Understanding the contribution of root traits for phosphorus responsiveness of wheat

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List of abbreviations

Al	Aluminum
AMF	Arbuscular Mycorrhizal Fungi
ANOVA	Analysis of Variance
ATP	Adenosine tri-phosphate
BLUEs	Best Linear Unbiased Estimates
BLUPs	Best Linear Unbiased Predictions
C	Carbon
Ca	Calcium
Cu	Copper
DAP	Di Ammonium Phosphate
DCP	Di Calcium Phosphate
D-LDH	D-Lactate Dehydrogenase
DH	Double Haploid
DM	Dry Matter
Fe	Iron
HAP	Hydroxyapatite
L-MDH	L-Malate Dehydrogenase
LOD	Logarithm of Odds
LR	Lateral Root
LRS	Likelihood Ratio Statistic
LSD	Least Significant Difference
MAP	Mono Ammonium Phosphate
MDH	Malate Dehydrogenase
Mg	Magnesium
N	Nitrogen
NADH	Nicotinamide Adenine Dinucleotide
NPK	Nitrogen Phosphorus Potassium
P	Phosphorus
PAE	Phosphorus Acquisition Efficiency
PUE	Phosphorus Uptake Efficiency
PR	Primary Root
QTL	Quantitative Trait Loci
RDW	Root Dry Weight
RO	Reverse Osmosis
RHL	Root Hair Length
S	Sulphur
SDW	Shoot Dry Weight
SNP	Single Nucleotide Polymorphism
SRA	Specific Root Area
SRL	Specific Root Length
SSP	Single Super Phosphate
TRL	Total Root Length
TSP	Triple Super Phosphate
Zn	Zinc

Abstract

Wheat is a major and widely-grown cereal crop around the world. Phosphorus (P) is a crucial element for plant growth and development, but the availability of soil P is very low. The low availability of soil P poses a serious nutritional constraint for plant growth. To combat the large difference between the P requirement for plant growth and the available soil P, plants have developed a number of root-based adaptive strategies to cope in low P environments. Crop improvement to increase P uptake efficiency will depend on exploiting one or more of these adaptive strategies.

To understand the contribution of a number of adaptive mechanisms of wheat varieties under P deficiency, a series of controlled environment experiments and some field studies were conducted. Ten bread wheat varieties were selected which have shown differential responses to applied P in a previous series of field trials over different sites and seasons. According to their response to P, varieties were categorised as non-responsive or responsive varieties. Non-responsiveness to applied P is indicative of high phosphorus use efficiency (PUE) which was considered to be the preferred trait. The study compared several root traits, which have been demonstrated to contribute to plant growth under P deficient conditions: seminal and crown root angle, root hair length, rhizosheath size, arbuscular mycorrhizal fungi (AMF) colonization and organic acid releasing capacity. Based on the results of these experiments, a further study was done to identify quantitative trait loci (QTL) for rhizosheath size and root hair length.

The findings of these experiments suggests that wide crown root angle, rather than seminal root angle, was associated with the non-P responsive varieties. These varieties benefit from shallow crown roots at later stages of their growth cycle when the demand for P increases. The non-responsive varieties also had longer root hairs regardless of

soil type or P treatments, and this was associated with a greater rhizosheath size. From these experiments, it was concluded that longer root hair length, greater rhizosheath size and shallow crown root are traits that contributed to the better performance in the field of the non-responsive varieties. Multivariate analysis for the all the traits also support this as most of the non-responsive varieties clustered together. Cluster analysis for shoot dry weight at nil P treatment and from two different soils in these experiments demonstrated that the ranking of varieties were similar to the ranking of varieties from the field based on the yield response.

QTL analysis was performed using a double haploid wheat population to understand the relation between root hair length and rhizosheath size. Despite the weak phenotypic correlation between root hair length and rhizosheath characteristics, co-located QTL were detected on chromosome 7A, a result consistent with reports from the literature supported. Four novel QTLs were detected for rhizosheath size from this study. Co-localization of other QTLs on chromosome 2A, 4B and 5A was also observed and information from available literature suggests that those chromosomal regions are important for yield and yield related components.

A significant difference among varieties was observed for AMF colonization, but it was not possible to relate this variation with the varietal P responsiveness. Varietal difference was also observed for the citric and malic acid concentration in the rhizosheath soil, but it was also not possible to relate that difference with the observed difference in varietal P responsiveness from field.

This study suggested that selection of varieties with more than one adaptive mechanisms to grow well under P deficient conditions is possible. Selection based on greater root hair length, greater rhizosheath size and wide crown root angle appears to

be most crucial adaptive mechanisms for growth and yield under P deficiency. Selection of varieties with more than one mechanisms will allow the variety to grow well under wide range of environmental conditions without compromising yield. The chromosomal region identified from this study can be selected for gaining further understanding on the genetic control of those traits and could be targeted for marker aided selection to improve wheat varieties. Future work should consider the genetic control and inheritance of these root traits to develop new varieties with less P dependency and greater capacity to acquire of soil P.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Kamrun Nahar…… **Date…….**

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Chapter 1 : General Introduction

Phosphorus (P) is one of the essential macronutrients for crop growth and development and after nitrogen it is the most important nutrient limiting crop growth. More than 40% of world's arable land is P deficient (Vance et al 2003) which limits agricultural productivity over large areas. The world's population is increasing and to meet the demands of a burgeoning population it is necessary to increase agricultural production and P fertilizer plays a key role in achieving this. However P fertilizer is a limited resource and it is being depleted at an alarming rate. According to some estimates the global P reserve will be completely exhausted by the year 2050 (Cordell et al. 2009). The price of P fertilizer will increase in future due to its increasing demand and production costs.

Phosphorus exists as phosphate minerals in nature and it is extremely reactive. There are at least 170 different phosphate minerals and they differ greatly in their solubility and as time goes the mineral forms of P tend to transform from sparingly soluble to increasingly insoluble forms (Holford 1997), but the rate is slow. Soil physical and chemical properties control the forms and solubility of the different P components. Soil properties such as pH, concentrations of iron (Fe), aluminium (Al), calcium (Ca) and the nature and surface areas of soil particles are important for the solubilization of P and its availability to plants. The total amount of soil P can be high, but its availability is very low which can cause important nutritional constraints to the growth of plant (Bates and Lynch 2000). Application of P fertilizer is the common agricultural practice to mitigate the low availability of P in agricultural soil (Ramaekers et al. 2010). However application of P fertilizer in excess of the requirement of plants can contribute to eutrophication and also put pressure on precious P fertilizer. The fate of applied P

depends on several processes such as uptake by plants, retention by soil or loss through leaching (Bolland 2000).

About 50-80% of total P in fertiliser is retained by the soil after its application (McBeath et al. 2012; McLaughlin et al. 1988), which makes P poorly available to plants and has resulted in substantial banks of soil P being built up. Improving the ability of crops to access this bank has the potential to significantly improve the profitability of farming systems. Identification of cultivars which are able to use nutrient efficiently is a desirable approach as there is no additional cost involved (Aziz et al. 2014).

Plants take up P from the soil as orthophosphate (Pi). The concentration of Pi in the soil solution is often very low and it rarely exceeds 10 µM (Schachtman et al. 1998). In addition, the movement of Pi in the soil solution is very slow because the diffusion is the most important process for the movement of P ions to the root surface (Marschner 1995; Syers et al. 2008). Plants have evolved a wide range of adaptive mechanisms to maintain P uptake and sustain growth when P supply is low. These mechanisms can be classified as acquisition efficiency and utilization efficiency (Rengel and Marschner 2005, Vance et al. 2003). Acquisition efficiency can be defined as the capacity to absorb sparingly soluble nutrients like P and utilization efficiency denotes to the capacity to produce greater biomass per unit of nutrient absorbed (Aziz et al. 2014).While improved utilization efficiency has often been suggested to be an important way of increasing P use efficiency, in many soils and especially soils that can fix P, uptake of sufficient amounts of P is often an important limitation to improved response to soil P. Enhanced root growth with modified root architecture (Bucher 2007; Gahoonia and Nielsen 2004; George and Richardson 2008; Lynch et al. 2005; Lynch and Brown 2001; Raghothama and Karthikeyan 2005; White and Hammond 2008), root hair development (Bates and

Lynch 1996; Ma et al. 2001) and enhanced expression of Pi transporters (Gilroy and Jones 2000), exudation of organic acids and phosphatases (Dakora and Phillips 2002; Gahoonia and Nielsen 2004; Gahoonia et al. 2000; Johnson and Loeppert 2006; Vance et al. 2003) and symbiosis with mycorrhizal fungi (Smith and Read 1997) are major adaptive mechanisms to enhance P uptake. There is substantial genetic variation in these adaptive mechanisms among and within crop species for efficient P use (Aziz et al. 2014). Understanding the underlying mechanisms of how plants sense and respond to P starvation might facilitate selection, breeding and GM approaches to improve crop production and reduced the reliance of non-renewable inorganic P (Hammond et al. 2004; Vance et al. 2003).

Uptake efficiency is related to root traits as plants take up all required nutrients primarily by their root system. Breeding for improved P uptake, by altering root architecture, has frequently been advocated as an important way of increasing crop P efficiency (Liao et al. 2004; Zhu and Lynch 2004). Root architectural changes consist of changes in root length, root branching, root hair formation and top soil foraging. The benefit of a large root system is that it increases the nutrient absorption area which is important for P absorption from soil (Gahoonia and Nielsen 1998; Lynch 2007).

Although a huge amount of work has been done to understand P uptake efficiency, the complexity of P nutrition makes it a difficult task and the relative importance of difference traits related to P efficiency is still not well understood. Targeting a single trait to improve P efficiency may not be always effective because the contribution of a particular trait can vary depending on the target environment (McDonald et al. 2015). For example, Liao et al (2004) detected several QTLs for rooting depth which were related to P acquisition efficiency (PAE, equivalent to P uptake efficiency) of common bean (*Phaseouls vulgaris*) and also observed several QTLs related to PAE that were not

related to root shallowness. They concluded that for a successful breeding programme it will be useful to select for multiple root traits rather than a single trait. Although much work has been done to assess the mechanisms and/or genetic controls of different adaptive mechanisms under P deficient conditions, the relative contribution of these different mechanisms towards varietal differences in PAE is not well understood. The complex nature of soil P and the environmental effects on root traits means that a single trait may not be effective under all conditions where P is limiting plant growth. Moreover traits identified as suitable for improved P acquisition under controlled environment may not work under field condition (Ryan et al. 2014). Thus more research is necessary to examine how different traits contribute towards varietal differences in P efficiency.

The problem associated with the low availability of P emphasises the need to identify efficient varieties that are able to grow well under P deficient condition or acquire more P from P fixing soil. In this study, a trait dissection approach was used to try to understand better the relative importance of the different mechanisms of P efficiency in wheat. Differences in P efficiency among wheat varieties were first identified in field trials and then selected varieties were assessed for specific root traits in controlled environment studies to understand the contribution of different adaptive traits that lead to the differences in P efficiency in the field experiments, but with a focus on root traits. This study examined several adaptive mechanisms, such as root architecture, rhizosheath size, root exudates and colonisation with arbuscular mycorrhizae (AM), to understand the relative contribution of these root traits and how several mechanisms work in parallel in the same plant towards its P efficiency.

The focus of this study was to understand the genetic basis of adaptive mechanisms and how these mechanisms can enhance P uptake at low P availability. The principal

objective of this thesis was to determine the effect of root traits to acquire P under low P conditions and to relate that with the observed field performance. A further aim was to understand genetic control of some of the root traits and to identify QTL from a mapping wheat population.

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Chapter 2 : Literature review

Introduction

After nitrogen (N), phosphorus (P) is the most limiting nutrient for crop production (Vance et al. 2003). Low P availability in soil is an important nutritional constraint for crop production in many soils (Bates and Lynch 2000) but irrespective of total P content of the soils, the low mobility of P in soil means that supply to the roots can be poor (Hinsinger 2001; Schachtman et al. 1998). Phosphorus plays a crucial role in plant productivity and substantial yield losses can occur when P availability is low. It is a major component of nucleic acids, phospholipids and ATP and is essential for photosynthesis (Schachtman et al. 1998). Phosphorus is also involved in carbon (C) and N metabolism (Huang et al. 2008), signal transduction cascades, photosynthetic and respiratory metabolisms and regulation of enzymes (Amtmann et al. 2005; Mimura 1999).

The importance of P to crop production means that in most cropping systems, P fertiliser is applied routinely to crops, although the rate and the frequency of application varies considerably. However, there are several concerns associated with the current global use of P fertilizer, which include (i) limitations of high quality phosphate rock which is the raw materials for P fertilizer, (ii) the low rates of P uptake by agricultural plants leading to low P use efficiency (PUE), (iii) poor uptake associated with high rates of application of P, which can lead to environmental pollution and (iv) the increasing cost associated with P fertilizer application as high quality reserves of rock phosphate are depleted. These issues of P supply and recovery are occurring at a time of growing

demand for P from an increasing world population and supply of food. To help improve the efficiency of P supply and sustainable P fertiliser use there is a strong argument to identify varieties which are able to acquire existing soil P and also which are able to use the acquired P efficiently to complement improvements in P management.

Plants have evolved several adaptive mechanisms to P deficiency and it is well documented that plant genotypes differ greatly in their adaptive mechanisms to P deficiency. The aim of the review is to provide an overview of the genetic differences and the importance of many of these adaptive mechanisms for P uptake with an emphasis on root architectural changes and symbiosis with mycorrhizal colonization of wheat.

Phosphorus in soil

The total amount of P in soil can be high, but the free P in soil solution is very low and often its concentration is of the order of 1 µM (Mimura 1999; Vance et al. 2003). The concentration of P in the cytoplasm of cereal plants are 10 times higher at around 10 µM or 0.2-1% of dry matter (Schachtman et al. 1998). This large gradient makes P the least available of the essential nutrients for plant growth in many agricultural systems (Lynch 1995; Schachtman et al. 1998; Shenoy and Kalagudi 2005).

In soil total P can be categorised as organic P (Po) and inorganic P (Pi). The transformation of soil P and different P pools in soil is showing in Figure 2.1.

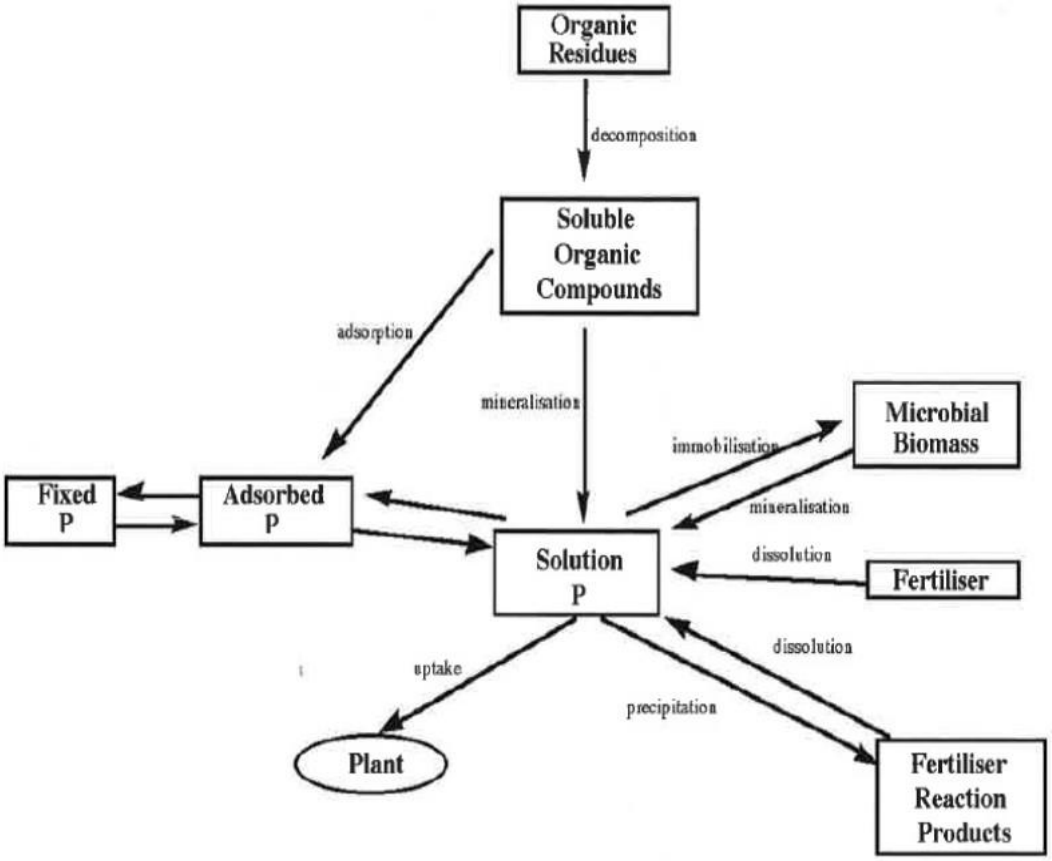

Figure 2.1. Phosphorus cycle in soil (adapted from Moody and Boland 1999).

