7
RGR.ht																									1

Figure 6.2: Correlation matrix of metrics and physiological characteristics of rice genotypes.

Table 6.1: The top ten genotypes for plant dry mass (P).

Name	**Sub-population**	**Mean Dry Mass (g)**
Zerawchanica Karatalski	*temperate japonica*	0.86
WIR 3764	*temperate japonica*	0.75
Sathi	*aus*	0.71
Nira	*indica*	0.66
Goria	*aus*	0.65
Bombilla	*temperate japonica*	0.65
Yodanya	*indica*	0.64
Agusita	*temperate japonica*	0.64
Tropical Rice	*temperate japonica*	0.64
Italica Carolina	*temperate japonica*	0.63

Table 6.2: The top ten genotypes for the potassium utilisation ratio (KUtR).

Name	**Sub-population**	**Mean KUtR (g mmol^{-1})**
O-Luen-Cheung	*indica*	13.7
PTB 30	*aus*	13.5
Sathi	*aus*	13.4
PR 304	*tropical japonica*	12.6
Sadri Belyi	*aromatic*	11.5
Arias	*tropical japonica*	11.2
Victoria F.A.	*temperate japonica*	11.2
TOg 7178	*admixed indica*	11.1
Keriting Tingii	*admixed japonica*	10.7
DM 59	*aus*	10.6

Table 6.3: The top ten genotypes for the plant potassium content (QP).

Name	**Sub-population**	**Mean QP (μmol)**
WIR 3764	*temperate japonica*	147
Zerawchanica Karatalski	*temperate japonica*	130
Goria	*aus*	120
Lady Wright Seln	*tropical japonica*	116
Agusita	*temperate japonica*	108
NSFTV-362	*tropical japonica*	103
Nira	*indica*	94
Aijiaonante	*indica*	94
Peh-Kuh-Tsao-Tu	*indica*	93
M. Blatec	*temperate japonica*	93

Table 6.4: The top ten genotypes for the relative plant mass (RP).

Name	**Sub-population**	**Mean RP (-)**
Okshitmayin	*admixed japonica*	1.04
OS6	*tropical japonica*	1.03
Leuang Hawn	*temperate japonica*	1.02
Kiuki No. 46	*temperate japonica*	1.01
Sitpwa	*temperate japonica*	1.00
Wanica	*tropical japonica*	1.00
Jambu	*tropical japonica*	0.99
Guineandao	*admixed japonica*	0.98
Tainan Iku 487	*temperate japonica*	0.97
O-Luen-Cheung	*indica*	0.97

Table 6.5: The top ten genotypes for the relative root potassium concentration (RCR).

Name	**Sub-population**	**Mean RCR (-)**
Djimoron	*admixed japonica*	1.25
Geumobyeo	*temperate japonica*	0.93
Leuang Hawn	*temperate japonica*	0.92
YRL-1	*admixed japonica*	0.81
Nortai	*admixed japonica*	0.78
B6616A4-22-Bk-5-4	*tropical japonica*	0.75
Sigadis	*indica*	0.75
WC 521	*admixed japonica*	0.72
OS6	*tropical japonica*	0.71

Table 6.6: The top ten genotypes for the relative shoot potassium concentration (RCS).

Name	**Sub-population**	**Mean RCS (-)**
Peh-Kuh-Tsao-Tu	*indica*	0.68
IITA 135	*tropical japonica*	0.60
Cybonnet	*tropical japonica*	0.58
Kaybonnet	*tropical japonica*	0.57
IR64	*indica*	0.53
Ghorbhai	*aus*	0.51
R 101	*tropical japonica*	0.50
Goria	*aus*	0.48
Jasmine85	*indica*	0.46
NSFTV-116	*tropical japonica*	0.45

Abbreviations

ADI: admixed indica

ADJ: admixed japonica

ADM: admixed

ANOVA: analysis of variance

AP: accumulative productivity

ARO: aromatic

BIC: Bayesian Information Criterion

bp: base pairs

CAGR: compound annual growth rate

Chrom.: chromosome

CR: root potassium concentration

CS: shoot potassium concentration

DW: dry weight

FAO: Food and Agricultural Organization of the United Nations

FW: fresh weight

$FWDW_{HT}$: high treatment fresh weight to dry weight ratio

$FWDW_{LT}$: low treatment fresh weight to dry weight ratio

g: grams

GO: gene ontology

GRiSP: Global Rice Science Partnership

GWAS: genome-wide association study

GY: Grain yield

HI: harvest index

IND: indica

IRGSP: International Rice Genome Sequencing Project

IRRI: International Rice Research Institute

KDI_{HT}: high treatment potassium distribution index

KDI_{LT}: low treatment potassium distribution index

KR_{HT}: high treatment root potassium concentration

KR_{LT}: low treatment root potassium concentration

KS_{HT}: high treatment shoot potassium concentration

KS_{LT}: low treatment shoot potassium concentration

KUE: potassium use efficiency

KUtI: potassium utilisation index

KUtR: Potassium utilisation ratio

LMM: linear mixed model

NaR$_{HT}$: high treatment root sodium concentration

NaR$_{LT}$: low treatment root sodium concentration

NaS$_{HT}$: high treatment shoot sodium concentration

NaS$_{LT}$: low treatment shoot sodium concentration

NP: nutrient productivity

P: plant mass

PC: principal component

PCA: principal component analysis

PE: physiological efficiency

PL: long-term plant mass

QP: plant potassium content

QR: root potassium content

QS: shoot potassium content

QTL: quantitative trait locus

RDP1: rice diversity panel 1

RDP2: rice diversity panel 2

R: root mass

RCR: relative root potassium concentration

RCS: relative shoot potassium concentration

RGR$_{HT}$: high treatment relative growth rate

RGR$_{LT}$: low treatment relative growth rate

RL: long-term root mass

RP: relative plant mass

RQP: relative plant potassium content

RQR: relative root potassium content

RQS: relative shoot potassium content

RR: relative root mass

RS: relative shoot mass

RSR$_{HT}$: high treatment root to shoot ratio

RSR$_{LT}$: low treatment root to shoot ratio

USGS: United States Geological Survey

S: shoot mass

SL: long-term shoot mass

SNP: single nucleotide polymorphism

t: time

TEJ: temperate japonica

TRJ: tropical japonica

U$_{HT}$: high treatment potassium uptake per gram of root

U_{LT}: low treatment potassium uptake per gram of root

W: plant mass

W_0: initial plant mass

References

Abbadi, J., Gerendás, J., and Sattelmacher, B. (2008) Effects of potassium supply on growth and yield of safflower as compared to sunflower. *Journal of Plant Nutrition and Soil Science*, **171**: 272-280

Adams, E. and Shin, R. (2014) Transport, signalling, and homeostasis of potassium and sodium in plants. *Journal of Integrative Plant Biology*, **56**: 231-249

Ahmad, I. and Maathuis, F. J. M. (2014) Cellular and tissues distribution of potassium: physiological relevance, mechanisms and regulation. *Journal of Plant Physiology*, **171**: 708-714

Ahmad, I. and Maathuis, F. J. M. (2016) Overexpression of the rice AKT1 potassium channel affects potassium nutrition and rice drought tolerance. *Journal of Experimental Botany*, **67**: 2689-2698

Ahn, S. J., Shin, R., and Schachtman, D. P. (2004) Expression of *KT/KUP* genes in *Arabidopsis* and the role of root hairs in K^+ uptake. *Plant Physiology*, **134**: 1135-1145

Ali, M. L., McClung, A. M., Jia, M. H., Kimball, J. A., McCouch, S. R., and Eizenga, G. C. (2011) A rice diversity panel evaluated for genetic and agro-morphological diversity between subpopulations and its geographic distribution. *Crop Science*, **51**: 2021-2035

Amtmann, A. and Blatt, M. R. (2009) Regulation of macronutrient transport. *New Phytologist*, **181**: 35-52

Amtmann, A., Troufflard, S., and Armengaud, P. (2008) The effects of potassium nutrition on pest and disease resistance in plants. *Physiologia Plantarum*, **133**: 682-691

Armengaud, P., Breitling, and Amtmann, A. (2004) The potassium-dependent transcriptome of *Arabidopsis* reveals a prominent role of jasmonic acid in nutrient signaling. *Plant Physiology*, **136**: 2556-2576

Asher, C. J. and Ozanne, P. G. (1967) Growth and potassium content of plants in solution cultures maintained at constant potassium concentration. *Soil Science*, **103**: 155-161

Aulchenko, Y. S., Ripke, S., Isaacs, A., van Duijn, C. M. (2007) GenABEL: an R library for genome-wide association analysis. *Bioinformatics*, **23**: 1294-1296