Organic P

Soil organic P (Po) is the P that is bound with organic compounds and must be mineralized before it can be taken by plants (Horst et al. 2001). The major form of Po in many soils is the orthophosphate monoester and with lesser amounts of phospholipid, nucleic acids, phosphonates and other compounds (Smernik and Dougherty 2007; Turner et al. 2005).

In Australian soils the amount of Po is typically in the range of 40-900 mg/kg (Stevenson 1999). The variation of the availability of Po is due to several factors such as soil texture, soil pH, temperature, organic C content, mineralization and

immobilization. According to Sanyal and Datta (1991) Po may be derived from plant residues, soil organic matter and microorganisms. Soil microorganisms play a vital role in the availability of soil P to the plants and the mineralization of Po in the soil largely depends on the soil microbial community (Horst et al 2001). A large number of studies have demonstrated that microorganisms are able to hydrolyze a wide range of organic P substrates when grown in culture and the rapid mineralization of different forms of soil organic P was observed when grow in soil (Adams and Pate 1992; Macklon et al. 1997).

Inorganic P

Inorganic P consists of poorly soluble phosphate salts, Ca phosphate in alkaline soil and Fe and Al phosphate in acid soil (Marschner 1995). Inorganic P is present in soil as orthophosphate (Pi) ions (H_2PO_4- and HPO_4^{2}-). The concentrations of Pi present in soil varies with soil pH, clay contents and mineral types (Brady and Weil 2000). Phosphorus fertilizer is the main source of Pi in agricultural ecosystems.

The total P in Australian soils is usually >250 mg P/kg in the top 0.10m (Richardson et al. 2009b). A large proportion of soil Pi can be adsorbed or fixed to clay minerals, Fe/ Al oxides, hydroxides or organic matter complexes (Hinsinger 2001). Clay minerals and Fe/ Al oxides provide a large number of adsorption sites as they have large specific surface area (Shen et al. 2011). With further reactions in Fe/Al oxides, P become occluded into nanopores and becomes unavailable to plants (Arai and Sparks 2007). Precipitation refers to a reaction of phosphate ions with metal cations, which forms a range of insoluble P minerals (Hinsinger et al 2001). The types of precipitated minerals depend on soil pH. Precipitation of phosphate with Ca generates di calcium phosphate

(DCP), which is available to plants. Eventually DCP is transformed into more stable forms such as octocalcium phosphate and hydroxyapatite (HAP) which are less available to plants (Arai and Sparks, 2007) and HAP can constitute up to 50% of total soil Pi (Shen et al 2011). Phosphate minerals can be divided in to primary and secondary minerals. Primary P minerals such as apatites, strengite, and variscite are very stable, in contrast the dissolution rate of secondary P minerals such as calcium (Ca), iron (Fe) and aluminium (Al) phosphate, which vary depending on the size of mineral particle and soil pH (Shen et al 2011).

Phosphorus uptake and translocation by plants

Phosphorus is taken up by plants Pi and the concentration of Pi in the soil solution is very low, rarely exceeding 10 µm (Schachtman et al. 1998). Plants have evolved a number of mechanisms to take up P at low availability. Movement of Pi in the soil to the roots is by diffusion rather than mass flow (Hinsinger 2001). At the root surface Pi is taken up rapidly resulting in a P depletion shell of 0.2-1.0mm around the root (Holford 1997). Kinetic analysis of Pi uptake shows that plants have both low and high affinity uptake systems (Vance et al. 2003). The high affinity uptake process is induced by P deficiency, whereas the low affinity system appears to be constitutive in plants (Raghothama 1999). The presence of these two systems operating at different concentrations means that plants can take up Pi over a wide range of concentrations.

Once plants take up Pi through the roots it is transported within the plant *via* specific Pi-transporters. A number of genes encoding Pi transporters have been cloned by Rausch and Bucher (2002) and the member of the Pht1 family are particularly important for Pi uptake (Mudge et al. 2002; Schünmann et al. 2004). Expression of specific Pht1

genes is localized in root epidermal cells and root hair cells and these PHT 1 proteins show high affinity Pi transport. These P transporters are induced by P deficiency and transport Pi across the plasma membrane against the steep electrochemical gradient of Pi that occurs between plant cells and the soil solution (Bieleski 1973; Schachtman et al. 1998).

Remobilization of internal P is important for plant growth besides P uptake by root. According to Schachtman et al (1998) the concentration of cytoplasmic Pi remains constant but the vacuolar concentration of Pi varies widely under P starvation (Schachtman et al 1998). Under P deficiency plants produce more roots for increased P uptake which retranslocates Pi from older leaves and depletes vacuolar Pi storage (Schachtman et al 1998). In Arabidopsis the AtPHO1 gene was found to be important for Pi transport from root to shoot (Venecklaas et al 2012).

Whatever the P status of soil, a very large proportion of P present in the vegetative parts of plants moves to the reproductive part (Veneklaas et al. 2012). For example, maize exports two third of its total acquired P to the harvested part, small grain crops such as soybean exports 80-100% to the harvested part (Vance et al 2003). For a profitable farming system it is important to produce crop with lower P export and because of nutritional and environmental reason large seed P concentration is not desirable. However, seed P content is important for seedling vigour and low grain P may adversely affect this.

Deficiency symptoms

Phosphorus is a phloem mobile nutrient and P deficiency first starts in older leaves. Notable changes due to P deficiency in plants are spindly growth habit, acute leaf angles, suppression of tillering and branching, prolonged dormancy, early senescence

and decreased size and number of flowers and buds (Marschner 1995). Among the first deficiency symptoms of P, development of dark green or blue green foliage is most common, but red, purple or brown pigments also develop in leaves, especially along veins as severity increases (White and Hammond 2008). Phosphorus deficiency reduces leaf area which reduces light interception and this becomes worse under severe deficiency as a result of chlorosis and necrosis. Severe P deficiency can cause chloroplast abnormalities which causes the reduction of grana and their morphology which adversely affects chloroplast function (White and Hammond 2008). Phosphorus deficiency also gradually reduces the rates of cell division, expansion, photosynthesis and respiration, and changes in the abundance of C, N and S metabolites and concentration of plant growth regulators (Marschner 1995).

Phosphorus deficient plants generally display stunted growth and increased root: shoot ratio (Lynch et al. 1991). Due to reduced leaf expansion and reduced leaf initiation, there is reduced shoot growth in P deficient plants and a change in partitioning of biomass (Lynch et al. 1991). For example, a significant increase in the proportion of assimilated carbon devoted to root growth and maintenance in common bean was observed at low P availability (Lynch et al. 1991; Nielsen et al. 1998; Nielsen et al. 2001).

Phosphorus use in Australian agriculture

Australian soils contain a relatively low amount of total P, and consequently applications of P fertilizer have been required to maintain productivity. The consumption of P fertilizer in Australian agriculture is relatively high compared to global rates of P consumption, but in recent years the use of P fertilizer has fallen, which

has been due to a combination of high fertilizer cost and drought (Bovill et al. 2013). In most parts of Australia P fertilizer is applied regularly and it is an important input into cereal production. Phosphorus fertilizer is often used in a P replacement strategy in which the rate of P fertilizer is equivalent to the removal of P by the previous crop, with some adjustment made for P recovery. Mono-ammonium phosphate (MAP) and di-ammonium phosphate (DAP) are more commonly used compared to single (9% P) or triple super phosphate forms (20% P) (Figure 2.2). The stable water soluble forms of P in MAP and DAP are suitable for making stable granulated, solid fertilizer for agricultural use (Bolland 2000) and there is the added benefit of appling N with the P. In recent years different forms of P fertilizer such as liquid P-fertilizer have become more popular in Australia on some highly P-fixing soils. Lombi et al (2004) found that compared to granular fertilizer, fluid fertilizer significantly increased P availability and diffusion in calcareous soil. According to McLaughlin et al (2011) at an equivalent rate of application, fluid source of P is 15 times more effective than a granular source on these soils. To reduce the offsite movement of applied P recently some new polymers and slow release coating of water soluble P is becoming popular (McLaughlin et al 2011). Accumulation of P in most of Australian agricultural soil is reported to be due to the application of P in excess of the amount of P exported in the grain (McLaughlin et al. 2011).

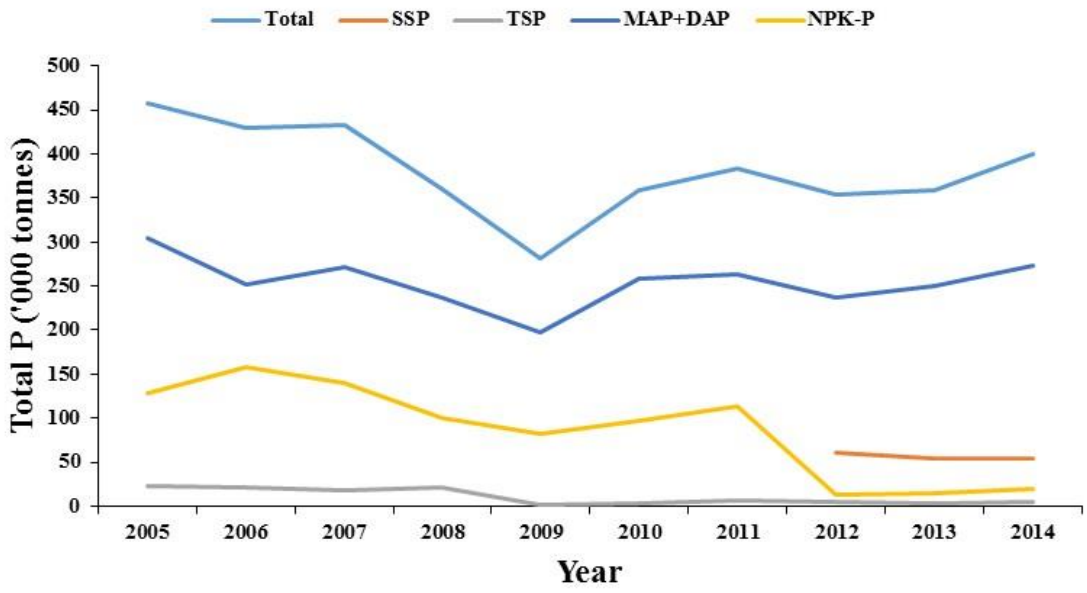

Figure 2.2. Total P from different forms of P fertilizers applied in Australia from 2005 to 2014. SSP: single super-phosphate; TSP: triple super phosphate MAP: mono-ammonium phosphate; DAP: di-ammonium phosphate; NPK-P: NPK compound fertilisers (source International Fertilizer Association).

Fixation of P reduces availability of P to the plant. It can be reduced by banding of P in the root zone, which involves placing the P 3-5cm under the seeds while sowing. It has two benefits: firstly, it localizes phosphate concentrates that reduces contact with soil constituents that cause fixation; and secondly, it increases P concentration in the soil solution near the root zone that increase P uptake by plants.

While the use of different P formulations and management practices can help to improve P availability and uptake, recovery of P applied as fertiliser is still often low and plants still rely largely on the uptake of residual P to meet their P requirements (McBeath et al. 2012). Improving the ability of plants to take up P or to use it more efficiently can contribute to the improvement in the overall efficiency of a cropping system.

Phosphorus efficiency

To select nutrient efficient varieties it is necessary to understand what nutrient efficiency is. Phosphorus efficiency can be considered in terms of acquisition efficiency and utilization efficiency (Rengel and Marschner 2005; Vance et al. 2003). Acquisition efficiency is the ability to take up a sparingly soluble nutrient such as Pi, while utilization efficiency can be defined as the capacity to produce greater biomass per unit of nutrient absorbed (Aziz et al. 2014). Root architecture, root morphology, mycorrhizal association, high affinity transporters and rhizosphere alteration are some of the mechanisms that could contribute to acquisition efficiency (Lambers et al. 2006). According to Siddiqi and Glass (1981) utilization efficiency can be define as the amount of biomass production or yield production per unit of nutrient present in biomass. Remobilization of internal P, metabolic modification that bypass P requiring steps or reduced consumption are the process that are involved in utilization efficiency (Fernandez et al. 2009). According to Shenoy and Kalagudi (2005) it is necessary to understand the physiological and molecular basis of mineral nutrient uptake and utilization in plants to develop better nutrient-efficient cultivars. Phosphorus efficiency is not an easy phenomenon to understand, as most of the parameters related to P efficiency vary according to growth conditions or environment and isolation of individual effects of P efficiency is not straightforward (Fernandez et al 2009).

Terminology can be a problem when discussing P efficiency. Many different terms are used in the literature to define P use efficiency (Table 2.1), and their use is often not consistent, which creates problems of identifying efficient genotypes. Gourley et al (1993) compared five commonly-used definitions of nutrient efficiency to rank the efficiency of different genotypes (Lucerne and white clover germplasms) and their

findings indicated that different results can be obtained from the same experimental data depending on the definition used. In wheat selection for P harvest index, as a criterion for P efficiency, was found not to be related to P efficiency (Jones et al. 1989). In order to improve P nutrition in cattle Miller et al (1987) end up selecting P inefficient alfalfa germplasm when considering total plant biomass production per unit nutrient absorbed as a definition of nutrient efficiency.

The terminology phosphorus use efficiency (PUE) is commonly used but less understood and P efficiency depends on the intended use of the result (Fixen 2006). According to Gourley et al (1994) difference in nutrient uptake per root length or root mass or root morphological character such as root: shoot ratio is able to identify mechanisms to P uptake but not able to distinguish between nutrient efficient of inefficient germplasms. Gourley et al (1994) concluded that screening for shoot dry mass production or yield may provide the best estimate of P efficiency in P limited condition.

Table 2.1. Some common definitions and terms used to describe phosphorus efficiency (adapted from Bovill et al 2013)

Term	Description	Reference
P utilization efficiency ratio	Grain yield production per unit of total P taken by plant. Total phosphorus uptake was calculated by grain DM multiplied with P concentration.	(Wang et al. 2005)
P efficiency ratio (1)	Grain yield divided by total P concentration of plant.	(Hammond et al. 2009)
P efficiency ratio (2)	Grain yield per unit of P uptake by grain.	(Yaseen and Malhi 2009)
Agronomic P efficiency	Yield increase per unit of P present in soil.	(White and Hammond 2008)
P utilization efficiency	Biomass production per unit P accrued.	(Wang et al. 2010)
P use efficiency(1)	Grain yield production per unit of available P.	(Manske et al. 2000)
P use efficiency(2)	Total P uptake of plant as a percentage of P applied	(Syers et al. 2008)
P uptake efficiency (1)	Accumulation of P per unit of root weight or per unit of root length.	(Liao et al. 2008)
P uptake efficiency (2)	Amount of P in plant per unit of P available.	(White and Hammond 2008)
P utilization efficiency	Shoot dry weight per unit P uptake.	(Osborne and Rengel 2002)
Shoot P utilization efficiency	Shoot dry weight per unit P uptake.	(Su et al. 2006)
P harvest index	Grain P uptake per unit of total P uptake (grain+ straw).	(Yaseen and Malhi 2009)

Most of the definitions of nutrient efficiency deal with the ability of a genotype to produce grain or biomass per unit of nutrient application, but excess application of nutrients has a potential impact on environment. If the plant genotype cannot use all the

applied nutrients it can increase soil retention and risk of loss (Mikkelsen 2005). Ideally, an efficient genotype will be one which not only can grow well under nutrient-limited conditions but also show response with nutrient application. Ozturk et al (2005) measured the variation in P efficiency of 73 bread and durum wheat. They compared dry matter production at two P level (P_{20} and P_{80}) and calculated P efficiency (defined as dry matter production at P_{20}/ dry matter production at P_{80} × 100) and selected genotypes as efficient or inefficient. The dry matter production of efficient and inefficient genotypes was similar at P_{80} but there was a huge difference in the dry matter production of the genotypes at P_{20}. Indeed the definition they used was helpful to understand P efficiency of wheat properly. However, from a commercial prospective, it is grain yield that is the most important parameter. While improvements in PUE based on responses in dry matter are helpful in characterising varieties, the grain yield responses needs to be assessed as well.

Adaptive mechanisms

Plants have evolved many different mechanisms to acquire P (Figure 2.3) such as modification of soil exploration by roots through increasing absorptive area, better symbiosis with mycorrhizal fungi, modification of rhizosphere by root exudation, increased production of phosphatases, and enhanced rate of P uptake (Shenoy and Kalagudi 2005). Among the different mechanisms, differences in root architecture that result in greater soil exploration to increase P absorption area by proliferation and extension of roots (Lynch et al. 2005) and improve formation of symbiotic relationship with arbuscular mycorrhizal fungi (Smith et al. 2003) can be the most significant for wheat and will be the focus of this review.

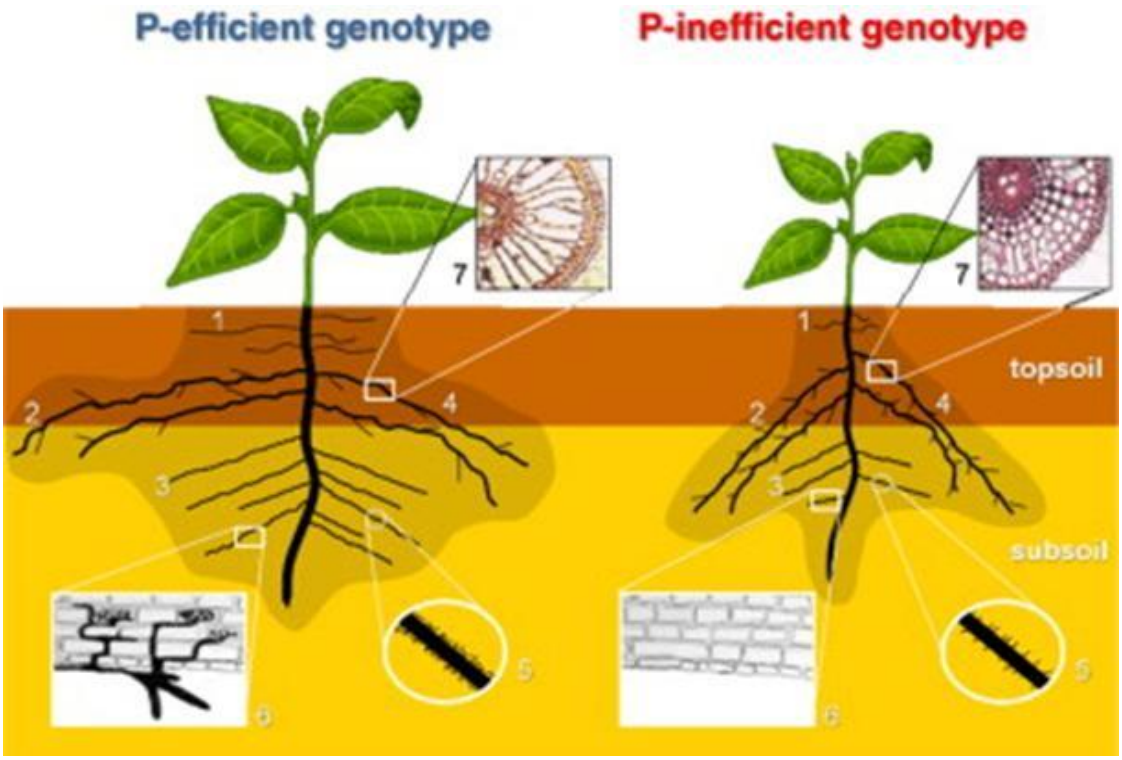

Figure 2.3. Plant traits and mechanisms for improving P uptake efficiency. P-efficient genotypes integrate different traits and mechanisms that contribute to adaptation to low P availability and are therefore more tolerant to P deficiency as compared to P-inefficient genotypes. Adaptations to low P availability include: (1) more and longer adventitious roots, (2) more horizontally oriented basal roots, (3) more taproot laterals, (4) more dispersed higher order laterals, (5) increased root hair density and length (together with increased organic acid exudation and more high-affinity P transporters), (6) greater association with mycorrhizae, and (7) greater formation of aerenchyma. Consequently, the soil volume explored by P-efficient genotypes is much larger compared to P-inefficient genotypes (adapted from Ramaekers et al 2010).

Root architecture

According to Lynch (1995) root architecture is the spatial configuration of a root system and this is important for P acquisition. The distribution of a root system shows a strong positive relationship with the P distribution of soil which is most strongly influenced by soil tillage, rhizosphere pH, fertilizer management and cultivation time (Andraski and Bundy 2003; Holanda et al. 1998; Vu et al. 2009). Root characteristics are an important feature for the development of new wheat germplasm with improved drought tolerance, nutrient and water uptake efficiency, lodging resistance and tolerance to mineral toxicity (Manske and Vlek 2002).

Cereal roots can be classified into two broad groups. One is the primary or seminal roots which emerge from the embryonic hypocotyl of the germinating caryopsis. In wheat, three to six seminal roots can emerge from the seed and these types of roots are fine with a diameter of 0.5mm (Setter 2000). Nodal or adventitious roots are the other root type which emerges from the coleoptilar nodes at the base of the apical culm and tillers. Adventitious roots are thicker (>1mm) than seminal roots and occupy the top soil layers. The number of adventitious roots correlates with the tillering ability of plant and is dependent on environmental factors such as soil moisture and fertility (Setter 2000). Root architectural adaptations are related to root branching pattern, root length and root hair formation (López-Bucio et al. 2002; Richardson and Simpson 2011; Trachsel et al. 2010). The importance of root architecture in P deficient conditions is well documented in the literature, and considerable amounts of work has been done in common bean (*Phaseolus vulgaris*) (Lynch and Brown 2001), *Arabidopsis thialiana* (Bates and Lynch 1996; Lynch 2011) and maize (Zhu et al. 2001). Genetic differences in root architecture exists (Lynch and Brown 2001), but little information on the contribution of root architecture to P efficiency in wheat, especially on genetic differences, is available. In

the following sections some important root architectural changes at low P availability will be discussed.

Root length, morphology and distribution

Root growth is central to P acquisition by plants and may affect PUE. Roots provide a large surface area for nutrient absorption and root length determines the root-soil contact and influences the length of the diffusive pathway over which Pi needs to travel to the root surface (Lynch 1995; Manske et al. 2000). According to Nielsen et al (2001) plants with a large root system with minimal overall carbon cost or with low root respiration cost, often will yield better under P deficient conditions. Root length is affected by the length of individual root axes as well as the degree of branching by roots.

Changes in root length are often a response to low P availability. In a field experiment with *Beta vulgaris* for example, a 25%, increase in calculated P uptake was observed at low P supply due to an increase in root length compared to plants growing with sufficient P supply (Steingrobe 2001). At low P supply a similar result was observed for *Hordeum vulgare* (Steingrobe et al. 2001). Phosphorus efficient bean genotypes had a vigorous root system with highly branched roots and a large number of root apices at low P availability and the variation in this trait contributed towards the genotypic variation in P uptake (Lynch and van Beem 1993). The relationship between the size of root and P uptake has been observed in many studies (Otani and Ae 1996; Wissuwa 2003; Wissuwa and Ae 2001).

The specific root length (SRL) is the length of root per unit of root mass and is a measure of the fineness of the roots. Plants are also known to change their SRL in response to P

supply: an increased SRL is associated with decrease P supply (Christie and Moorby 1975; Schroeder and Janos 2005). No genetic variation of wheat and barley for SRL was observed at P deficient condition by Løes and Gahoonia (2004). There are not many reported data on genetic variation of SRL and its relationship with P efficiency. Work with soybean (*Glycine max*) identified that higher SRL was associated with P-inefficient genotype and was negatively correlated with biomass and P content and as well as other root traits (Ao et al. 2010).

Plants can achieve a large root surface area by reducing mean root diameter and by producing relatively thinner roots (Fitter et al. 2002). Root diameter is an important trait as it can determine the volume of soil that can be explored by the root system (Fitter 1991; Gahoonia et al. 2006). According to Fitter et al (1991) plants with smaller root diameter (a high SRA) can explore more soil per unit of root surface area and can take up P efficiently from low P environment (Gahoonia and Nielsen 2004). Fernandez et al (2009) observed a contrasting result in their experiment with maize, soybean and sunflower. Maize roots showed greater diameter and explored more soil per root length compared to soybean and sunflower, but had no benefit in P uptake compared to the two other plant species.

Low P availability also changes the distribution among different root types (Hodge 2009). Work with *Arabidopsis thaliana* and various rapeseed cultivars showed the root system becomes highly branched with reduced primary root (PR) and an increase in the number and length of lateral roots (LR) when plants were grown under low soil P (Akhtar et al. 2008; Pérez-Torres et al. 2008). Genetic variability exists within cultivars of maize for lateral rooting under P stress condition. Zhu and Lynch (2004) found that maize genotypes with increased lateral root development had a better ability to acquire P and maintained growth better under P deficiency than genotypes with a less branched

root system. In common bean the growth of the main root system (primary and basal roots) under P deficiency was maintained, but the initiation of lateral roots was reduced which reduced the lateral root density (Borch et al. 1999). Reduced lateral root density will reduce P uptake which will affect P efficiency. Maize also shows substantial genetic variation in lateral rooting among genotypes (Ramaekers et al. 2010). Several QTLs for lateral root number, length and plasticity of maize were identified at contrasting P supply (Zhu et al. 2005b).

Increased root growth will benefit P efficiency but may reduce overall shoot growth (Lynch and Brown 2008). According to Fernandez et al (2009) root length is not necessarily related to nutrient efficiency of plants compared to other root morphological traits such as SRL. Otani and Ae (1996) concluded that in terms of P uptake, plants with a longer root system are not necessarily more efficient. QTL analysis of root traits and P accumulation of common bean have revealed close relationship between root morph-architecture traits (i.e. root length, root surface area, root architecture) with P efficiency (Beebe et al. 2006; Liao et al. 2004). Environmental conditions such as soil texture and pH can affect root growth greatly and contradictory results were observed for root growth and its relation to P uptake. Based on the literature analysis it can be concluded that root length or root distribution pattern alone cannot be a selection criteria for P efficiency. However, plant varieties will benefit in terms of P uptake by root system as it is the root by which plants absorbs nutrients and water for growth.

Length and density of root hairs

Root hairs are the tubular shaped structures that arise from the epidermal cells of roots (trichoblasts) and which are specialized for nutrient uptake. Root hairs increase

substantially the root-soil contact. According to Parker et al (2000) root hairs can form as much as 77% of the root surface area of field crops. Gierson et al (2001) reported that at least 40 genes in Arabidopsis affect root hair initiation and development and many of these may be responsive to P deficiency.

Horst et al (1993) studied genotypic differences in PUE of wheat. They compared an old and a modern wheat cultivar and assessed their responses to different P levels. They found significant differences in SRL between the two cultivars at tillering, shooting and anthesis. The modern wheat cultivar had longer root hairs than the older one and root hair length tended to be lower at a high phosphorus level. They suggested from their results that the modern cultivar is agronomically efficient in P use due to efficient use of assimilates for root growth characteristics (small root diameter and longer root hair) which enhanced P acquisition. As only two varieties were used by Horst et al (1993), further work is required to confirm their conclusions.

Bates and Lynch (1996) showed that in *Arabidopsis thaliana* P stress induced an increase in root hair elongation, lateral roots and root hair density but decrease in total root length. Gahoonia et al (1997) found that cereal cultivars varied widely in root hair formation and depletion of P from their rhizosphere. Gahoonia et al (2001) worked with a hairless root barley mutant and the poorer P uptake by the mutant illustrated the importance of root hairs in uptake of P. Their results showed that the variety with root hairs (Pallas) depleted almost two times more P than the mutant without root hairs and that it had higher acid phosphatase (Apase) activity near its root, which suggests a relationship between root hair formation and Apase activity. An increased Apase activity was also observed by Liu et al (2004) under P deficiency for efficient maize genotypes, which had a larger root system. The work with hairless mutants illustrates

the importance of the presence of root hairs to P uptake, but it does not infer anything about the importance of differences in root hair length among genotypes to P uptake.

Root hairs may also help to disperse organic acid throughout the rhizosphere which has the potential to improve the bioavailability of P in many soil (Hinsinger 2001; Ryan et al. 2001). Root hairs are particularly important for non-mycorrhizal plants, since mycorrhizal hyphae can fulfill some of the same functions as root hairs. In maize, common bean and barley the genotypic variation of root hair length and density was mapped to several major QTLs suggesting the potential importance of this trait in selection for improved P efficiency in breeding programs through marker aided selection, as well as through direct phenotypic selection (George et al. 2014; Yan et al. 2004; Zhu et al. 2005a).

The rhizosheath is the amount of soil with the root system which remain attached when the root is removed from the surrounding soil (Watt et al. 1994). Root hair length is known to be important for rhizosheath formation and there is a positive relationship between root hair length and rhizosheath size in some species such as wheat (Delhaize et al. 2012; Delhaize et al. 2015; Hailing et al 2010). Rhizosheath size is also considered to be important for the regulation of plant soil water relations, nutrient acquisition, soil aggregation and microbial activity (McCully 1999).

Rhizosheath formation is influenced by environmental conditions and may help with maintaining growth under different stresses, not only P stress. The development of the rhizosheath was associated with plant size in sandy soil conditions (Duell and Peacock 1985). According to Watt et al (1994) in maize a decrease in soil water caused higher root hair growth and stable rhizosheath production. An extensive and stable rhizosheath may help plants to acquire nutrients in dry soil (Watt et al. 1994). Root hairs, plant and

microbial mucilage and repeated wet-dry cycles are the proposed factors that play an important role in formation of rhizosheath (Watt et al. 1993). Rhizosheath of wheat was described by Goodchild and Myers (1987) from field-grown root and they speculated the importance of the rhizosheath for nutrient uptake and dry matter production. In acid soil, significant genetic variation was observed in formation of rhizosheath of wheat (Haling et al. 2010). Rhizosheath size of wheat seedling grown on acid soil was strongly correlated with root hair length and was used as a surrogate for root hair length to develop germplasms by Delhaize et al. (2012). James et al. (2016) also showed a significant correlation between rhizosheath size and root hair length and suggested that phenotypic screening for rhizosheath size as a surrogate for root hair length is possible. Av strong correlation between rhizosheath size and root hair length of wheat was also observed by Delhaize et al. (2015). George et al (2014) observed genetic differences in rhizosheath production in barley which was related to P uptake in dry soil, but in contrast the studies with wheat there was a poor correlation with root hair length.