Bach, M. and Frede, H. -G. (1998) Agricultural nitrogen, phosphorus and potassium balances in Germany - Methodology and trends 1970 to 1995. *Journal of Plant Nutrition and Soil Science*, **161**: 385-393

Baligar, V. C., Fageria, N. K., and He, Z. L. (2001) Nutrient use efficiency in plants. *Communications in Soil Science and Plant Analysis*, **32**: 921-950

Balkos, K. D., Britto, D. T., and Kronzuker, H. J. (2010) Optimization of ammonium acquisition and metabolism by potassium in rice (*Oryza sativa* L cv. IR-72). *Plant, Cell and Environment*, **33**: 23-34

Banuelos, M. A., Garciadeblas, B., Cubero, B., and Rodrìguez-Navarro (2002) Inventory nd functional characterization of the HAK potassium transporters in rice. *Plant Physiology*, **130**: 784-795

Barragán, V., Leidi, E. O., Andrés, Z., Rubio, L., De Luca, A., Fernández, J. A., Cubero, B., and Pardo, J. M. (2012) Ion exchangers NHX1 and NHX2 mediate active potassium uptake into vacuoles to regulate cell turgor and stomatal function in *Arabidopsis*. *The Plant Cell*, **24**: 1127-1142

Benito, B., Haro, R., Amtmann, A., Cuin, T. A., and Dreyer, I. (2014) The twins K^+ and Na^+. *Journal of Plant Physiology*, **171**: 723 -731

Benjamini, Y. and Hochberg, Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society, Series B (Methodological)*, **57**: 289-300

Besford, R. T. and Maw, G. A. (1974) Uptake and distribution of potassium in tomato plants. *Plant and Soil*, **41**: 601-618

Blatt, M. R. (2000) Cellular signaling and volume control in stomatal movements in plants. *Annual Review of Cell and Developmental Biology*, **16**: 221-241

Botha, C. E. J., Aoki, N., Scofield, G. N., Liu, L., Furbank, R. T., and White, R. G. (2008) A xylem sap retrieval pathway in rice leaf blades: evidence of a role for endocytosis. *Journal of Experimental Botany*, **59**: 2945-2954

Brentup, F. and Pallière, C. (2008) *Energy efficiency and greenhouse gas emissions* *in European nitrogen fertilizer production and use.* York: International Fertiliser Society, pp. 1-22

Britto, D. T., Siddiqi, M. Y., Glass, A. D., and Kronzucker, H. J. (2001) Futile transmembrane NH_4^+ cycling: a cellular hypothesis to explain ammonium toxicity in plants. *Proceedings of the National Academy of Sciences of the United States of America*, **98**: 4255-4258

Brohi, A. R., Karaman, M. R., Topbas, M. R., Aktas, A., and Savasli, A. E. (2000) Effect of potassium and magnesium fertilization on yield and nutrient content of rice crop grown on artificial siltation soil. *Turkish Journal of Agriculture and Forestry*, **24**: 429-435

Cai, H., Xie, W., and Lian, X. (2013) Comparative analysis of differentially expressed genes in rice under nitrogen and phosphorus starvation stress conditions. *Plant Molecular Biology Reporter*, **31**: 160-173

Cakmak, I. (2005) The role of potassium in alleviating detrimental effects of abiotic stresses in plants. *Journal of Plant Nutrition and Soil Science*, **168**: 521-530

Campbell,M. T., Bandillo, N., Razzaq, F., Al Shiblawi, A., Sharma, S., Liu, K., Du, Q., Schmitz, A. J., Zhang, C., Véry, A., Lorenz, A. J., and Walia, H. (2017) Allelic variants of *OsHKT1;1* underlie the divergence between *indica* and *japonica* subspecies of rice (*Oryza sativa*) for root sodium content. *PLoS Genetics*, **13**: e1006823

Cellier, F., Conéjéro, G., Ricaud, L., Luu, D. T., Lepetit, M., Gosti, F., and Casse, F. (2004) Characterization of *AtCHX17*, a member of the cation/H^+ exchangers, CHX family, from *Arabidopsis thaliana* suggests a role in K^+ homeostasis. *The Plant Journal*, **39**: 834-846

Chao, D. -Y., Chin, Y., Chen, J., Shi, S., Chen, Z., Wang, C., Danku, J. M., Zhao, F. -J., and Salt, D. E. (2014) Genome-wide association mapping identifies a new arsenate reductase enzyme critical for limiting arsenic accumulation in plants. *PLoS Biology*, **12**: e1002009

Chardon, F., Berthélémy, J., Daniel-Vedele, F., and Masclaux-Daubresse, C. (2010) Natural variation of nitrate uptake and nitrogen use efficiency in *Arabidopsis thaliana* cultivated with limiting and ample nitrogen supply. *Journal of Experimental Botany*, **61**: 2293-2302

Chen, J. and Gabelman, W. H. (2000) Morphological and physiological characteristics of tomato roots associated with potassium-acquisition efficiency. *Scientia Horticulturae*, **83**: 213-225

Chen, G., Hu, Q., Luo, L., Yang, T., Zhang, S., Hu, Y., Yu, L., and Xu, G.

(2015) Rice potassium transporter OsHAK1 is essential for maintaining potassium-mediated growth and functions in salt tolerance over low and high potassium concentration ranges. *Plant, Cell and Environment*, **38**: 2747-2765

Chen, Z. H., Hills, A., Baetz, U., Amtmann, A., Lew, V. L., Blatt, M., R. (2012) Systems dynamic modeling of the stomatal guard cell predicts emergent behaviors in transport, signalling, and volume control. *Plant Physiology*, **159**: 1235-1251

Chytrỳ, M., Hejeman, M., Hennekens, S. M., and Jürgen, S. (2009) Changes in vegetation types and Ellenbrg indicator values after 65 years of fertilizer application in the Rengen Grassland Experiment, Germany. *Applied Vegetation Science*, **12**: 167-176

Ciceri, D., Manning, D. A. C., and Allanore, A. (2015) Historical and technical developments of potassium resources. *Science of the Total Environment*, **502**: 590-601

Claasen, N., Syring, K. M., and Jungk, A. (1986) Verification of a mathematical model by simulating potassium uptake from soil. *Plant and Soil*, **95**: 209-220

Clarkson, D. T. and Hanson, J. B. (1980) The mineral nutrition of higher plants. *Annual Review of Plant Physiology*, **31**: 239-298

Crowell, S., Korniliev, P., Falc̆ao, A., Ismail, A., Gregorio, G., Mezey, J., and McCouch, S. R. (2016) Genome-wide association and high-resolution phenotyping link *Oryza sativa* panicle traits to numerous trait-specific QTL clusters. *Nature Communications*, **7**: 10527

Dai, X., Ouyang, Z., Li, Y., and Wang, H. (2013) Variation in yield gap induced by nitrogen, phosphorus and potassium fertilizer in North China Plain. *PLoS One*, **8**: e82147

Damon, P. M., Osborne, L. D., and Rengel, Z. (2007) Canola genotypes differ in potassium efficiency during vegetative growth. *Euphytica*, **156**: 387-397

Damon, P. M., and Rengel, Z. (2007) Wheat genotypes differ in potassium efficiency under glasshouse and field conditions. *Australian Journal of Agricultural Research*, **58**: 816-825

Davies, J. M., Rea, P. A., and Sanders (1991) Vacuolar proton-pumping pyrophosphatase in *Beta vulgaris* shows vectorial activation by potassium. *FEBS Letters*, **278**: 66-68

Deeken, R., Geiger, D., Fromm, J., Koroleva, O., Ache, P., Langenfeld-Heyser, R., Sauer, N., May, S. T., Hedrich, R. (2002) Loss of the AKT2/3 potassium channel affects sugar loading into the phloem of *Arabidopsis*. *Planta*, **216**: 334-344

Devlin, B. and Roeder, K. (1999) Genomic control for association studies. *Biometrics*, **55**: 997-1004

Dewar, R. C. (1993) A root-shoot partitioning model based on carbon-nitrogen-water interactions and Munch phloem flow. *Functional Ecology*, **7**: 356-368

Dobermann, A., Cassman, K. G., Mamaril, C. P., and Sheehy, J. E. (1998) Management of phosphorus, potassium, and sulfur in intensive, irrigated lowland rice. *Field Crops Research*, **56**: 113-138

Drew, M. C. (1975) Comparison of the effects of a localized supply of phosphate, nitrate, ammonium and potassium on the growth of the seminal root system, and the shoot, in barley. *New Phytologist*, **75**: 479-490