Topsoil foraging and root angle

Most soil contains the greatest amount of bioavailable P in the upper layers (Ramaekers et al. 2010). Root systems that can increase top soil foraging may be able to acquire more P from the soil. Wide root angles are associated with greater root growth in the topsoil layers and roots can increase top soil foraging and P acquisition by reducing competition among the same plant's roots (Lynch 2011). Work in maize, bean and soybean, found that wide root angles were important for P acquisition Lynch (2011). Liao et al (2001) studied common bean and found that P availability changed the

shallowness of basal root length (basal roots originate from a narrow region of the hypocotyl which is the meeting point of the tap root with the hypocotyl) and found that roots of P efficient genotypes became shallower with P stress. Their results showed that basal root length in both sand and soil culture and relative basal root length in soil culture in the upper 0-3 cm layer were significantly correlated with plant shoot biomass and P uptake. Basal root growth angle was reduced (when measuring from horizontal line) with P stress for three genotypes and for the other two was unaffected. In a P efficient genotype (G19833), P stress reduced the growth angle from 24 to only 3°. They suggested that the variation of root gravitropic responses is indeed related to P-acquisition efficiency and that it is also varies among genotypes. Zhu et al (2005c) observed that P deficiency increased total root length and relative root length in the top soil of P efficient maize cultivars.

Adventitious roots are common in many plants and they can be an important element for top soil foraging as they arise from the hypocotyl in dicots and from tillers in cereals, and grow horizontally just below the soil surface. In bean adventitious roots have greater SRL than other roots and according to Lynch and Ho (2005) they are important for top soil foraging because they reduce the metabolic investment in root tissue for large volume of soil exploration. Several QTLs were identified for adventitious rooting of bean at low P environment suggesting the possibilities of selecting this trait for crop breeding (Ochoa et al. 2006).

Although much work has been done to measure root shallowness in control environments using sand culture or growth pouch, there are not many studies done under field condition and few with wheat. Shallow rooted genotypes are not suitable for regions where water is limited and in shallow root system the mortality of fine roots can be higher than deep rooted genotypes (Liao et al. 2004). All these constraints of root

shallowness makes it difficult to understand the utility of root shallowness for PUE and genetic differences for root shallowness needs to be understand properly.

Root biomass and root: shoot ratio

A common response to P deficiency is an increase in root: shoot dry weight ratio (Hermans et al. 2006). Under a P deficient treatment maize genotypes, for example, had higher root: shoot ratios compared to the P sufficient treatment (Mollier and Pellerin 1999) while Moorby et al (1988) found P deprivation in rape plants affected shoot weight more than root weight. Shoot weight was reduced by 60% whereas the root weight reduction was 30%, resulting in increased root: shoot ratio in plants grown under limiting P supply. Phosphorus deficiency increases carbohydrate accumulation in roots, which increases the root: shoot ratio of plants (Cakmak et al. 1994; Hermans et al. 2006). The changes in the relative growth of roots and shoots may be related to genetic differences in PUE. As P in soil is relatively immobile, greater allocation to the root is beneficial if it improves the plant's ability to scavenge for P, but overall plant growth can be slowed due to increased respiratory burden to root tissue (Lynch and Ho 2005; Zhu and Lynch 2004).

A number of studies have shown that root: shoot ratio is related to a genotype's P efficiency. Nielsen et al (2001) studied common bean and compared P-efficient genotypes with P-inefficient genotypes and found that P-efficient genotypes maintained a higher root: shoot ratio during their growth at low P. However the connection between P efficiency and higher root: shoot ratio may not be a universal relationship. Some highly P efficient *Lupinus* species showed little change in biomass partitioning to the root at low P supply (Keerthisinghe et al. 1998; Pearse et al. 2006). In Chinese wheat

higher PUE was associated with higher root: shoot ratio (Davies et al. 2002). Although under P stress greater allocation to the root may be desirable to maintain root growth and soil exploration (Anghinoni and Barber 1980), there are several reports in the literature indicating that P efficient cultivars do not maintain high root: shoot ratio. Dechassa et al (2003) and Dechassa and Schenk (2004) compared carrot, potato and cabbage and found that cabbage had the lowest root: shoot ratio but also had highest P uptake rate per unit root length. Similar results were observed for rape and spinach (Föhse et al. 1988) and in maize (Gill et al. 2005). For soybean and sunflower no clear difference in root-shoot ratio was observed and field results were different to the glasshouse results (Fernandez et al 2009).

Contrasting results and environmental effect makes difficult to evaluate P efficiency considering root: shoot ratio as a targeted trait. It may be a response to low P but there is conflicting evidenced that it is important in explaining genetic differences in P efficiency. More research is necessary to understand this mechanism.

Root exudates

Organic acids/ carboxylates

Organic anion exudation into the rhizosphere is a common response to various nutritional stresses including P, Fe deficiency and Al toxicity. The concentration of different organic anions is typically greater in the rhizosphere (around 10 fold) compared with that in bulk soil. Numerous studies with white lupin (*Lupinus albus*), which exudes significant amounts of citrate from cluster roots that are formed in the response of P deficiency, have highlighted the importance of organic anions in

mobilization of P from soil, (Richardson et al. 2009a; Vance et al. 2003). The effect of carboxylates and other exudates on mobilizing soil organic and inorganic P is shown in Figure 2.4. A number of plant species (such as rice, barley and maize) are known to increase P acquisition by releasing organic acid anions from their roots. Organic acid anions are thought to be particularly important in P fixing soils because they could increase the bioavailability of P in the rhizosphere (Lynch 2007).

Plant roots can secrete organic acids such as citric, iso-citric, oxalic, malic, fumaric, succinic, α-ketoglutaric, aconitic, formic, lactic, piscidic, shikimic acids and also protons (Shenoy and Kalagudi 2005). Among them citric, malic and oxalic acids are the most important. Citrate is important as it can mobilize P from Al-P and Fe-P complexes in acid soil and Ca-P in calcareous soils or from rock phosphorus (Richardson et al. 2009b). In the species which form cluster roots, a correlation between formation of cluster roots and a high rate of organic anion release has been reported in response to P deficiency (Roelofs et al. 2001). Release of organic acid anions varies within different parts of the root: for example it is higher in the young region near to the root tip than in the older part of root of rapeseed plant (Hoffland et al. 1989). However the ability to excrete organic anions to improve P

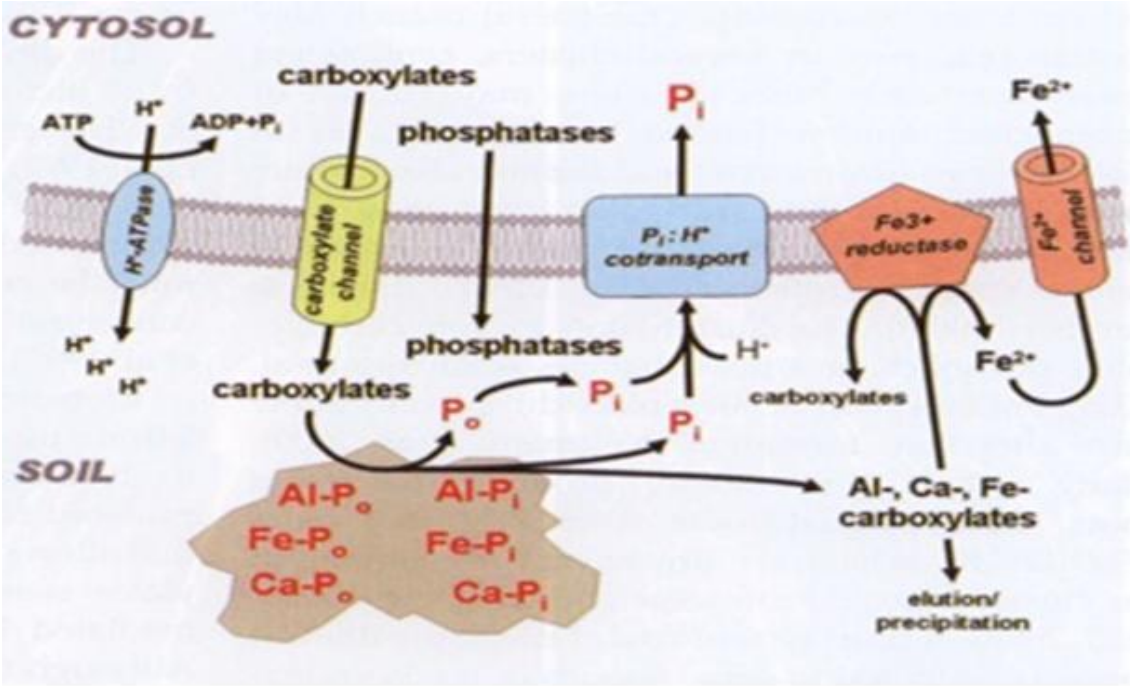

Figure 2.4. Effects of carboxylates (and other exudates) on inorganic (Pi) and organic (Po) mobilization in soil. Carboxylates are thought to be released via an anion channel. The exact way which phosphatases are released is not known. Carboxylates mobilize both inorganic and organic phosphorus. Phosphatases hydrolyse organic phosphorus compounds, once these have been mobilized by carboxylates. Carboxylates will also mobilize some of the cations that bind P. some of these cations (especially Fe) move to the root surface for uptake by roots. Sourced from Lambers et al 2006.

availability does not come without a cost: a significant amount of C is associated with the root exudation (Maschner 1995) and the exudation varies with plant species (Lesuffleur et al. 2007), development stage (Gransee and Wittenmayer 2000) and nutritional status (Hinsinger 2001; Marschener 1998), as well as soil structure (Berg and Smalla 2009).

Increased exudation of organic acids is a common response to P starvation. In P deficient soil citrate secretion by common bean was found to be effective in mobilizing P from Al-P and Fe-P compounds (Shen et al. 2002). Some lowland rice genotypes showed an 81% increase in their organic acid exudation capacity under P deficiency (Hoffland et al. 2006). Similar results were demonstrated by Shen et al (2002) for common bean genotypes which had a two- to threefold increase in organic acid exudation after 7 days of P starvation.

Although numerous works on root exudation have been done, direct evidence to relate P uptake and root exudation has yet to be established, especially for plant species which do not form cluster roots. A recent study by Duputel et al (2013) showed that citrate efflux could decrease P availability in certain soil type and Ryan et al (2014) did not find any relationship between citrate efflux and P uptake in near isogenic lines of wheat.

Phosphatases and other exudates

Under P deficient conditions plant roots are known to increase the acid phosphatases activity which helps P solubilization in the rhizosphere (Yun and Kaeppler 2001). The importance of phosphatases for P nutrition under P deficiency is well documented, although the importance varies with species, cropping system and the form of P that is present in the soil (George et al. 2005; Yun and Kaeppler 2001).

Other than carboxylates and phosphatases plant roots are also known to exudate phenolic and mucilage under P deficiency. The exudation of both phenolic and mucilage can be enhanced under P deficiency and this can also enhance the availability of P in the soil (Lambers et al. 2006). According to Guppy et al (2005) both phenolics and mucilage act similarly to carboxylates, but tend to be less effective than carboxylates.

Whether genetic differences in exudation of these compounds exists in wheat and contributes to differences in PUE has yet to be demonstrated.

Aerenchyma formation

Root cortical aerenchyma are enlarged gas spaces in the root cortex that form through either cell death or cell separation (Evans 2004). Aerenchyma commonly form as a response to hypoxia (Jackson and Armstrong 1999) but they can also be induced by other stresses including P deficiency (Bouranis et al. 2003; Fan et al. 2003). Aerenchyma formation involves the replacement of metabolically active cortical tissues with air spaces, which reduces the energy cost of root growth and increases the proportion of root mass occupied by non-respiring tissues (Brown et al. 2013). The additional P available due to lower energy requirements as a result of aerenchyma formation contributes to the P economy and the physiological P utilization efficiency of plants (Fan et al. 2003; Koide et al. 2000; Lu et al. 1999). In maize Fan et al (2003) observed that root segments with 20% of their cross-sectional area as aerenchyma respired at half the rate of roots without aerenchyma. Brown et al (2013) suggested that targeting root hair zones of root with aerenchyma may improve the efficiency of root to take up P. Postma and Lynch (2011) reported that root cortical aerenchyma in lateral root would benefit nutrient deficient plants.

Although some research has identified the importance of aerenchyma formation in nutrient uptake, genetic variation and contribution towards PUE in still not known. More research is necessary to understand the utility of this trait.

Mycorrhizal colonization

Soil fungi which infect roots of higher plants and form a symbiotic relationship are known as mycorrhizae. According to Smith and Smith (1990) this symbiotic relationship results in bidirectional nutrient transfer: the plant supplies sugars to the fungus and the fungus provides immobile nutrients such as P and other nutrients such as zinc (Zn), calcium (Ca), and magnesium (Mg) to the plant (Smith et al. 2003). The symbiotic relationship between the plant and the mycorrhizal fungi may change the physiology of the plant including composition of mineral nutrients in tissues, hormonal balance of the plant and carbon allocation patterns (Richardson et al. 2009a). The chemical composition of root exudates can be altered by the fungus and the developing mycelium in the soil can act as a C source for the soil microbial communities and introduce physical modification of the soil environment, which can alter the soil microbial population both qualitatively and quantitatively.

Following infection of the root and colonization of the root cortex by mycorrhizal fungi and development of external hyphae into the surrounding soil, there is an increase in the nutrient absorbing area (Richardson et al. 2009a). Hyphae of arbuscular mycorrhizal fungi (AMF) can enhance the nutrient absorbing area considerably because the hyphae are thinner than root hairs and they can enter into the soil pores where root hairs cannot (Manske and Vlek 2002). Smith and Smith (1990) reported that the hyphae of AMF are 5-10 times thinner than root hairs and it can exceed the nutrient depletion zone of uninfected roots. Root colonization of wheat by AMF depends on a number of different factors including soil type, cropping practices and fertiliser use (Graham and Abbott 2000).

AMF can also absorb orthophosphate from the soil solution at lower concentrations than roots, but it is still not clear that this contribution has a significant advantage for the P nutrition of plant (Richardson et al. 2009a). Expression of a high affinity phosphate transporter in the extra-radical mycelium of AMF has been measured. Plants infected with AMF have two pathways for P uptake from soil (Figure 2.5). Direct uptake occurs by the plant root system (such as root epidermal cells and by root hairs) and uptake by AM pathway occurs by the hyphal network that can exceed the root absorption area. Thus by using the AM pathway, the P depletion zone can be extended and P can be translocated rapidly to the root cells by Pi transporter genes (Smith et al. 2011). The length of root colonized by AMF does not necessarily always represent the amount of active fungi which can transfer nutrients to the plant (Smith and Gianinazzi-Pearson 1990). The nutrient uptake by AMF mostly depends on the length of active internal fungus that is transferring P to the plant and also active external hyphae which can take up the available form of P from the soil. Molecular genetics studies support the operation of a mycorrhizal pathway for P uptake by demonstrating that plant P transporter operating in the mycorrhizal uptake pathway are induced by AMF (Karandashov et al. 2004). In wheat mycorrhizal inducible P transporter genes have been reported by Glassop et al (2005).