Eizenga, G. C., Ali, M. L., Bryant, R. J., Yeater, K. M., McClung, A. M., and McCouch, S. R. (2014) Registration of the Rice Diversity Panel 1 for genomewide association studies. *Journal of Plant Registrations*, **8**: 109-116

Epstein, E., Rains, D. W., and Evans, O. E. (1963) Resolution of dual mechanisms of potassium absorption by barley roots. *Proceedings of the National Academy of Sciences of the United States of America*, **49**: 684-692

Evans, H. J. and Sorger, G. J. (1966) Role of mineral elements with emphasis on the univalent cations. *Annual Review of Plant Physiology*, **17**: 47-76

Fageria, N. K. (1976) Influence of potassium concentration on growth and potassium uptake by rice plants. *Plant and Soil*, **44**: 567-573

Fageria, N. K. (2014) Growth, nutrient uptake, and use efficiency in dry bean in tropical upland soil. *Journal of Plant Nutrition*, **37**: 2085-2093

Fageria, N. K. and Melo, L. C. (2014) Agronomic evaluation of dry bean genotypes for potassium use efficiency. *Journal of Plant Nutrition*, **37**: 1899-1912

Fageria, N. K., Barbosa Filho, M. P., and da Costa, J. G. C. (2001) Potassium-use efficiency in common bean genotypes. *Journal of Plant Nutrition*, **24**: 1937-1945

Fageria, N. K., Moreira, A., Ferreira, E. P. B., and Knupp, A. M. (2013) Potassium-use efficiency in upland rice genotypes. *Communications in Soil Science and Plant Analysis*, **44**: 2656-2665

Famoso, A. N., Zhao, K., Clark, R. T., Tung, C. -W., Wright, M. H., Bustamante, C., Kochian, L. V., and McCouch, S. R. (2011) Genetic architecture of aluminium tolerance in rice (*Oryza sativa*) determined through genome-wide association analysis and QTL mapping. *PLoS Genetics*, **7**: e1002221

Fan, M., Bie, Z., Xie, H., Zhang, F., Zhao, S., and Zhang, H. (2013) Genotypic variation for potassium efficiency in wild and domesticated watermelons under ample and limited potassium supply. *Journal of Plant Nutrition and Soil Science*, **176**: 466-473

Fang, Y., Wu, W., Zhang, X., Jiang, H., Lu, W., Pan, J., Hu, J., Guo, L., Zeng, D., and Xue, D. (2015) Identification of quantitative trait loci associated with tolerance of low potassium and related ions concentrations at seedling stage in rice (*Oryza sativa* L.). *Plant Growth Regulation*, **77**: 157-166

FAO (2015) *World fertilizer outlook to 2018.* Rome: FAO, pp. 1-53

FAO (2016) *World fertilizer trends and outlook to 2019.* Rome: FAO, pp. 1-29

FAO (2017) http://www.fao.org/faostat/en/#data/QC [accessed 4th July 2017]

Farooq, M., Aziz, T., Cheema, Z. A., Hussain, M., and Khaliq, A. (2008) Activation of antioxidant system by KCl improves the chilling tolerance in hybrid maize. *Journal of Agronomy and Crop Science*, **194**: 438-448

Flowers, T. J. and Dalmond, D. (1992) Protein synthesis in halophytes: the influence of potassium, sodium and magnesium *in vitro. Plant and Soil*, **146**: 153-161

Fox, H. and Weisburg, S. (2011) *An R companion to applied regression.* Thousand Oaks, California: Sage, pp. 1-472

Foy, R. H., Bailey, J. S., and Lennox, S. D. (2002) Mineral balances for the use of phosphorus and other nutrients by agriculture in Northern Ireland from 1925 to 2000 - methodology, trends and impacts of losses to water. *Irish Journal of Agricultural and Food Research*, **41**: 247-263

Friedman, J., Hastie, T., Tibshirani, R. (2010) Regularization paths for generalized linear models via coordinate descent. *Journal of Statistical Software*, **33**: 1-22

Gajdanowicz,P., Michard, E., Sandmann, M., Rocha, M., Corrêa, L. G. G., Ramìrez-Aguilar, S. J., Gomez-Porras, J . L., Gonzàlez, W., Thibaud, J. -B., van Dongen, J. T., Dreyer, I. (2011) Potassium gradients serve as a mobile energy source in plant vascular tissues. *Proceedings of the National Academy of Science of the United States of America*, **108**: 864-869

Garcia-Oliveira, A. L., Tan, L., Fu, Y., and Sun, C. (2009) Genetic identification of quantitative trait loci for contents of mineral nutrients in rice grain. *Journal of*

Integrative Plant Biology, **51**: 84-92

Garris, A. J., Tai, T. H., Coburn, J., Kresovich, S., and McCouch, S. R. (2005) Genetic structure and diversity in *Oryza sativa* L. *Genetics*, **169**: 1631-1638

Gattward, J. N., Almeida, A. -A., F., Souza Jr, J. O., Gomes, F. P., and Kronzucker, H. K. (2012) Sodium-potassium synergism in *Theobroma cacao*: stimulation of photosynthesis, water-use efficiency and mineral nutrition. *Physiologia Plantarum*, **146**: 350-362

Gaymard, F., Pilot, G., Lacombe, B., Bouchez, D., Bruneau, D., Boucherez, J., Michaux-Ferrière, Thibaud, J. -B, Sentenac, H. (1998) Identification and disruption of a plant Shaker-like outward channel involved in K^+ release into the xylem sap. *Cell*, **94**: 647-655

George, M. S., Lu, G., and Zhou, W. (2002) Genotypic variation for potassium uptake and utilization efficiency in sweet potato (*Ipomoea batatas* L.). *Field Crops Research*, **77**: 7-15

Gerendàs, J., Heuser, F., and Sattelmacher, B. (2007) Influences of nitrogen and potassium supply on content of acrylamide precursors in potato tubers and on acrylamide accumulation in french fries. *Journal of Plant Nutrition*, **30**: 1499-1516

Glaszmann, J. C. (1987) Isozymes and classification of Asian rice varieties. *Theoretical and Applied Genetics*, **74**: 21-30

Gobert, A., Isayenkov, S., Voelker, C., Czempinski, K., and Maathuis, F. J. M. (2007) The two-pore channel *TPK1* gene encodes the vacuolar K^+ conductance and plays a role in K^+ homeostasis. *Proceedings of the National Academy of Sciences of the United States of America*, **104**: 10726-10731

Goff, S. A., Ricke, D., Lan, T. -H., Presting, G., Wang, R., Dunn, M., Glazebrook, J., Sessions, A., Oeller, P., Varma, H., Hadley, D., Hutchison, D., Martin, C., Katagiri, F., Lange, B. M., Moughamer, T., Xia, Y., Budworth, P., Zhong, J., Miguel, T., Paszkowski, U., Zhang, S., Colbert, M., Sun, W. -L., Chen, L., Cooper, B., Park, S., Wood, T. C., Mao, L., Quail, P., Wing, R., Dean, R., Yu, Y., Zharkikh, A., Shen, R., Sahasrabudhe, S., Thomas, A., Cannings, R., Gutin, A., Pruss, D., Reid, J., Tavtigan, S., Mitchell, J., Eldredge, G., Scholl, T., Miller, R. M., Bhatnagar, S., Adey, N., Rubano, T., Tusneem, N., Robinson, R., Feldhaus, J., Macalma, T., Oliphant, A., and Briggs, S. (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*. *Science*, **296**: 92-100

Good, A. G., Shrawat, A. K., and Muench, D. G. (2004) Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? *Trends in Plant Science*, **9**: 597-605

GRiSP (2013) *Rice almanac.* Los Baños, Philippines: International Rice Research Institute, pp. 1-283

Grömping, U. (2006) Relative importance for linear regression in R: the package relaimpo. *Journal of Statistical Software*, **17**: 1-27

Guo, Y., Kong, F. -M., Xu, Y. -F., Zhao, Y., Liang, X., Wang, Y. -Y., An, D. -G., Li, S. -S. (2012) QTL mapping for seedling traits in wheat grown under varying concentrations of N, P and K nutrients. *Theoretical and Applied Genetics*, **124**: 851-865

Guoping, Z., Jingxing, C., and Tirore, A. (1999) Genotypic variation for potassium uptake and utilization efficiency in wheat. *Nutrient Cycling in Agroecosystems*, **54**: 41-48