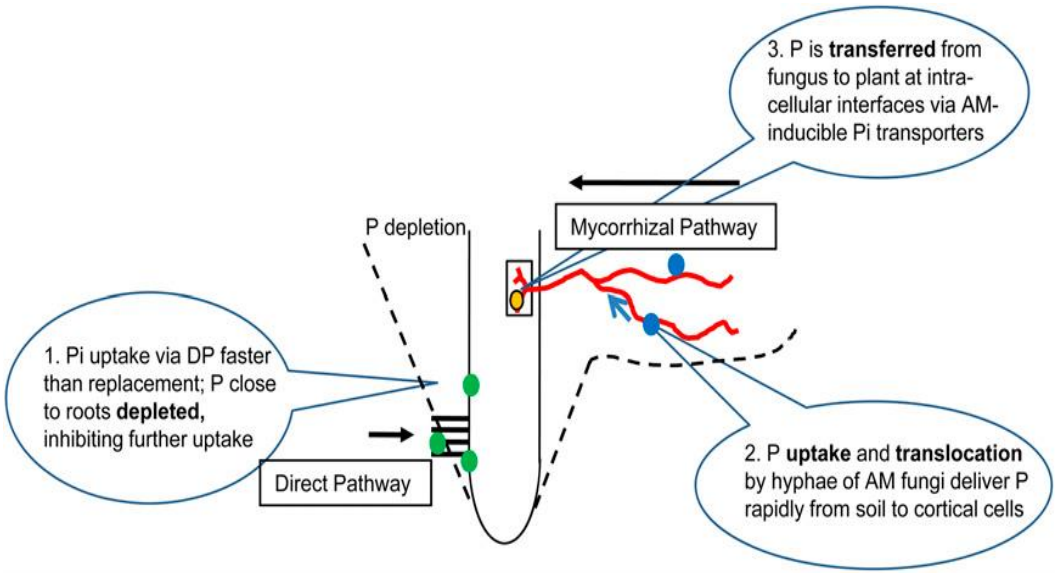

Figure 2.5. The two pathways of AM plants for P uptake (taken from Smith et al 2011).

Differences in the extent of root colonization exist among plant species. Some plants such as clover generally show a high degree of colonization (Smith et al. 1986) while others such as wheat commonly show low colonization, which ranges between 10-30% of total root length (Mäder et al. 2000). Wheat genotypes vary in AMF colonization (Zhu et al. 2001) and mycorrhizal dependency is probably controlled by plant genes (Hetrick et al. 1993). Work with other plant species revealed the involvement of several genes for AMF establishment but there is little knowledge about wheat (Marsh and Schultze 2001). Functional diversity also exists within fungal species. Colonization by the same AMF does not result in similar growth response in different plant species and plants also can have preference for particular AMF species which can result different growth response (Smith et al. 2011). Smith et al (2004) worked with tomato, flax and medic and colonized these three plant species with three different AMF species. They achieved varying results in responsiveness for each of the three plant species. Zhu et al (2001) worked with some modern and old wheat cultivars and found significant

difference in mycorrhizal responsiveness among the cultivars. Modern wheat cultivars showed lower mycorrhizal dependency compared to older cultivars, suggesting that modern breeding practices have reduced the mycorrhizal dependency of wheat cultivars. However, a contrasting result was observed by Koide et al (1988) in oats, who found that AMF colonization was more beneficial for cultivated oat compared to wild oat. Genetic variation of mycorrhizal dependency within different genotypes of barley and maize was observed by An et al (2010) and Jakobsen et al (2005).

The most important function of mycorrhizal symbiosis is to take up immobile nutrients such as P. The symbiosis can result in increases of either total P uptake or P concentration in the plant tissues. Al-Karaki and Clark (1998) suggested that uptake of other nutrients such as Zn, Ca, Mg, and copper (Cu) by wheat can also be improved by AMF. It has also been suggested that AMF can increase activity of phosphatase enzymes (Dodd et al. 1987; Tarafdar and Marschner 1994) and the solubilisation of rock phosphate or other forms of P (Omar 1998). However increases in P concentration in AM plants is not always accompanied with growth increase, sometimes it can be associated with growth depressions (Zhu and Smith 2001; Zhu et al. 2001) as AMF receive C from the plants. However, the cost of AMF will not be harmful if plant growth is not limited by C (Smith et al. 2011). The value of the symbiotic relationship will depend on whether the benefits from improvements in uptake of nutrients form the soil by the AMF outweighs the cost of supporting the growth of the hyphae.

Grain yield can be affected by AMF colonization. Mohammad et al (1998) observed that wheat which had roots colonized by AMF had significantly higher yield, kernel number/head, grain number/spike and 1000-grain weight/g than non-mycorrhizal plants. AMF colonization may also affect a plant's seed size and seed nutrient content and nutrient composition. AMF colonized wheat have larger seed size and high nutrient

(e.g. N, P or Zn) contents than non-mycorrhizal plants (Mohammad et al. 1998; Ryan and Angus 2003). However, from the above discussion it is clear that AMF help the host plants to take up P from soil and there is genetic variation in infection by AMF, but the contribution of mycorrhizal fungi in PUE is still unclear. Most of the work with AMF has focused on the fungal diversity by selecting two or more different plant species (Jansa et al. 2008; Smith et al. 2003) but there is less information of the contribution of AMF on the diversity within varieties of a single plant species. Even in the presence of mycorrhiza, a strong correlation between root traits such as root hair length and root shallowness and P uptake is observed, which suggested that mycorrhizal P uptake can be supplemented by other root traits (Lynch and Brown 2008). The presence of AMF did not change growth pattern of maize, soybean and common bean (Lynch and Brown 2008) compared to their growth in the absence of mycorrhizal fungi, which suggest that AMF colonization alone cannot be a selection criteria.

Furthermore, the contribution of AMF symbiosis can depend on environmental condition such as available soil P, availability of light and CO_2 to the plants, plants density and other factors. Depending on this factors AMF symbiosis can vary from highly beneficial to apparently parasitic (Jansa et al. 2011). After the application of water soluble P fertilizer which enhance the availability of soil P, plant can gain access to the P through their root system. So when soil P is high (>50 mg/kg, extractable with 0.5 M $NaHCO_3$) plants are not dependent on AMF symbiosis and the root colonization will be reduced (Bolan et al. 1984; Jansa et al. 2009). Therefore, in farming systems where regular application of P fertilizer is necessary to maintain plant yields, the significance of AMF colonization towards plants P uptake remains questionable. More research is needed to understand the genetic responsiveness of plants to AMF and the contribution of AMF to improve PUE of plants.

Remobilization / internal utilization of P

Movement of nutrients within the plant body and utilization of acquired P can be another mechanism of plant species adaptation to P deficiency. Nutrient efficiency depends on efficient redistribution and reutilization of nutrients from deficient or senescent plant parts (Aziz et al. 2014). As deficiency increased, an increase in the rate of absorption and translocation of P to leaves was observed (Adu-Gyamfi et al. 1989). Under P deficiency it was also observed that-efficient cultivars retained a lower proportion of total P in roots and stems and higher proportion was translocated to leaves compared to inefficient cultivars (Snapp and Lynch 1996).

Substantial differences in P utilization efficiency among varieties have been reported in maize, rice, field bean, and groundnut (Shen and Ae 2001; Wissuwa and Ae 1999). One of the internal physiological adaptations to P starvation is to remobilize P from older leaves and vacuolar stores, induction of metabolic bypasses of adenylate and Pi dependent reactions in the respiratory pathways (Schachtman et al. 1998) and replacement of phospholipids with non- phosphorus galactolipids (Härtel et al 2000). Rearrangement of cell wall components was also induced by P deficiency, as for lipid, genes involved for the synthesis of galacto and sulpholipids were strongly induced by P deficiency. Mission et al (2005) observed induction of genes for sulfate transporters and an increase content of sulfur which possibly meets the increases demand of sulfolipids synthesis under P deficiency.

Summary

Wheat is an essential and important crop for the world. Phosphorus is a critical element for crop growth and development, although the availability of P in soil is generally limited. The limited availability of P in soil poses an important constraint for crop production and the fertiliser P that is applied is often used inefficiently and recovery is low. Plants develop complex adaptive strategies to cope with the low P environments to combat the large difference between P requirement for growth and the P availability in the soil and improvements in crop PUE will be based on exploiting one or more of these adaptive strategies. Root architecture plays an important role for acquisition of P for different plants such as common bean and maize (Lynch and Brown 2001; Zhu et al. 2005c). Plants with larger root systems with longer and denser root hairs have been shown to explore larger soil volumes of soil and acquire more soil P (Bates and Lynch 2001; Gahoonia et al. 2001; Manske et al. 2000). However, while these differences have often been demonstrated under controlled conditions, there is little consideration of the contribution these mechanisms make to P efficiency in field situations.

Manipulating symbiosis with mycorrhizal fungi may also be beneficial for crop plants such as wheat. Mycorrhizal fungi can extract P from highly P-fixing soils and deliver that P to the plant in return for C (Smith et al. 2011). However, mycorrhizal association is a complex mechanism that depends on several factors such as plant age, rate of root growth, root hairs and plant tissue P concentration.

Much work has been done on each of these traits individually with the aim of improving the PUE of crops. While there has been some success in crops such as maize, in wheat there have been no improvement in PUE. While we know a considerable amount about

some specific traits, the relatively importance of them to the PUE of a variety is not well understood. Without a better understanding of plants mechanisms to improve P uptake under P deficient conditions, it will be difficult to improve yield. There is little, and often contrasting, information about genetic variation for root architecture and mycorrhizal colonization of wheat. Further research effort is needed on these two aspects to improve phosphorus use efficiency of wheat.

Aim and objective

The aim to improve PUE will vary depending on the targeted environment where plants will be grown. For in instant agricultural system were P fertiliser application is either maximal or near maximal plants will not be P starved. So the traits related to PUE from these environments will differ from those where P application is insufficient. The primary aim of the proposed work is to improve the profitability and productivity of wheat by improving our understanding of mechanisms of PUE in this crop species. Importantly, this work will focus upon varieties of wheat which have been shown to differ in phosphorus use efficiency that has been demonstrated under commercial growing conditions, from data obtained from three sites and seasons from a GRDC project currently managed by the Principal Supervisor.

The specific aims of the work are:

I. To understand the contribution of root morphological and architectural traits for P responsiveness of wheat varieties.

II. To evaluate genetic differences among the varieties and to understand the regulation of several mechanisms in the variety itself

III. To understand the contribution of mycorrhizal colonization towards varietal P responsiveness and to evaluate genetic differences for AMF colonization

IV. To understand the contribution of AMF at field and how the inoculation of AMF works compare to native soil

V. To understand the genetic variation of root exudation of wheat varieties and how it relates with their P responsiveness.

VI. To understand the relationship between root hair length and rhizosheath size and to identify chromosomal region associated with root hair length and rhizosheath size.

The outcome of this thesis will provide more details about adaptive mechanisms of wheat at P deficiency and how they are regulated in same cultivar, which can be used for further improvement of wheat.

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Chapter 3 : Root angle, total root length and root hair length: combined contribution for phosphorus responsiveness of wheat

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Name of Principal Author (Candidate)	Kamrun Nahar
Contribution to the Paper	Managed experiments and performed analysis on all samples, interpreted data, wrote manuscript.
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
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Abstract

Background and Aims: Various root traits have been suggested to be important to P uptake and response to P but there has been no comparison of the importance of various traits. The aim of this study was to assess the relative importance of various root traits among wheat varieties with different responses to P.

Methods: Seedlings of 10 bread wheat varieties with different yield responses to P were characterised for root angle, root length, root hair length and rhizosheath size. Two experiments were conducted in a loamy-sand (Halidon soil) and one experiment was done with two soils (Halidon soil and a loam from Mallala) that were low in available P. The effect of P availability on root traits was also assessed by using two different rates of P addition.

Results: Root length and root diameter were not consistently related to P responsiveness. Crown root angle rather than the seminal root angle was associated with the varietal differences in responsiveness to P, with non-responsive wheat varieties having a wide crown root angle. Compared to the responsive varieties, non-responsive wheat varieties had longer root hairs and larger rhizosheath size regardless of the soil type and these differences were observed at different rates of P. Seedling biomass and shoot P uptake of non-responsive varieties was significantly different to that of the responsive varieties. Relatively high broad sense heritability of crown root angle, root hair length and rhizosheath size suggests that these traits could be targeted for future wheat breeding programs.

Conclusion: No single root trait was uniquely associated with P responsiveness. Varieties non-responsive to P possessed several root traits which can explain why they performed better than responsive varieties under field condition. High heritability of

these traits also demonstrates the potential for selecting these varieties for future breeding efforts.

Key words: phosphorus efficiency, root angle, rhizosheath, root architecture

Introduction

Phosphorus (P) is an important macronutrient for plant growth and development. Although the total P content in soil is often high, it is the least available macronutrient and limited availability of P is a key nutritional constraint to the growth of many crop plants (Bates and Lynch 2000; Ramaekers et al. 2010; Schachtman et al. 1998). To mitigate this problem and to maintain yield a universal response by farmers is to apply P fertiliser. However, after fertiliser application, P is rapidly transformed to poorly available forms (Vance et al. 2003) and as a consequence the efficiency of P fertiliser use is often low with less than 30% of the P being recovered by crops in the year in which the fertiliser is applied (McBeath et al. 2012). Phosphorus is a non-renewable resource and it is expected that high quality P reserves will be exhausted within the next 80-100 years and that the cost of P will increase globally (Cordell et al. 2009; Van Vuuren et al. 2010). The problems associated with low P availability and poor fertiliser recovery emphasise the need to develop cultivars that can acquire and utilize applied P more efficiently. Developing plant genotypes with a greater ability to grow and yield in soil with low P availability is an important goal in plant breeding (Hash et al. 2002; Wissuwa et al. 2002; Yan et al. 2004) but which has yet to be realised in wheat. Deployment of P efficient genotypes in both high and low input farming systems to improve recovery of P or to reduce the amount of P required may help to reduce P fertiliser application costs, minimize the risk of environmental pollution associated with

P fertiliser application and slow the depletion of global P reserves (Cakmak 2002; Vance et al. 2003).

Plants have evolved many different mechanisms to acquire P such as greater soil exploration by roots through increasing absorptive area and this can be achieved by increased branching, longer root systems and production of longer root hairs. Other mechanisms include better symbiosis with mycorrhizal fungi, modification of the rhizosphere by root exudation, increased production of phosphatases, and enhanced rate of P uptake (Shenoy and Kalagudi 2005). Although adaptations such as these have been shown to be important under controlled conditions, their usefulness in conferring greater P uptake under field conditions for wheat (*Triticum aestivum*) is still unclear.

Genotypic variation and tolerance to P deficiency in wheat has been widely reported (Fageria and Baligar 1999; Gahoonia et al. 1999; Gunes et al. 2006; Manske et al. 2000; Osborne and Rengel 2002; Ozturk et al. 2005; Wang et al. 2005), although the relative importance of the underlying mechanisms for the P efficiency of wheat remains unclear. The focus of this paper is on root architecture which is defined as a spatial configuration of root system, and which has been shown to be important for P acquisition (Lynch 1995). A common response to suboptimal nutrient availability is to increase allocation of carbohydrates to roots increasing root biomass. A larger root system is particularly important for P acquisition as it increases root-soil contact. Top soil foraging is another important adaption of plants for P acquisition, as the most bioavailable P occurs in the top layer of soil. Wide root angles, which is an important aspect of root architecture, can increase top soil foraging and P acquisition (Lynch 2011). Previous work has been conducted on the importance of basal root angle for P acquisition of bean (Liao et al. 2004) and maize (*Zea mays*) (Zhu et al. 2005) and this has demonstrated that there is some advantage of shallow roots angles to P uptake in these crops. Although there is

some information available on the intra specific difference of seminal root angle for drought adaptation of wheat (Manschadi et al. 2008), very limited information is available about the importance of this trait for P acquisition. While seminal roots are important for early development and nutrient acquisition, crown (or adventitious) roots may also be important for P acquisition. Crown roots are shallower than seminal roots and by staying in the top layers of soil they can be important for top soil exploration. Miller et al (2003) proposed that adventitious roots of common bean explore the top soil more efficiently than other root types suggesting it can be a useful trait under P deficient conditions. However to date there are no data on variation in crown root angle of wheat and its potential contribution for acquiring P.