Haines, J. L., Hauser, M. A., Schmidt, S., Scott, W. K., Olson, L. M., Gallins, P., Spencer, K. L., Kwan, S. Y., Noureddine, M., Gilbert, J. R., Schnetz-Boutaud, N., Agarwal, A., Postel, E. A., and Pericak-Vance, M. (2005) Complement factor H variant increases the risk of age-related macular degeneration. *Science*, **308**: 419-421

Harada, H. and Leigh, R. A. (2006) Genetic mapping of natural variation in potassium concentrations in shoots of *Arabidopsis thaliana. Journal of Experimental Botany*, **57**:953-960

Hartley, T. N. and Maathuis, F. J. M. (2016) Allelic variation in the vacuolar TPK1 channel affects its calcium dependence and may impact on stomatal conductance. *FEBS Letters*, **590**: 110-117

Hawkesford, M. J. (2012) Improving nutrient use efficiency in crops. In *eLS*, Chichester, UK: John Wiley and Sons, pp. 1-7

Hernandez, M., Fernandez-Garcia, N., Garcia-Garma, J., Rubio-Asensio, J. S., Rubio, F., and Olmos, R. (2012) Root potassium starvation induces oxidative stress in *Solanum lycopersicum* L. roots. *Journal of Plant Physiology*, **169**: 1366-1374

Hills, A., Chen, Z. H., Amtmann, A., Blatt, M. R., and Lew, V. L. (2012) OnGuard, a computational platform for for quantitative kinetic modeling of guard cell physiology. *Plant Physiology*, **159**: 1026-1042

Hirose, N., Makita, N., Kojima, M., Kamada-Nobusada, T., and Sakakibara, H. (2007) Overexpression of a type-A response regulator alters rice morphology and cytokinin metabolism. *Plant and Cell Physiology*, **48**: 523-539

Hirsch, R. E., Lewis, B. D., Spalding, E. P., and Sussman, M. R. (1998) The role of AKT1 potassium channel in plant nutrition. *Science*, **280**: 918-921

Hoa, N. Y., Janssen, B. H., Oenema, O., and Dobermann, A. (2006) Comparison of partial and complete soil K budgets under intensive rice cropping in the Mekong Delta, Vietnam. *Agriculture, Ecosystems and Environment*, **116**: 121-131

Hoffman, W. A. and Poorter, H. (2002) Avoiding bias in calculations of relative growth rates. *Annals of Botany*, **80**: 37-42

Høgh-Jensen, H. and Pedersen, M. B. (2003) Morphological plasticity by crop plants and their potassium use efficiency. *Journal of Plant Nutrition*, **26**: 969-984

Holt, J., Davis, S., and Leirs, H. (2006) A model of Leptospirosis infection in an African rodent to determine risk to humans: seasonal fluctuations and the impact of rodent control. *Acta Tropica*, **99**: 218-225

Hölttä, T., Mencuccini, M., and Nikinmaa, E. (2009) Linking phloem function to structure: analysis with a coupled xylem-phloem transport model. *Journal of Theoretical Biology*, **259**: 325-337

Hommels, C. H., Kuiper, P. J. C.m and de Haan, A. (1989) Response to internal potassium ion concentrations of two *Taraxacum* microspecies of contrasting mineral ecology: the role of inorganic ions in growth. *Physiologia Plantarum*, **77**: 562-568

Hong, J. -P., Takeshi, Y., Kondou, Y., Schachtman, D. P., Matsui, M., Shin, R. (2013) Identification and characterization of transcription factors regulating *Arabidopsis HAK5. Plant and Cell Physiology*, **54**: 1478-1490

Horie, T., Costa, A., Kim, T. H., Hang, M. J., Horie, R., Leung, H. -Y., Miyao, A., Hirochika, H., An., G., and Schroeder, J. I. (2007) Rice OsHKT2;1 transporter mediates large Na^+ influx component into K^+-starved roots for growth. *The EMBO Journal*, **26**: 3003-3014

Huang, S., Pan, X., Sun, Y., Zhang, Y., Hang, X., Yu, X., and Zhang, W. (2013) Effects of long-term fertilization on the weed growth and community composition in a double-rice ecosystem during the fallow period. *Weed Biology and Management*, **13**: 10-18

Hwang, H., Yoon, J., Kim, H. Y., Min, M. K., Kim, J. -A., Choi, E. -H., Lan, W., Bae, Y/ -M., Luan, S., Cho, H., and Kim, B. -G. (2013) Unique features of two potassium channels, OsKAT2 and OsKAT3 expressed in rice guard cells. *PLoS One*, **8**: e72541

Ingestad, T. and Ågren, G. I. (1988) Nutrient uptake and allocation at steady-state nutrition. *Physiologia Plantarum*, **72**: 450-459

IRGSP (2005) The map-based sequence of the rice genome. *Nature*, **436**: 793-800

IRRI (2017) http://www.knowledgebank.irri.org/ [accessed 4th July 2017]

Jabnoune, M., Espeout, S., Mieulet, D., Fizames, C., Verdeil, J. -L., Conéjéro, G., Rodríguez-Navarro, A., Sentenac, H., Guiderdoni, E., Abdelly, C., and Véry, A. -A. (2009) *Plant Physiology*, **150**: 1955-1971

Jaquinod, M., Villiers, F., Kieffer-Jacquinod, S., Hugouvieux, V., Bruley, C., Garin, J., and Bourguignon, J. (2007) A proteomics dissection of *Arabidopsis thaliana* vacuoles isolated from cell culture. *Molecular and Cellular Proteomics*, **6**: 394-412

Jeschke, W. D., Kirkby, E. A., Peuke, A. D., Pate, J. S., and Hartung, W. (1997) Effects of P deficiency on assimilation and transport of nitrate and phosphate in intact plants of castor bean (*Ricinus communis* L.). *Journal of Experimental Botany*, **48**: 75-91

Jeschke, W. D., Wolf, O., and Hartung, W. (1992) Effect of NaCl salinity on flows and partitioning of C, N, and mineral ions in whole plants of white lupin, *Lupinus albus* L. *Journal of Experimental Botany*, **43**: 777-788

Jia, Y. -B., Yang, X., Feng, Y., and Jilani, G. (2008) Differential response of root morphology to potassium deficient stress among rice genotypes varying in potassium efficiency. *Journal of Zhejiang University Science B*, **9**: 427-434

Jiang, C. -C., Hau, Y. -S., Wang, X. -L., Wang, D., and Lei, J. (2013) Effect of K on dry matter accumulation and distribution and changes of root-zone K in different cotton genotypes. *Journal of Food, Agriculture and Environment*, **11**: 604-608

Johnston, A. E. and Milford, G. F. J. (2012) *Potassium and nitrogen interactions in crops.* http://www.pda.org.uk/technical-potash-notes/ [accessed 14th August 2017]

Jones, E., Oliphant, T., and Peterson, P. (2001) *SciPy: open source scientific tools for Python.* http://www.scipy.org/ [accessed 13th March 2017]

Jordan-Meille, L. and Pellerin, S. (2008) Shoot and root growth of hydroponic maize (*Zea mays* L.) as influenced by K deficiency. *Plant and Soil*, **304**: 157-168

Ju, H., Liu, J., Gao, J., Fang, Y., Bian, M., and Yang, Z. (2014) Potassium uptake characteristics of two maize hybrids and their parents under the stress from a low amount of potassium. *Journal of Plant Nutrition*, **37**: 123-135

Jung, J. -Y., Shin, R., and Schachtman, D. P. (2009) Ethylene mediates responses and tolerance to potassium deprivation in *Arabidopsis*. *The Plant Cell*, **21**: 607-621

Kellermeier, F., Chardon, F., and Amtmann, A. (2013) Natural variation of *Arabidopsis* root architecture reveals complementing adaptive strategies to potassium starvation. *Plant Physiology*, **161**:1421-1432

Keunecke, M., Lindner, B., Seydel, U., Schulz, A., and Hansen, U. P. (2001) Bundles sheath cells of small veins in maize leaves are the location of uptake from the xylem. *Journal of Experimental Botany*, **52**: 709-714

Kim, M. J., Ruzicka, D., Shin, R., and Schachtman, D. P. (2012) The *Arabidopsis* AP2/ERF transcription factor RAP2.11 modulates plant response to low-potassium conditions. *Molecular Plant*, **5**: 1042-1057

Klein, R. J., Zeiss, C., Chew, E. Y., Tsai, J. -Y., Sackler, R. S., Haynes, C., Henning, A. K., SanGiovanni, J. P., Mane, S. M., Mayne, S. T., Bracken, M. B., Ferris, F. L., Ott, J., Barnstable, C., and Hoh, J. (2005) Complement factor H polymorphism in age-related macular degeneration. *Science*, **308**: 385-389