Root hair length is an important trait for P acquisition for many plant species (Gahoonia and Nielsen 1998; Gahoonia et al. 2001) and it contributes up to 80% of P uptake by increasing the root to soil contact (Jungk 2001). There is significant intra- and inter-specific variation for root hair traits and varieties with longer and denser root hairs have greater P uptake and plant growth under P deficient conditions (Brown et al. 2012; Gahoonia and Nielsen 1997; Gahoonia et al. 2001). Root hair length is important for rhizosheath formation. The rhizosheath is important for the regulation of plant soil water relations, nutrient acquisition, soil aggregation and microbial activity (McCully 1999), and an extensive and stable rhizosheath may help plants acquire nutrients in dry soil (Watt et al. 1994). Root hairs, plant and microbial mucilage and repeated wet-dry cycles are important factors in rhizosheath formation (Watt et al. 1993). Rhizosheath of wheat was described by Goodchild and Myers (1987) from field grown plants and they speculated about the importance of rhizosheath for nutrient uptake and dry matter production. In acid soil significant genetic variation was observed in formation of rhizosheath of wheat (Delhaize et al. 2012; Haling et al. 2010; James et al. 2016).

Despite the large number of studies, there is no general mechanism to explain P efficiency and to date there is very limited information on how several adaptive mechanisms could work in parallel towards varietal responsiveness to P. This emphasises the need for more research to identify the underlying mechanism for P efficiency and try to assess their relative contribution to P efficiency. Most of the previously reported work has dealt with single traits, but there is little information on relative importance of different root architectural traits for P efficiency. The field environment is spatially heterogeneous and a single trait may not always be helpful to confer P efficiency, especially if it is associated with particular growth conditions or environments.

Much of the previous work has examined a trait under controlled conditions with no or limited field evaluation. We took a different approach: our aim was to dissect the importance of root traits among genotypes that have shown differences in yield response to P in the field as a means of inferring the value of specific root traits. Four root traits – seminal and crown root angles, rhizosheath size and root hair length - were targeted to explore genetic variation of wheat varieties and the relationship with P responsiveness under field condition. Genotypes of wheat that had shown differences in responsiveness to P in the field were selected. It was hypothesised that if roots traits were important to the observed genotypic differences in P responsiveness then there would be consistent differences in one or more root traits between P- responsive and non-P responsive genotypes. The aim of this study was to assess the contribution of a number of root traits towards varietal differences in P responsiveness in wheat.

Materials and methods

Soil and plant materials

Soil with low P availability was collected from the 0-15cm layer from two field sites at Halidon and Mallala, South Australia. The soil from Halidon is classified as a tenosol (Isbell 1996) and was a loamy-sand, with pH 7.0 (1:5 soil: water) and Colwell P of 8 mg/kg. The Mallala soil is classified as a calcarosol and was a loam with pH 8.2 with a Colwell P of 18 mg/kg. The soils were air dried and passed through a 2 mm sieve prior to being used in the experiments.

Ten bread wheat genotypes differing in their yield response to P (Figure 3.1) from recent multi-site field experiments in South Australia (McDonald et al. 2015) were selected for this study. These varieties were grown with and without P fertiliser at Mallala and Tumby Bay, South Australia. Compared to Mallala, Tumby Bay is an acidic soil (pH~ 6) and is a higher rainfall environment. The old varieties Warigal, Carazinho and Trintecinco were low yielding when no P was applied, which reflected their low yield potential compared to the recently-released varieties. The analysis of P response took account of these differences in yield potential (McDonald et al 2015) and while there are effects of site and seasons on the P responsiveness, the varieties were classified as relatively responsive to applied P (Wyalkatchem, Krichauff and BTSchomburgk), and non-responsive (Axe, Carazinho, Correll, Gladius, RAC875, and Warigal) based on the overall consistency of the responses across sites and years. There was only a single year's data for Trintecenco, but it was included among the non-responsive genotypes.

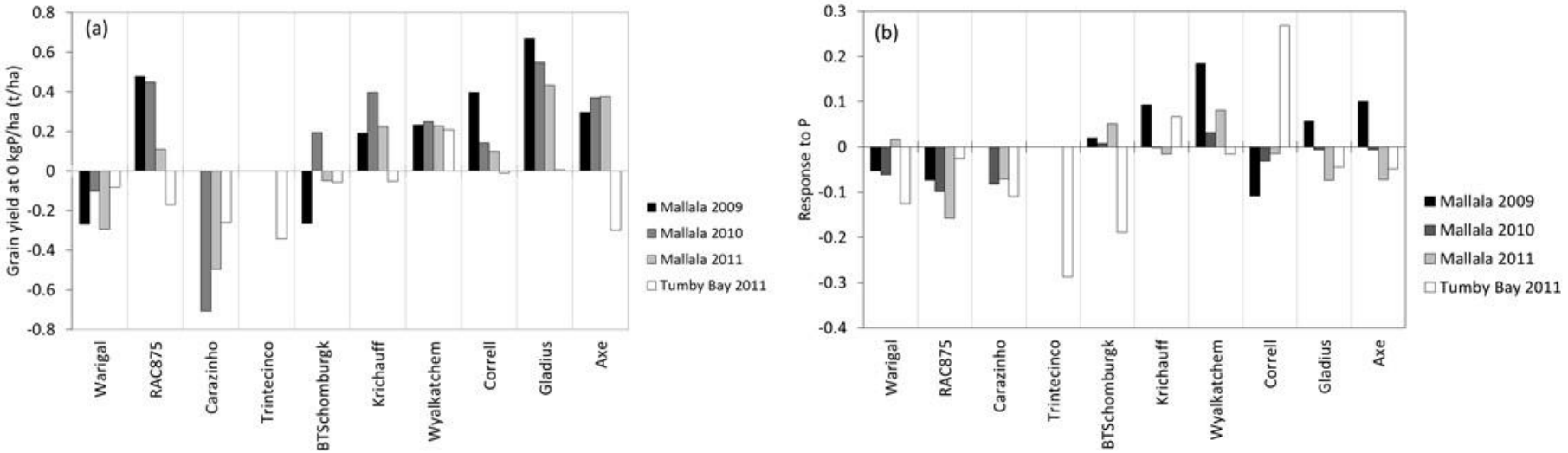

Figure 3.1. The Best Linear Unbiased Predictions (BLUPs) for (a) the grain yield with no applied P and (b) the response to 30 kgP/ha for 10 wheat varieties based on a meta-analysis of a series of P response trials involving 50 genotypes of wheat. A negative yield indicates the variety's yield is lower than average. A negative response to P is lower than the average and the variety is considered relatively non-responsive to P and a positive response indicates a variety is more responsive than average and is considered to be relatively responsive to P. Mallala has an alkaline calcareous soil, Tumby Bay is a relatively acidic soil (pH~6). (Adapted from Mc Donald et al. 2015)

Growth conditions and measurements

Root angle

Experiment 1: To measure the seminal root angle seedlings were grown in germination pouches made from sheets of filter paper. Seeds were first germinated on moistened filter paper in Petri dishes at 20°C in an incubator. Evenly germinated seedlings with a primary root of 2-3 mm long were then placed between two sheets of germination paper suspended in a square tub (30×17×19 cm) containing reverse osmosis (RO) water. One piece of filter paper had a cut in the middle of the top edge that held the pre-germinated seed and allowed the seminal roots to grow between the filter paper sheets. Seedlings were kept in an incubator at 20°C for 7 days and were photographed by a digital camera for root angle measurement.

Experiment 2: To measure the crown root angle, pre-germinated seeds were sown in a Perspex root box (23.5cm×23.5cm×1.5cm) containing 0.95 kg dry Halidon soil. Soil was watered to 100% field capacity (15% w/w) with a basal macronutrient solution containing 9.5mL $Ca(NO_3)_2.4H_2O$ (final concentration 918mg/kg), 9.5mL of K_2SO_4 (250 mg/kg) and $MgSO_4$ (150 mg/kg), 4.75mL solution of $ZnSO_4.7H_2O$ (26 mg/kg), $CuSO_4.5H_20$ (9 mg/kg), $FeSO_4.7H_20$ (17 mg/kg), $MnSO_4$ (5 mg/kg) and $Na_2MoO_4.2H_2O$ (0.1 mg/kg). Phosphorus was applied as $Ca\ (H_2PO_4).H_2O$ placed in a concentrated zone 5 cm below the seed to simulate the placement of P in a commercial crop. There were three P treatments, equivalent to 0, 3 and 30 kg P/ha. Plants were grown in a growth room at 20°C/18°C day/night temperature and a 14/10 h photoperiod for 5 weeks. The intensity of PAR in the growth room was 300-400 μmol quanta/m^2/s.

The plants were watered regularly with reverse osmosis (RO) water to return the sand to field capacity. The root boxes were contained within a plastic crate in which they were placed at an angle of approximately 20°.

At the end of the experiment, the number of tillers and crown roots were counted on each plant. Photographs of the root system were taken at harvest to measure the angles of the seminal and crown roots. Both seminal and crown root angles were measured using ImageJ software (version 1.46, http://imagej.net/). Seminal root angle was measured on both the first and second pair of roots and are reported as the internal angle between the roots. To help overcome curvature and crookedness in the roots, the angle was based on the axes of the roots drawn from the point of attachment to the seed. Crown root angle was measured by considering the outer pair of crown roots.

A completely randomized design was followed for all the experiments. There were six replicates per variety to measure seminal root angle in Experiment 1 and there were three replicates per variety in Experiment 2.

Rhizosheath size

Two experiments (Experiments 3 and 4) were conducted to characterise rhizosheath size among the 10 wheat varieties. The seedlings were grown in soil from Halidon; Experiment 3 examined the effect of P and Experiment 4 compared different types of soil. In both experiments seed was sown in white plastic pots 10.5 cm long and 7.0 cm in diameter which contained 355 g of dry soil. A basal nutrient solution which contained 3.55 mL of Ca $(NO_3)_2.4H_2O$ (final concentration 918 mg/kg soil), 3.55 mL of K_2SO_4 (250 kg/kg) and $MgSO_4$ (150 mg/kg) and 1.78 mL of micronutrients consisting of $ZnSO_4.7H_2O$(26 mg/kg), $CuSO_4.5H_20$(9 km/kg), $FeSO_4.7H_20$(17 mg/kg), $MnSO_4$ (5 mg/kg) and $Na_2MoO_4.2H_2O$(0.1 mg/kg) was added and all pots were watered to 75%

field capacity. In each pot three seeds were planted and seedlings were grown until the plants were two weeks old. No additional water was added to the pots during the growing period.

In Experiment 3, rhizosheath size and root architecture were measured in two P treatments (low P=3 kg P/ha and high P=30 kg P/ha). To see the effect of soil type on root architecture and rhizosheath size, Experiment 4 was set up to compare root growth in Halidon and Mallala soil. Halidon soil had a lower concentration of available P than the Mallala soil and without some additional P the seedlings become severely P-deficient, whereas this does not occur in Mallala soil. Therefore a small amount of P, equivalent to 3 kg P/ha, was added to Halidon soil. In both experiments the seedlings were grown in a controlled environment at 20°C/18°C day/night temperature and a 14/10 h photoperiod. The intensity of PAR in the growth room was 300-400 μmol quanta/m^2/s. A completely randomized block design was followed with five replicates per variety.

The rhizosheath size was measured using the method of Hailing et al (2010). The soil was removed from the pots and the roots were separated carefully from the soil. Roots and shoots were separated and then the roots with the adhering soil were transferred to a plastic tube containing 20 mL of deionised water and shaken to remove the soil. The roots were removed and retained to measure root length and diameter. The tubes were then left for the soil to settle to the bottom and the excess water was poured out. The tubes then transferred to an oven (80°C) for 48 h to dry and weighed. The shoots were dried at 80°C for 48 h to determine their dry weights.

To measure seedling root length and related traits, the soil was shaken from the roots, and they were gently washed to remove any debris that still adhered to them. Once the

roots were cleaned of soil and they were floated on water in a plastic Petri dish and scanned using an A3 Epson Expression-10000 XL scanner. Images were analysed using WinRHIZO 2005 software to record total root length. Root samples were dried in an oven at 80°C for 4 days and the root dry weight measured. Rhizosheath size was estimated as the weight of dry soil per meter of root length.

A dissecting microscope was used to measure root hair length at 3-5 cm from the root tip of the longest seminal root. Ten measurements per sample were taken from each root (2× eyepiece magnification) and the root hair length was reported in millimetres.

Total shoot phosphorus concentration

The phosphorus concentration was measured using phosphovanado-molybdate method (Hanson 1950). Dried, whole plant shoots were cut finely and placed in 3 mL of concentrated HNO_3 (69.8 wt%) overnight and then digested in a hotblock at 140°C for 4-5 hours. The P concentration was measured using a spectrometer at an absorbance of 390 nm after 1 h following the addition of 0.275mL H_2O, 0.01mL of sample and 0.025mL of colour reagent. The colour reagent was made by adding 1L concentrated nitric acid, 1L 0.25% ammonium vanadate (2.5g NH_4VO_3/L) and 1L 5.0% ammonium molybdite (50g $(NH_4)_6MO_7O_{24}$/L). The P concentrations of the samples were estimated from a standard curve using 20ugP/ml Ortho P standard.

Data analysis

Data was analysed with general analysis of variance menu on Genstat. The assumptions of the analysis of variance were checked during the analyses and no transformations of the data were necessary. Orthogonal contrasts (or single degree of freedom contrasts;

Steele and Torrie 1960) were used to compare the measurements among the two groups of genotypes (responsive and non-responsive). Simple linear correlations were used to examine relationships between variables. All analyses were done using GenStat 11th edition. When comparisons were conducted using two soil types, broad sense heritability (h^2) for all the traits was calculated following the method described by Toker (2004).

Results

Root angle

Experiment 1. Genotypes differed significantly in root angle for both the first and second pair of seminal roots (Figure 3.2). The angles for the first pair of seminal roots ranged from 149.9° in variety Axe to 99.8° in variety Warigal and the angle for the second pair of seminal roots ranged from 147.7° in variety BT Schomburgk to 92.4° in variety Gladius. Single degree of freedom contrast found the responsive varieties exhibited significantly larger seminal root angles compared to the non-responsive varieties, with the first pair of seminal roots showing the greater difference (Figure 3.2). A summary of the ANOVA of this experiment is presented in Appendix 1.