Kochian, L. V. and Lucas, W. J. (1982) Potassium transport in corn roots. *Plant Physiology*, **70**: 1723-1731

Kong, F. -M., Guo, Y., Liang, X., Wu, C. -H., Wang, Y. -Y., Zhao, Y., and Li, S. -S. (2013) Potassium (K) effects and QTL mapping for K efficiency traits at seedling and adult stages in wheat. *Plant and Soil*, **373**: 877-892

Korte, A. and Farlow, A. (2013) The advantages and limitations of trait analysis with GWAS: a review. *Plant Methods*, **9**: 29

Koyama, M. L., Levesley, A., Koebner, R. M. D., Flowersm T. J., and Yeo, A. R. (2001) Quantitative trait loci for component physiological traits determining salt tolerance in rice. *Plant Physiology*: **125**: 406-422

Krishnasamy, K., Bell, R., and Ma, Q. (2014) Wheat responses to sodium vary with potassium use efficiency of cultivars. *Frontiers in Plant Science*, **5**: 631

Kronzucker, H. J., Britto, D. T., Davenport, R. J., and Tester, M. (2001) Ammonium toxicity and the real cost of transport. *Trends in Plant Science*, **6**: 335-337

Kumar, V., Singh, A., Mithra, S. V. A., Krishnamurthy, S. L., Parida, S. K., Jain, S. Tiwari, K. K., Kumar, P., Rao, A. R., Sharma, S. K., Khurana, J. P., Singh, N. K., and Mohapatra, T. (2015) Genome-wide association mapping salinity tolerance in rice (*Oryza sativa*). *DNA Research*, **22**: 133-145

Kushawa, U. K. S. (2016) Rice. In Kushawa, U. K. S. (ed.) *Black rice: research, history and development.* Berlin: Springer, pp. 1-19

Lee, S. C., Lan, W. -Z., Kim, B. -G., Li, L., Cheong, Y. H., Pandey, G. K., Lu, G., Buchanan, D., and Luan, S. (2007) A protein phosphorylation/dephosphorylation network regulates a plant potassium channel. *Proceeding of the National Academy of Science of the United States of America*, **104**: 15959-15964

Leigh, R. A. (2001) Potassium homeostasis and membrane transport. *Journal of Plant Nutrition and Soil Science*, **164**: 193-198

Leigh, R. A., Chater, M., Storey, R., and Johnston, A. E. (1986) Accumulation and subcellular distribution of cations in relation to the growth of potassium-deficient barley. *Plant, Cell and Environment*, **9**: 595-604

Leigh, R. A. and Wyn Jones, R. G. (1984) A hypothesis relating critical potassium concentrations for growth to the distribution and functions of this ion in the plant cell. *New Phytologist*, **97**: 1-13

Leonardos, O. H., Theodoro, S. H., and Assad, M. L. (2000) Remineralization for sustainable agriculture: a tropical perspective from a Brazilian viewpoint. *Nutrient Cycling in Agroecosystems*, **56**: 3-9

Li, L., Kim, B. -G., Cheong, Y. H., Pandey, G. K., and Luan, S. (2006) A Ca^{2+} signaling pathway regulates K^+ channel for low-K response in *Arabidopsis. Proceedings of the National Academy of Sciences of the United States of America*, **103**: 12625-12630

Liebersbach, H., Steingrobe, B., and Claasen, N. (2004) Roots regulate ion transport in the rhizosphere to counteract reduced mobility in dry soil *Plant and Soil*, **260**: 79-88

Lin, H. X., Zhu, M. Z., Yano, M., Gao, J. P., Liang, Z. W., Su, W. A., Hu, X. H., Ren, Z. H., and Chao, D. Y. (2004) QTLs for Na^+ and K^+ uptake of the shoots and roots controlling rice salt tolerance. *Theoretical and Applied Genetics*, **108**: 253-260

Liu, G., Li, Y., and Porterfield, D. M. (2009) Genotypic differences in potassium nutrition in lowland rice hybrids. *Communications in Soil Science and Plant Analysis*, **40**: 1803-1821

Lumley, T. (2017) *leaps: regression subset selection.* https://CRAN.R-project.org/package=leaps [accessed 25th July 2017]

Ma, Q., Scanlan, C., Bell, R., and Brennan, R. (2013) The dynamics of potassium uptake and use, leaf gas exchange and root growth throughout plant phenological development and its effects on seed yield in wheat (Triticum aestivum) on a low-K sandy soil. *Plant and Soil*, **373**: 373-384

Ma, T. -L., Wu, W. -H., and Wang, Y. (2012) Transcriptome analysis of root responses to potassium deficiency. *BMC Plant Biology*, **12**: 161

Maathuis, F. J. M. (2009) Physiological functions of mineral macronutrients. *Current Opinion in Plant Biology*, **12**: 250-258

Maathuis, F. J. M. and Amtmann, A. (1999) K^+ nutrition and Na^+ toxicity: the basis of cellular K^+/Na^+ ratios. *Annals of Botany*, **84**: 123-133

Maathuis, F. J. M., Filatov, V., Harzyk, P., Krijger, G. C., Axelsen, K. B., Chen, S., Green, B. J., Li, Y., Madagan, K. L., Sánchez,-Fernández, R., Forde, B. G., Pamgren, M. G., Rea, P. A., Williams, L. E., Sanders, D., and Amtmann, A. (2003) Transcriptome analysis of root transporters reveals participation of multiple gene families in the response to cation stress. *The Plant Journal*, **35**: 675-692

Maathuis, F. J. M. and Sanders, D. (1995) Contrasting roles in ion transport of two K^+-channel types in root cells of *Arabidopsis thaliana*. *Planta*, **197**: 456-464

Maathuis, F. J. M. and Sanders, D. (1996) Mechanisms of potassium absorption by higher plants. *Physiologia Plantarum*, **96**: 158-168

Manning, D. A. C. (2010) Mineral sources of potassium for plant nutrition. A review. *Agronomy for Sustainable Development*, **30**: 281-294

Marschner, H., Kirkby, E. A., and Cakmak, I. (1996) Effect of mineral nutritional status on shoot-root partitioning of photoassimilates and cycling of mineral nutrients. *Journal of Experimental Botany*, **47**: 1255-1263

Marschner, H., Kirkby, E. A., and Engels, C. (1997) Importance of cycling and recycling of mineral nutrients within plants for growth and development. *Botanica Acta*, **110**: 265-273

Martìnez-Cordero, M. A., Martìnez, V., and Rubio, F. (2004) Cloning and functional characterization of the high-affinity K^+ transporter HAK1 in pepper. *Plant Molecular Biology*, **56**: 413-421

McCouch, S. R., Wright, M. H., Tung, C. -W., Maron, L. G., McNally, K. L., Fitzgerald, M., Singh, N., DeClerck, G., Agosto-Perez, F., Korniliev, P., Greenberg, A. J., Naredo, M. E. B., Mercado, S. M. Q., Harington, S. E., Shi, Y., Branchini, D. A., Kuser-Falčao, P. R., Leung, H., Ebana, K., Yano, M., Eizenga, G., McClung, A., and Mezey, J. (2016) Open access resources for genome-wide association mapping in rice. *Nature Communications*, **7**:10532

McNally, K. L., Childs, K. L., Bohnert, R., Davidson, R. M., Zhao, K., Ulat, V., Zeller, G., Clark, R. M., Hoen, D. R., Bureau, T. E., Stokowski, R., Ballinger, D. G., Frazer, K. A., Cox, D. R., Padhukasahasram, B., Bustamante, C. D., Weigel, D., Mackill, D. J., Bruskiewich, R. M., Rätsch, G., Buell, C. R., Leung, H., and Leach, J. E. (2009) Genomewide SNP variation reveals relationships among landraces and modern rice varieties of rice. *Proceedings of the National Academy of Sciences of the United States of America*, **106**: 12273-12278

Memon, A. R., Saccomani, M., and Glass, A. D. M. (1985) Efficiency of potassium utilization by barley varieties: the role of subcellular compartmentation. *Journal of Experimental Botany*, **36**: 1860-1875

Mengel, K. and Steffens, D. (1985) Potassium uptake of rye-grass (*Lollium perenne*) and red clover (*Trifolium pratense*) as related to root parameters. *Biology and Fertility of Soils*, **1**: 53-58

Miyamoto, T., Ochiai, K., Nonoue, Y., Matsubara, K., Yano, M., and Matoh, T. (2015) Expression level of the sodium transporter gene *OsHKT2;1* determines sodium accumulation of rice cultivars under potassium-deficient conditions. *Soil Science and Plant Nutrition*, **61**: 481-492