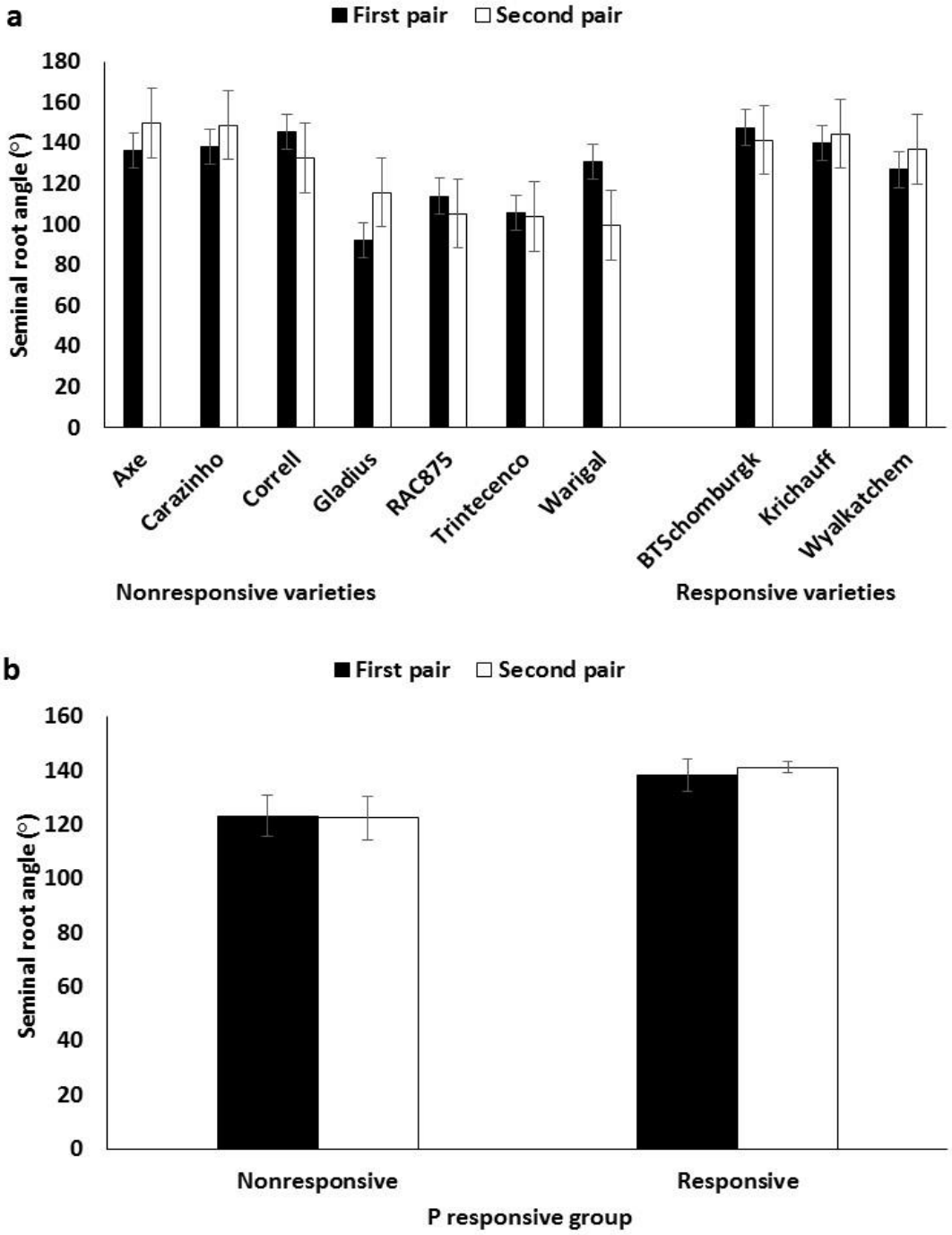

Figure 3.2. Experiment 1: (a) First and second pair of seminal root angle of ten wheat varieties. (b) first and second pair of seminal root angle of two groups of wheat varieties grown on germination paper. Root angle is the internal angle subtending the roots. The responsive group represents the mean of three varieties and nonresponsive group represents mean of seven varieties. Error bar represents standard error of mean.

Experiment 2. Crown root angle was affected by P rate and differed among the varieties, but there was no P rate × Genotype interaction (summary of ANOVA is presented in Appendix 2). Analysis using orthogonal contrasts suggested the non-responsive varieties had wider crown root angles and responded differently to P treatments compared to the non-responsive varieties (Figure 3.3, Appendix 2). The largest difference between the two groups occurred in the low P treatment. Mean crown root angle (averaged over all P rates) of the non-responsive varieties was 96.5° compared to the responsive varieties 84.8°. Crown root angles were smallest at the lowest P rate in the non-responsive varieties and progressively increased as the P rate increased. The non-responsive varieties showing a smaller response to increasing P fertiliser than the responsive group.

Tillering was promoted by addition of P fertiliser and varied among the genotypes (data not presented). Tiller number ranged from 3 tillers/plant in Axe to 5 tillers/plant in BT Schomburgk. BT Schomburgk also produced the largest number of crown roots (13/plant) while Axe and Carazinho produced the fewest number of crown roots (8/plant). There was a strong positive correlation between tiller number and crown root number ($r = 0.90$, $n = 10$, $P < 0.001$). There was no significant correlation between tiller number and crown root angle ($r = 0.26$, $n = 10$), nor between crown root angle and crown root number ($r = 0.40$, $n = 10$).

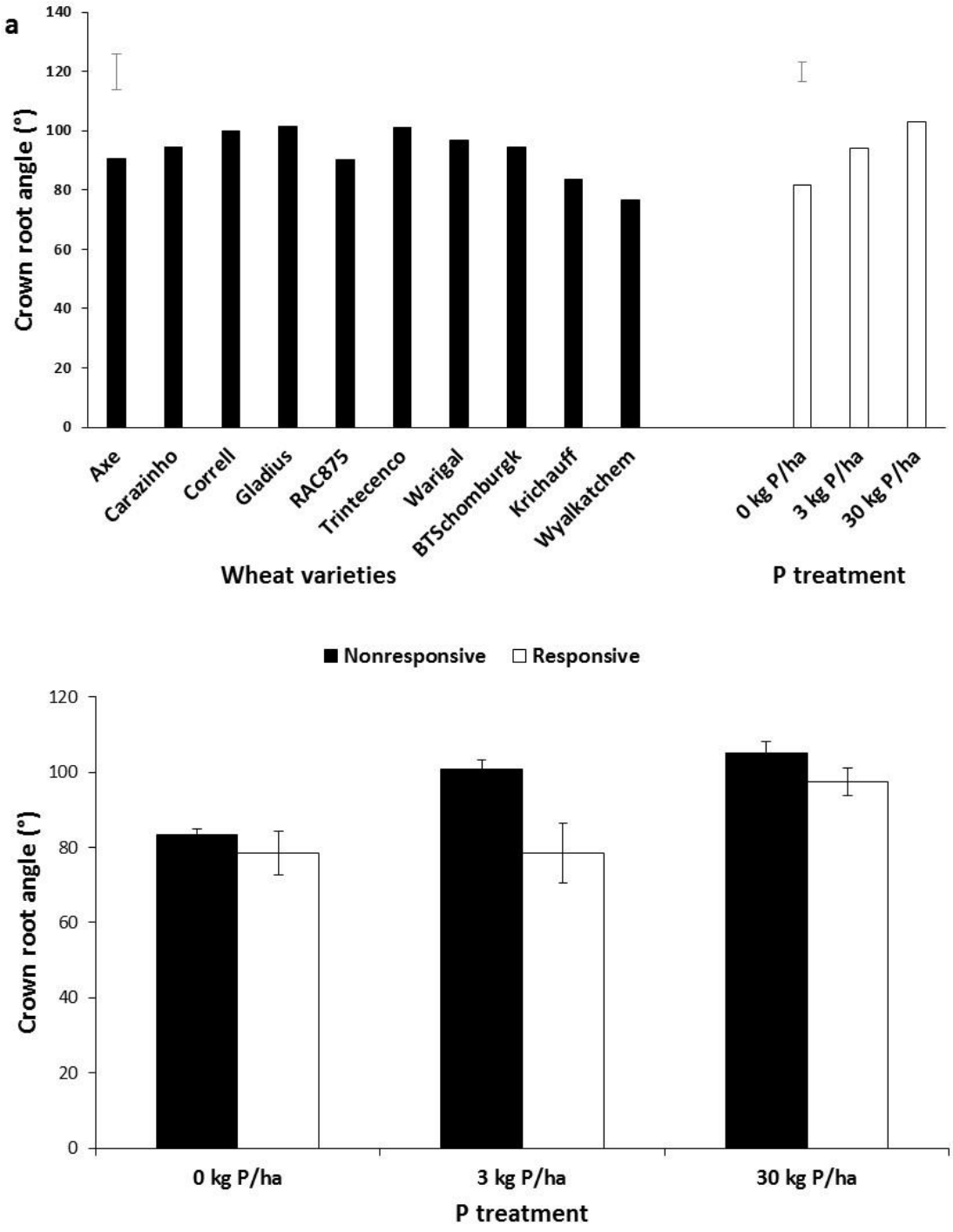

Figure 3.3. Experiment 2: (a) Crown root angle of ten wheat varieties. (b) Crown root angle at three P treatment of two groups of wheat varieties grown on Halidon soil. Responsive group represents the mean of three varieties and nonresponsive group represents mean of seven varieties. Error bar represents lsd for Figure 3a and standard error of mean for the two group of varieties for Figure 3b.

Root length: Genotypes differed significantly (P<0.001) in their total root length in Experiment 3 (Table 3.1). The two Brazilian wheat varieties (Carazinho and Trinticenco) had the greatest total root length (mean total root length =194 cm) compared to the Australian varieties (mean total root length = 159 cm). There was no significant Variety × P treatment interaction or effect of P treatment on root length. There was no significant difference between the responsive and non-responsive varieties in root length or response to P (Appendix 3 and 4). In Experiment 4 no significant Variety, Treatment or Variety x Treatment effects were observed for total root length due to variety and soil type (Table 3.1).

Root diameter: Varieties differed significantly (*P*=0.002) in their average root diameter in Experiment 3, but no significant effect of P treatment was observed (Table 3.1). Orthogonal contrast suggests that the roots of the non-responsive varieties were significantly thinner (*P*=0.03) than roots of the responsive wheat varieties irrespective of P treatment (Table 3.1, Appendix 3).

In Experiment 4, average root diameter was greater in Mallala soil (mean diameter of 10 varieties = 0.50 ± 0.011 mm) than Halidon soil (0.47 ± 0.009 mm), but there was a significant (*P*<0.001) variety × soil type interaction (Table 3.1). Most of the varieties exhibited a similar or greater root diameter in Mallala soil except Warigal. The Genotype ×Soil interaction was also significant between responsive and non-responsive varieties when the orthogonal contrast was conducted (Appendix 5). In this experiment average root diameter of the non-responsive varieties did not differ significantly

Table 3.1. Total root length and average root diameter of ten wheat varieties in Experiment 3 and 4. Means for Experiment 3 are the averages of the two P rates as there was no significant effect of P treatment or significant variety × P rate interaction. Mean values for the P-responsive and non-responsive varieties are shown as mean± standard error of mean. The levels of significance are: * P<0.05; ** P<0.01 and *** P<0.001; NS -non significant

Variety	Experiment 3		Experiment 4			
	Total root length (cm)	Average root diameter (mm)	Total root length (cm)		Average root diameter (mm)	
			Halidon	Mallala	Halidon	Mallala
Non Responsive						
Axe	182	0.45	190	179	0.47	0.49
Carazinho	178	0.43	187	168	0.43	0.48
Correll	148	0.43	156	153	0.45	0.50
Gladius	154	0.50	183	201	0.50	0.53
RAC875	131	0.45	217	166	0.52	0.51
Trintecenco	210	0.45	205	176	0.47	0.48
Warigal	180	0.45	177	142	0.46	0.41
Mean	169±10.0	0.45±0.009	188±7.41	169±7.18	0.47±0.01	0.50±0.01
Responsive						
BTSchomburgk	159	0.42	197	129	0.44	0.47
Krichauff	177	0.43	181	199	0.44	0.55
Wyalkatchem	180	0.45	150	205	0.49	0.49
Mean	172.0±6.6	0.43±0.009	175.9±13.9	177.9±24.5	0.46±0.02	0.50±0.02
LSD (P=0.05)						
Variety	24.8***	0.04**	36.7_{NS}		0.03***	
Treatment	11.1_{NS}	0.02_{NS}	16.4_{NS}		0.01**	
Variety*Treatment	35.1_{NS}	0.05_{NS}	52.0_{NS}		0.05***	
CV (%)	16.4	9.6	23.2		7.5	

between the two soil types whereas the responsive varieties had thicker roots in Mallala soil.

Root hair length

Root hair length of ten wheat varieties was measured in Experiment 4 and significant differences ($P< 0.001$) were observed due to variety and soil type and there was a significant variety × soil type interaction (Appendix 6). Root hairs were generally longer when seedlings were grown in Halidon soil and the non-responsive varieties showed a greater difference between the two soils compared with the responsive varieties (Figure 3.4). In Mallala soil there was no significant difference between the two groups of varieties. (Figure 3.4b). In Halidon soil the responsive variety Wyalkatchem exhibited the smallest root hair length and the non-responsive varieties Carazinho, RAC875 and Trintecenco had the greatest root hair length. In Mallala soil, differences among the varieties were much smaller and only Carazinho and RAC875 had significantly longer root hairs.

Rhizosheath size

There were significant differences in rhizosheath size among the 10 varieties and the differences were not significantly affected by P treatment or soil type (Appendix 3 and 5). In Experiment 3 the rhizosheath size of the responsive varieties (1.5 g/m) was significantly smaller than that of the non-responsive varieties (1.8 g/m). The rhizosheath was lower in Mallala soil (1.6 g/m) than Halidon soil (2.25 g/m). In both soils non-

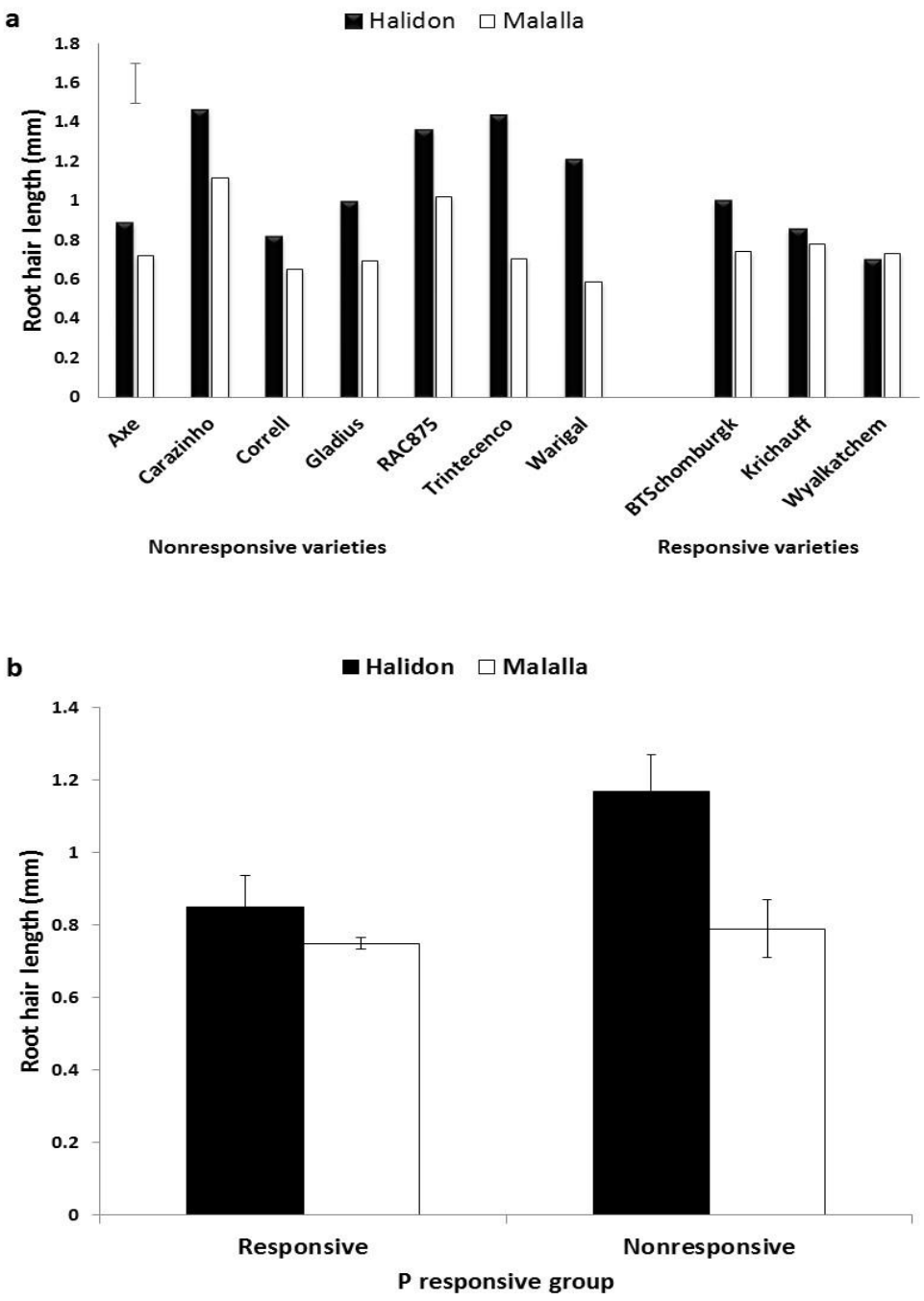

Figure 3.4. Experiment 4: (a) Root hair length (mm) of ten wheat varieties. (b) Root hair length (mm) of two groups of wheat varieties in two soil types. Responsive group represents mean of three varieties and nonresponsive group represents mean of three varieties and nonresponsive group represents mean of seven varieties. Error bar represents lsd for Figure 3.4a and standard error of mean for Figure 3.4b.