Miyamoto, T., Ochiai, K., Takeshita, S., and Matoh, T. (2012) Identificaiton of quantitative trait loci associated with shoot sodium accumulation under low potassium conditions in rice plants. *Soil Science and Plant Nutrition*, **58**: 728-736

Moriconi, J. I. and Santa-María, G. E. (2013) A theoretical framework to study potassium utilization efficiency in response to withdrawal of potassium. *Journal of Experimental Botany*, **64**: 4289-4299

Murata, T. and Akazawa, T. (1969) Stimulative effect of potassium ion on starch synthetase of different plant origins. *Plant and Cell Physiology*, **10**: 457-460

Nieves-Cordones, M., Alemán, F., Martinéz, V., and Rubio, F. (2014) K^+ uptake in plant roots. The systems involved, their regulation and parallels in other organisms. *Journal of Plant Physiology*, **171**:688-695

Nieves-Cordones, M., Martínez, V., Benito, B., and Rubio, F. (2016) Comparison between *Arabidopsis* and rice main pathways for K^+ and Na^+ uptake by roots. *Frontiers in Plant Science*, **7**: 992

Nitsos, R. E. and Evans, H. J. (1969) Effects of univalent cations on the activity of particulate starch synthetase. *Plant Physiology*, **44**: 126-1266

O'Brien, R. M. (2007) A caution regarding rules of thumb for variance inflation factors. *Quality and Quantity*, **41**: 673-690

Ohnishi, M., Fakada-Tanaka, S., Hoshino, A., Takada, J., Inagaki, Y., and Iida, S. (2005) Characterization of a novel Na^+/H^+ antiporter gene *InNHX2* and comparison of *InNHX2* with *InNHX1*, which is responsible for blue flower coloration by increasing the vacuolar pH in the Japanese morning glory. *Plant and Cell Physiology*, **46**: 259-267

Oomen, R. J. F. J., Benito, B., Sentenac, H., Rodríguez-Navarro, A., Talón, M., Véry, A. -A., and Domingo, C. (2012) HKT2;2/1, a K^+-permeable transporter identified in a salt-tolerant rice cultivar through surveys of natural genetic polymorphism. *The Plant Journal*, **71**: 750-762

Oosterhuis, D. M., Loka, D. A., and Raper, T. P. (2013) Potassium and stress alleviation: physiological functions and management of cotton. *Journal of Plant Nutrition and Soil Science*, **176**: 331-343

Outlaw, W. H. (1983) Current concepts on the role of potassium in stomatal movements. *Physiologia Plantarum*, **59**: 302-311

Pathak, H., Mohanty, S., Jain, N., and Bhatia, A. (2010) Nitrogen, phosphorus, and potassium budgets in Indian agriculture. *Nutrient Cycling in Agroecosystems*, **86**: 287-299

Patishtan, J., Hartley, T. N., de Carvalho, R. F., and Maathuis, F. J. M. (2017) Genome-wide association studies to identify rice salt-tolerance markers. *Plant, Cell and Environment*, in press.

Patterson, H. D. and Williams, E. R. (1976) A new class of resolvable incomplete block designs. *Biometrika*, **63**: 83-92

Patterson, H. D., Williams, and Hunter, E. A. (1978) Block designs for variety trials. *Journal of Agricultural Science*, **90**: 395-400

Peiter, E., Maathuis, F. J. M., Mills, L. N., Knight, H., Pelloux, J., Hetherington, A. M., and Sanders, D. (2005) The vacuolar Ca^{2+}-activated channel TPC1 regulates germination and stomatal movement. *Nature*, **434**: 404-408

Perenoud, S. (1990) *Potassium and Plant Health.* Bern: IPI, pp. 1-365

Perevz, H., Ashraf, M., and Makhdum, M. I. (2005) Effects of potassium rates and sources on fiber quality parameters in four cultivars of cotton grown in aridisols. *Journal of Plant Nutrition*, **27**: 2235-2257

Pettigrew, W. T. (1999) Potassium deficiency increases specific leaf weights and leaf glucose levels in field-grown cotton. *Agronomy Journal*, **91**: 962-968

Peuke, A. D. (2010) Correlations in concentrations, xylem and phloem flows, and partitioning of elements and ions in intact plants. A summary and statistical re-evaluation of modelling experiments in *Ricinus communis*. *Journal of Experimental Botany*, **61**: 635-655

Peuke, A. D., Jeschke, W. D., and Hartung, W. (2002) Flows of elements, ions and abscisic acid in *Ricins communis* and site of nitrate reduction under potassium limitation. *Journal of Experimental Botany*, **53**: 241-250

Phillipar, K., Büchsenschütz, K., Abshagen, M., Fuchs, I., Geiger, D., Lacombe, B., and Hedrich, R. (2003) The K^+ channel KZM1 mediates potassium uptake in the phloem and guard cells of the C_4 grass *Zea mays*. *The Journal of Biological Chemistry*, **278**: 16973-16981

Prinzenberg, A. E., Barbier, H., Salt, D. E., Stich, B., and Reymond, M. (2010) Relationships between growth, growth response to nutrient supply, and ion content using a recombinant inbred line population in *Arabidopsis*. *Plant Physiology*, **154**: 1361-1371

Python Software Foundation (2017) https://www.python.org/ [accessed 13th March 2017]

Quampah, A., Wang, R. -M., Shamsi, I. H., Jilani, G., Zhang, Q., Hua, S., and Xu, H. (2011) Improving water productivity by potassium application in various rice genotypes. *International Journal of Agriculture and Biology*, **13**: 9-17

R Core Team (2017) *R: a language and environment for statistical computing.* https://www.R-project.org/ [accessed 25th July 2017]

Ragel, P., Ródenas, R., Garcìa-Martìn, Andrés, Z., Villalta, I., Nieves-Cordones, M., Rivera, R. M., Martìnez, V., Pardo, J. M., Quintero, F. J., and Rubio, F. (2015) A CBL-interacting protein kinase CIPK23 regulates HAK5-mediated high-affinity K^+ uptake in *Arabidopsis* roots. *Plant Physiology*, **169**: 2863-2873

Rengel, Z. and Damon, P. M. (2008) Crops and genotypes differ in efficiency of potassium uptake and use. *Physiologia Plantarum*, **133**: 624-636

Römheld, V. and Kirkby, E. A. (2010) Research on potassium in agriculture: needs and prospects. *Plant and Soil*, **335**: 155-180

Roose, T., Fowler, A. C., and Darrah, P. R. (2001) A mathematical model of plant nutrient uptake. *Journal of Mathematical Biology*, **42**: 347-360

Rose, T. J., Rose, M. T., Pariasca-Tanaka, J., Heuer, S., and Wissuwa, M. (2011) The frustration with utilization: why have improvements in internal phosphorus utilization efficiency in crops remained so elusive? *Frontiers in Plant Science*, **2**: 73

Roshani, G. A., Narayanasamy, G., and Datta, S. C. (2009) Modelling potassium uptake by wheat. *International Journal of Plant Production*, **3**: 55-68

Rubio, F., Nieves-Cordones, M., Alemàn, F., and Martìnez, V. (2008) Relative contribution of AtHAK5 and AtAKT1 to K^+ uptake in the high-affinity range of concentrations. *Physiologia Plantarum*, **134**: 598-608

Sale, P. W. G. and Campbell, L. C. (1986) Yield and composition of soybean seeds as a function of potassium supply. *Plant and Soil*, **96**: 317-325

Samal, D., Kovar, J. L., Steingrobe, B., Sadana, U. S., Bhadoria, P. S., and Claasen, N. (2010) Potassium uptake efficiency and dynamics in the rhizosphere of maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), and sugar beet (*Beta vulgaris* L.) evaluated with a mechanistic model. *Plant and Soil*, **332**: 105-121

Santa-María, G. E., Moriconi, J. I., and Oliferuk, S. (2015) Internal efficiency of nutrient utilization: what is it and how to measure it during vegetative plant growth. *Journal of Experimental Botany*, **66**: 3011-3018

Sauvage, F., Langalis, M., Yoccoz, N. G., Pontier, D. (2003) Modelling hantavirus in fluctuating populations of bank value: the role of indirect transmission on virus persistence. *Journal of Animal Ecology*, **72**: 1-13

Sardans, J. and Peñuelas, J. (2015) Potassium: a neglected nutrient in global change. *Global Ecology and Biogeography*, **24**: 261-275

Satter, R. L. and Galston, A. W. (1981) Mechanisms of control of leaf movements. *Annual Review of Plant Physiology*, **32**: 83-110

Schortemeyer, M., Stamp, P., and Feil, B. (1997) Ammonium tolerance and carbohydrate status in maize cultivars. *Annals of Botany*, **79**: 25-30

Schroeder, J. I., Allen, G. J., Hugouvieux, V., Kwak, J. M., and Waner, D. (2001) Guard cell signal transduction. *Annual Review of Plant Physiology and Plant Molecular Biology*, **52**: 627-658

Schwarz, G. (1978) Estimating the dimension of a model. *The Annals of Statistics*, **6**: 461-464

Shabla, S. and Cuin, T. A. (2007) Potassium transport and plant salt tolerance. *Physiologia Plantarum*, **133**: 651-669

Shankar, A., Singh, A., Kanwar, P., Srivastava, A. K., Pandey, A., Suprasanna, P., Kapoor, S., and Pandey, G. K. (2013) Gene expression analysis of rice seedling under potassium deprivation reveals major changes in metabolism and signaling components. *PLoS One*, **8**: e70321

Sheehy, J. E., Mitchell, P. L., and Ferrer, A. B. (2004) Bi-phasic growth patterns in rice. *Annals of Botany*, **94**: 811-817

Sheldrick, W. F. and Lingard, J. (2002) The use of nutrient audits to determine nutrient balances in Africa. *Food Policy*, **29**: 61-98

Sheldrick, W. F., Syers, J. K., and Lingard, J. (2003) Soil nutrient audits for China to estimate nutrient balances and output/input relationships. *Agriculture, Ecosystems and Environment*, **94**: 341-354

Shin, R. (2014) Strategies for improving potassium use efficiency in plants. *Molecules and Cells*, **37**: 575-584

Shin, R., Berg, R. H., and Schachtman, D. P. (2005) Reactive oxygen species and root hairs in *Arabidopsis* root response to nitrogen, phosphorus, and potassium deficiency. *Plant and Cell Physiology*, **46**: 1350-1357

Siddiqi, M. Y. and Glass, A. D. M. (1982) Simultaneous consideration of tissue and substrate potassium concentration in K^+ uptake kinetics: a model. *Plant Physiology*, **69**: 283-285

Silberbush, M., Sorek, S., and Yakirevich, A. (1993) K^+ uptake by root systems grown in soil under salinity: I. A mathematical model. *Transport in Porous Media*, **11**: 101-116

Spear, S. N., Asher, C. J., and Edwards, D. G. (1978a) Response of cassava, sunflower, and maize to potassium concentration in solution. I. Growth and plant potassium concentration. *Field Crops Research*, **1**: 347-361

Spear, S. N., Asher, C. J., and Edwards, D. G. (1978b) Response of cassava, sunflower, and maize to potassium concentration in solution. II. Potassium absorption and its relation to growth. *Field Crops Research*, **1**: 363-373

Spiess, E. (2011) Nitrogen, phosphorus and potassium balances and cycles of Swiss agriculture from 1975 to 2008. *Nutrient Cycling in Agroecosystems*, **91**: 351-365

Subbarao, G. V., Wheeler, R. M., Stutte, G. W., and Levine, L. H. (1999) How far can sodium substitute for potassium in red beet? *Journal of Plant Nutrition*, **22**: 1745-1761

Svishcheva, G. R., Axenovich, T. I., Belonogova, N. M., van Duijn, C. M., and Aulchenko, Y. (2012) Rapid variance components-based method for whole-genome association analysis. *Nature Genetics*, **44**: 1166-1170

Szczerba, M. W., Britto, D. T., and Kronzucker, H. J. (2006) Rapid, futile K^+ cycling and pool-size dynamics define low-affinity potassium transport in barley. *Plant Physiology*, **141**: 1494-1507

Takehisa, H., Sato, Y., Antonio, B. A., and Nagamura, Y. (2013) Global transcriptome profile of rice root in response to essential macronutrient deficiency. *Plant Signaling and Behaviour*, **6**: e24409

Tanaka, A. and Yoshida, S. (1965) *Nutritional disorders of the rice plant in Asia.* Los Baños, Philippines: IRRI, pp. 1-51

ten Hoopen, F., Cuin, T. A., Pedas, P., Hegelund, J. N., Shabala, S., Schjoerring, J. K., and Jahn, T. P. (2010) Competition between uptake of ammonium and potassium in barley and *Arabidopsis* roots: molecular mechanisms and physiological consequences. *Journal of Experimental Botany*, **61**: 2303-2315

Thornley, J. H. M. (1969) A model to describe the partitioning of photosynthate during vegetative plant growth. *Annals of Botany*, **33**: 419-430

Thornley, J. H. M. (1972) A balanced quantitative model for root:shoot ratios in vegetative plants. *Annals of Botany*, **36**: 431-441

Tibshirani, R. (1996) Regression shrinkage and selection via the lasso. *Journal of the Royal Statistical Society. Series B (Methodological*, **58**: 267-288

Timsina, J., Singh, V. K., and Majumdar, K. (2013) Potassium management in rice-maize systems in South Asia. *Journal of Plant Nutrition and Soil Science*, **176**: 317-330

Travis, A. J., Norton, G. J., Datta, D., Sarma, R., Dasgupta, T., Savio, F. L., Macaulay, M., Hedley, P. E., McNally, K. L., Sumon, M. H., Islam, M. R., and Price, A. H. (2015) Assessing the genetic diversity of rice originating from Bangladesh, Assam and West Bengal. *Rice*, **8**: 35

Trehan, S. P. and Claassen, N. (1998) External K requirement of young plants of potato, sugar beet, and wheat in flowing solution culture resulting from different internal requirements and uptake efficiency. *Potato Research*, **41**: 229-237

Trehan, S. P., El Dessougi, H., and Claassen, N. (2005) Potassium efficiency of 10 potato cultivars as related to their capability to use nonexchangeable soil potassium by chemical mobilization. *Communications in Soil Science and Plant Analysis*, **36**: 1809-1822

USGS (2016) *Mineral commodity summaries 2016.* Reston, Virginia: USGS, pp. 1-201

USGS (2017) *Mineral commodity summaries 2017.* Reston, Virgina: USGS, pp. 1-202

van der Wiel, C. C. M., van der Linden, C. G., and Scholten, O. E. (2016) Improving phosphorus use efficiency in agriculture: opportunities for breeding. *Euphytica*,

207: 1-22

Venables, W. N. and Ripley, B. D. (2002) Modern applied statistics with S. New York: Springer, pp. 1-495

Walker, D. J., Black, C. R., and Miller, A. J. (1998) The role of cytosolic potassium and pH in the growth of barley roots. *Plant Physiology*, **118**: 957-964

Walker, D. J., Leigh, R. A., and Miller, A. J. (1996) Potassium homoeostasis in vacuolate plant cells. *Proceedings of the National Academy of Sciences of the United States of America*, **93**: 10510-10514

Wang, C., Chen, H., Hao, Q., Sha, A., Shan, Z., Chen, L., Zhou, R., Zhi, H., and Zhou, X. (2012) Transcript profile of the response of two soybean genotypes to potassium deficiency. *PLoS One*, **7**: e39856

Wang, H. -Y., Shen, Q. -H., Zhou, J. -M., Wang, J., Du, C. -W., and Chen, X. -Q. (2011) Plants use alternative strategies to utilize nonexchangeable potassium in minerals. *Plant and Soil*, **343**: 209-220

Wang, J. G., Zhang, F. S., Cao, Y. P., and Zhang, X. L. (2000) Effect of plant types on release of mineral potassium from gneiss. *Nutrient Cycling in Agroecosystems*, **56**: 37-44

Wang, M., Zheng, Q., Shen, Q., and Guo, S. (2013) The critical role of potassium in plant stress response. *International Journal of Molecular Sciences*, **14**: 7370-7390

Wang, X. -P., Chen, L. -M., Liu, W. -X., Shen, L. -K., Wang, F. -L., Zhou, Y., Zhang, Z., Wu, W. -H., and Wang, Y. (2016) AtKC1 and CIPK23 synergistically modulate AKT1-mediated low-potassium stress responses in *Arabidopsis*. *Plant Physiology*, **170**: 2264-2277

Wang, Y., He, L., Li, H. -D., Xu, J., and Wu, W. -H. (2010) Potassium channel α-subunit AtKC1 negatively regulates AKT1-mediated K^+ uptake in *Arabidopsis* roots under low-K^+ stress. *Cell Research*, **20**: 826-837

Wang, Y. and Wu, W. -H. (2015) Genetic approaches for improvement of the crop potassium acquisition and utilization efficiency. *Current Opinion in Plant Biology*, **25**: 46-52

White, P. J. (1993) Relationship between the development and growth of Rye (*Secale cereale* L.) and the potassium concentration in solution. *Annals of Botany*, **72**: 349-358

White, P. J. (1997) The regulation of K^+ influx into roots of rye (*Secale cereale* L.) seedlings by negative feedback *via* the K^+ flux from shoot to root in the phloem.

Journal of Experimental Botany. **48**: 2063-2073

White, P. J. (2013) Improving potassium acquisition and utilisation by crop plants. *Journal of Plant Nutrition and Soil Science*, **176**: 305-316

White, P. J. and Karley, A. J. (2010) Potassium. In Hell, R. H. and Mendel, R. -R. (eds.) *Cell Biology of Metals and Nutrients.* Berlin: Springer, pp. 199-224

White, P. J., Hammond, J. P., King, G. J., Bowen, H. C., Hayden, R. M., Meacham, M. C., Spracklen, W. P., and Broadley, M. R. (2010) Genetic analysis of potassium use efficiency in *Brassica oleracea. Annals of Botany*, **105**: 1199-1210

Williams, R. F. (1948) The effects of phosphorus supply on the rates of intake of phosphorus and nitrogen and upon certain aspects of phosphorus metabolism in *Gramineous* plants. *Australian Journal of Biological Sciences*, **1**: 333-361

Woodend, J. J. and Glass, A. D. M. (1993) Genotype-environment interaction and correlation between vegetative and grain production measures of potassium use-efficiency in wheat (*T. aestivum*, L.) grown under potassium stress. *Plant and Soil*, **151**: 39-44

World Bank (2017) http://www.worldbank.org/en/research/commodity-markets#1 [accessed 3$^{\text{rd}}$ August 2017]

Wu, P., Ni, J. J., and Luo, A. C. (1998) QTLs underlying rice tolerance to low-potassium stress in rice seedlings. *Crop Science*, **38**: 1458-1462

Xia, Y., Jiang, C., Chen, F., Lu, J., and Wang, Y. (2011) Differences in growth and potassium-use efficiency of two cotton genotypes. *Communication in Soil Science and Plant Analysis*, **42**: 132-143

Xu, J., Li, H. -D., Chen, L. -Q., Wang, Y., Liu, L. -L., He, L., and Wu, W. -H. (2006) A protein kinase, interacting with two calcineurin B-like proteins, regulates K^+ transporter AKT1 in *Arabidopsis. Cell*, **125**: 1347-1360

Xu, X., Liu, X., He, P. Johnston, A. M., Zhao, S., Qiu, S., and Zhou, W. (2015) Yield gap, indigenous nutrient supply and nutrient use efficiency for maize in China. *PLoS One*, **10**: e0140767

Yamaguchi, T. and Blumwald, E. (2005) Developing salt-tolerant crop plants: challenges and opportunities. *Trends in Plant Science*, **10**: 615-620

Yang, F., Du, M., Tian, X., Eneji, A. E., Duan, L., and Li, Z. (2014) Plant growth regulation enhanced potassium uptake and use efficiency in cotton. *Field Crops Research*, **163**: 109-118

Yang, J. -S., Hu, W., Zhao, W., Meng, Y., Chen, B., Wang, Y., and Zhou, Z. (2016) Soil potassium deficiency reduces cotton fiber strength by accelerating and shortening fiber development. *Scientific Reports*, **6**: 28856

Yang, T., Zhang, S., Hu, Y., Wu, F., Hu, Q., Chen, G., Cai, J., Wu, T., Moran, M., Yu, L., and Xu, G. (2014) The role of potassium transporter OsHAK5 in potassium acquisition and transport from roots to shoots in rice at low potassium supply levels. *Plant Physiology*, **166**: 945-959

Yang, X. E., Li, H., Kirk, G. J. D., and Dobermann, A. (2005) Room-induced changes of potassium in the rhizosphere of lowland rice. *Communications in Soil Science and Plant Analysis*, **36**: 1947-1963

Yang, X. E., Liu, J. X., Wang, W. M., Li, H., Luo, A. C., Ye, Z. Q., and Yang, Y. (2003) Genotypic differences and some associated plant traits in potassium internal use efficiency of lowland rice (*Oryza sativa* L.). *Nutrient Cycling in Agroecosystems*, **67**: 273-282

Yang, X., Liu, J. X., Wang, W. M., Ye, Z. Q., and Luo, A. C. (2004) Potassium internal use efficiency relative to growth vigor, potassium distribution, and carbohydrate allocation in rice genotypes. *Journal of Plant Nutrition*, **27**: 837-852

Yoshida, S. (1981) *Fundamentals of Rice Crop Science.* Los Baños, Philippines: IRRI, pp. 1-269

Yoshida, S., Forno, D. A., Cock, J. H., and Gomez, K. A. (1976) *Laboratory manual for physiological studies of rice.* Los Baños, Philippines: International Rice Research Institute, pp. 1-83

Yousaf, M., Li, J., Ren, T., Cong, R., Fahad, S., and Li, X. (2017) Effects of fertilization on crop production and nutrient-supplying capacity under rice-oilseed rape rotation system. *Scientific reports*, **7**: 1270

Yu, J., Hu, S., Wang, J., Wong, G. K. -S., Li, S., Liu, B., Deng, Y., Dai, L., Zhou, Y., Zhang, X., Cao, M., Liu, J., Sun, J., Tang, J., Chen , Y., Huang, X., Lin, W., Ye, C., Tong, W., Cong, L., Geng, J., Han, Y., Li, L., Li, W., Hu, G., Huang, X., Li, W., Li, J., Liu, Z., Li, L., Liu, J., Qi, Q., Liu, J., Li, L., Li, T., Wang, X., Lu, H., Wu, T., Zhu, M., Ni, P., Han, H., Dong, W., Ren, X., Feng, X., Cui, P., Li, X., Wang, H., Xu, X., Zhai, W., Xu, Z., Zhang, J., He., S., Zhang, J., Xu, J., Zhang, K., Zheng, X., Dong, J., Zeng, W., Tao, L., Ye, J., Tan, J., Ren, X., Chen, X., He, J., Liu, D., Tian, W., Tian, C., Wang, J., Zhao, W., Li, P., Chen, W., Wang, X., Zhang, Y., Hu, J., Wang, J., Liu, S., Yang, J., Zhang, G., Xiong, Y., Li, Z., Mao, L., Zhou, C., Zhu, Z., Chen, S., Guo, W., Li, G., Liu, S., Tao, M., Wang, J., Zhuo, L., Yuan, L., and Yang, H. (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*). *Science*: **296**: 79-92

Zeng, J., He, X., Wu, D., Zhu, B., Cai, S., Nadira, U. A., Jabeen, Z., and Zhang, G. (2014) Comparative transcriptome profiling of two Tibetan wild barley genotypes in responses to low potassium. *PloS One*, **9**: e100567

Zhang, X., Jiang, H., Wang, H., Cui, J., Wang, J., Hu, J., Guo, L., Qian, Q., and Xue, D. (2017) Transcriptome analysis of rice seedling roots in response to potassium deficiency. *Scientific Reports*, **7**: 5523

Zhao, K., Tung, C. -W., Eizenga, G. C., Wright, M. H., Ali, M. L., Price, A. H., Norton, G. J., Rafiqul, I., Reynolds, A., Mezey, J., McClung, A. M., Bustamante, C. D., and McCouch, S. R. (2011) Genome-wide association mapping reveals a rich genetic architecture of complex traits in *Oryza sativa*. *Nature Communications*, **2**: 467

Zhao, K., Wright, M., Kimball, J., Eizenga, G., McClung, A., Kovach, M., Tyagi, W., Ali, M. L., Tung, C. -W., Reynolds, A., Bustamante, C. D., and McCouch, S. R. (2010) Genomic diversity and introgression in *O. sativa* reveal the impact of domestication and breeding on the rice genome. *PloS One*, **5**: e10780

Zhao, Y., Li, X. -Y., Zhang, S. -H., Wang, J., Yang, X. -F., Tian, J. -C., Hai, Y., and Yang, X. -J. (2014) Mapping QTLs for potassium-deficiency tolerance at the seedling stage in wheat (*Triticum aestivum*, L.). *Euphytica*, **198**: 185-198

Zörb, C., Senbayram, M., and Peiter, E. (2014) Potassium in agriculture - status and perspectives. *Journal of Plant Physiology*, **171**: 656-669

Zuur, A. F., Ieno, E. N., Elphick, C. S. (2010) A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, **1**: 3-14