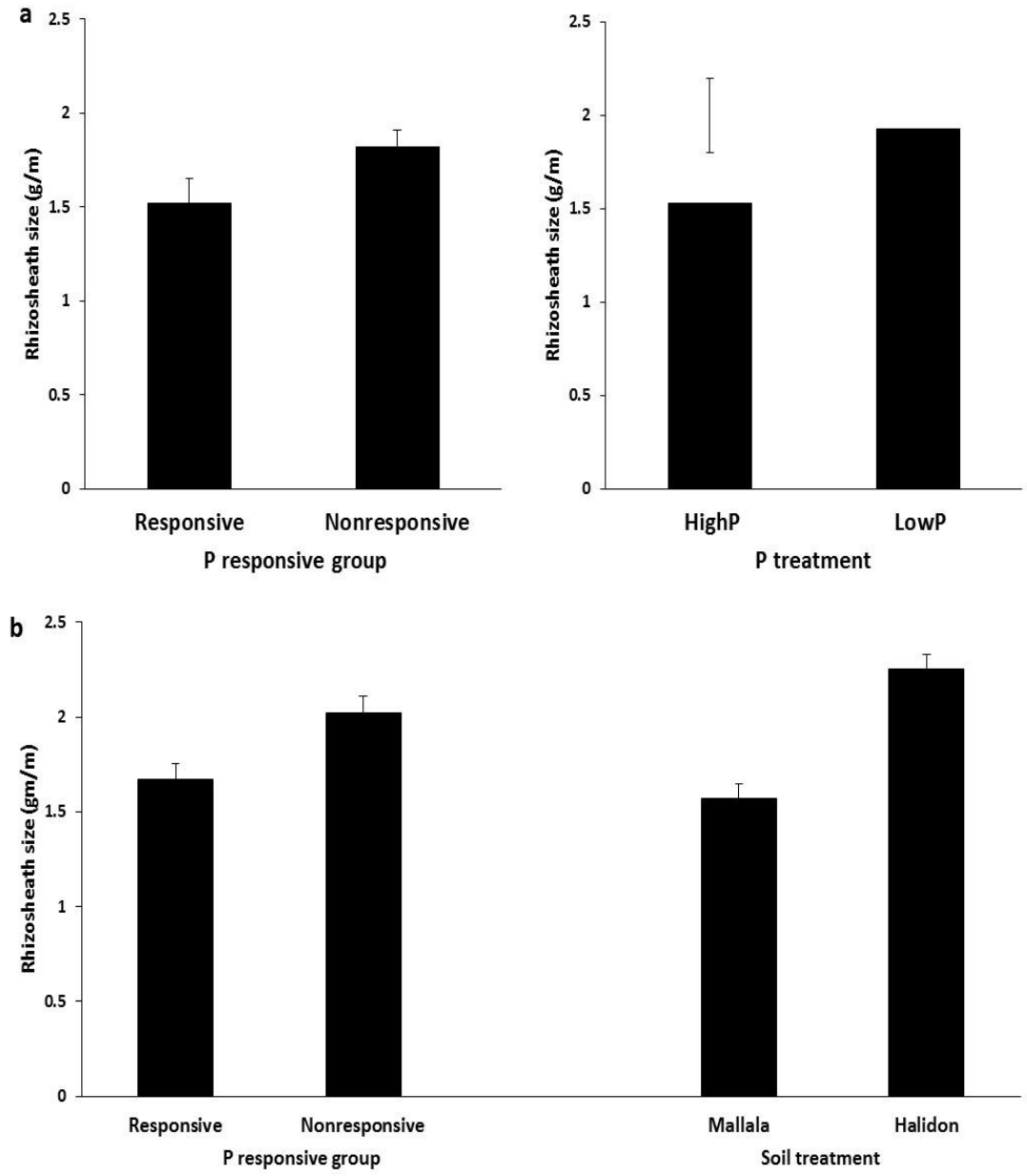

Figure 3.5. (a) Experiment 3: Rhizosheath size (g/m) of two groups of wheat varieties (see Fig 3.2) and the effect of two different P treatments grown in Halidon soil. (b) Experiment 4: Rhizosheath size (g/m) of two representative groups of wheat varieties in two soil types.

Table 3.2. Shoot dry weight (SDW) and root dry weight (RDW) of ten wheat varieties in Experiment 3 and 4. Mean values for the P-responsive and non-responsive varieties are shown as mean ± standard error of mean. The levels of significance are: * P<0.05; **; P<0.01 and ***; P<0.001; NS –non significant

Responsiveness group and Variety	Experiment 3				Experiment 4			
	SDW(mg)		RDW(mg)		SDW(mg)		RDW(mg)	
	Low P	High P	Low P	High P	Halidon	Mallala	Halidon	Mallala
Non Responsive								
Axe	28.0	37.0	13.8	13.0	50.4	39.6	15.0	12.6
Carazinho	32.4	28.6	12.6	11.0	46.6	34.6	12.4	10.8
Correll	41.0	23.0	10.6	8.9	33.2	33.0	10.7	11.6
Gladius	30.6	25.6	14.0	11.0	41.0	40.2	13.1	13.4
RAC875	24.6	33.0	10.3	8.4	56.8	38.0	18.4	14.8
Trintecenco	25.8	27.2	13.2	12.6	48.2	37.4	12.6	12.4
Warigal	35.4	21.9	11.6	9.2	39.8	27.4	12.3	6.8
Mean	31.1 ±2.17	28.0 ±2.05	12.3 ±0.57	10.6 ±0.69	45.1 ±2.94	35.7 ±1.69	13.5 ±0.95	11.8 ±0.96
Responsive								
BTSchomburgk	28.1	35.4	12.0	9.4	37.4	20.6	12.8	9.4
Krichauff	24.8	33.2	11.6	11.4	36.0	28.8	11.9	11.4
Wyalkatchem	26.4	25.4	11.6	13.3	33.2	32.6	12.6	12.2
Mean	26.4 ±0.95	31.3 ±3.03	11.7 ±0.13	11.4 ±1.13	35.5 ±1.23	27.3 ±3.54	12.5 ±0.27	11.0 ±0.83
LSD (P=0.05)								
Variety	1.6***		1.7***		7.6***		2.6***	
Treatment	1.5_{NS}		0.7**		3.4***		1.2**	
Variety*Treat.	4.6***		2.4_{NS}		10.7_{NS}		3.7_{NS}	
CV (%)	12.5		16.2		22.6		23.6	

responsive varieties had larger rhizosheaths (Appendix 5, Figure 3.5), especially the nonresponsive variety Carazinho had consistently greater rhizosheath size in both soil types (2.58 g/m in Halidon and 2.10 g/m in Mallala soil).

Dry matter production

In Experiment 3 there was a significant Variety × Phosphorus interaction when plants were grown with two P levels (Table 3.2) and the mean response to P of the responsive varieties was higher than that of the non-responsive varieties (Appendix 3).

There was no significant difference in average shoot dry weight between the two P treatments for the non-responsive varieties whereas there was a significant increase in average shoot dry weight in the responsive group (Table 3.2).

In Experiment 4 soil type had a significant influence on shoot dry weight (SDW) (Table 3.2), being greater in Halidon soil (mean of 10 varieties = 42.3±2.5 mg) than in Mallala soil (33.2±2.0 mg). The results of the orthogonal contrast suggested that the responsive varieties produced significantly lower SDW (average of two soils = 31 mg) than non-responsive varieties (mean from both soil types is 40 mg). Shoot dry weight was greater in Halidon soil in both groups but the non-responsive varieties showed a greater difference

Root dry weight (RDW) was reduced with the addition of P (Table 3.2) and both the responsive and non-responsive varieties responded similarly. In Experiment 4 RDW (Table 3.2) was affected by soil type as it was lower in Mallala soil (11.5 mg) than Halidon soil (13.2 mg). There was no difference in RDW between the responsive and

non-responsive varieties and both groups of varieties showed similar differences between the soils (Appendix 5)

Table 3.3. Root to shoot ratio of ten wheat varieties in Experiments 3 and 4 (± standard error of mean). (* P<0.05; ** P<0.01 and *** P<0.001 NS means non-significant)

	Experiment 3		Experiment 4	
	Low P	High P	Halidon	Mallala
Non Responsive				
Axe	0.50	0.35	0.23	0.32
Carazinho	0.39	0.39	0.27	0.33
Correll	0.26	0.46	0.38	0.49
Gladius	0.45	0.43	0.32	0.33
RAC875	0.42	0.26	0.33	0.41
Trintecenco	0.51	0.47	0.26	0.33
Warigal	0.34	0.51	0.31	0.24
Mean	0.41±0.03	0.41±0.03	0.30±0.02	0.35±0.03
Responsive				
BTSchomburgk	0.44	0.27	0.35	0.45
Krichauff	0.47	0.36	0.33	0.41
Wyalkatchem	0.44	0.53	0.39	0.38
Mean	0.45±0.01	0.39±0.08	0.36±0.02	0.41±0.02
LSD				
Variety	0.08***		0.10*	
Treatment	0.03_{NS}		0.04*	
Variety*Treatment	0.11***		0.13_{NS}	
CV (%)	20.4		32.0	

A significant (*P*<0.001) Variety × Phosphorus interaction was observed for root: shoot ratio in Experiment 3 (Table 3.3), but there was no significant difference in response to P between the two groups of varieties. There was no consistent response to P among the varieties, with higher and lower ratios as well as no change with the addition of P being observed. Root: shoot ratio was greater in Mallala soil (Table 3.3) and non-

responsive varieties had significantly (P=0.006) lower ratios than the responsive varieties.

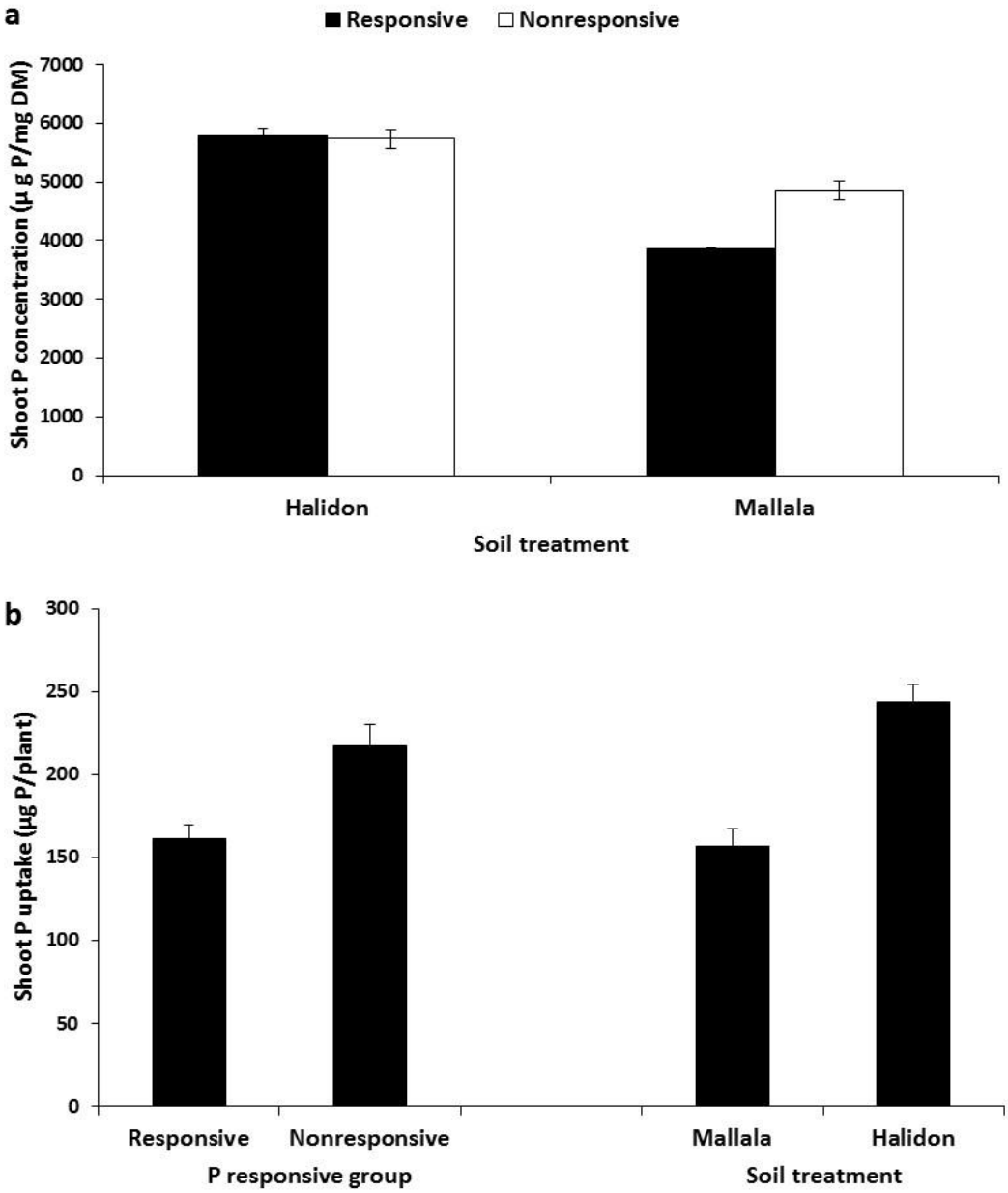

Figure 3.6. Experiment 4: (a) Shoot P concentration (µg P/g DM) and (b) total P uptake by shoot (µg P/plant) of two groups of wheat varieties (see Fig. 3.2) in two soil types. Responsive group represents mean of three varieties each and nonresponsive group represents mean of seven varieties. Error bar represents standard error of mean for the group of variety.

Total shoot P uptake

A significant interaction between the P-responsiveness and soil type was observed for shoot P concentration (Fig 3.6; Appendix 5). Shoot P concentration was equivalent for responsive and non-responsive groups when grown in Halidon soil, but the P concentration of the responsive group was significantly lower than that of the non-responsive group in Mallala soil (Figure 3.6a). Both groups had lower P concentration when grown in Mallala soil.

Total shoot P uptake per plant was measured in both soil types and as a group the responsive and non-responsive varieties differed significantly (Figure 3.6b). The total shoot P uptake per plant was higher when grown in Halidon soil (244 µg P/plant) than in Mallala soil (157 µg P/plant). The P- responsive wheat varieties had significantly less P (161 µg P/plant) compared to the non-responsive varieties (217 µg P/plant) (Figure 3.6b). This was consistent with the difference seen in the Mallala soil (Figure 3.6a). There was a significant positive linear correlation ($r = 0.81$, $n = 10$ $P < 0.01$) between total shoot P uptake and total root length (Figure 3.7a) and root hair length (Figure 3.7b) in Halidon soil, but not in Mallala soil.

Heritability and correlation of root traits and shoot P uptake

With the exception of root diameter in Experiment 4, broad sense heritabilities were above 60% (Table 3.4) which suggests high genetic control of the traits studied here. Phenotypic correlations among the root traits for Experiment 3 are presented in Table 3.5.

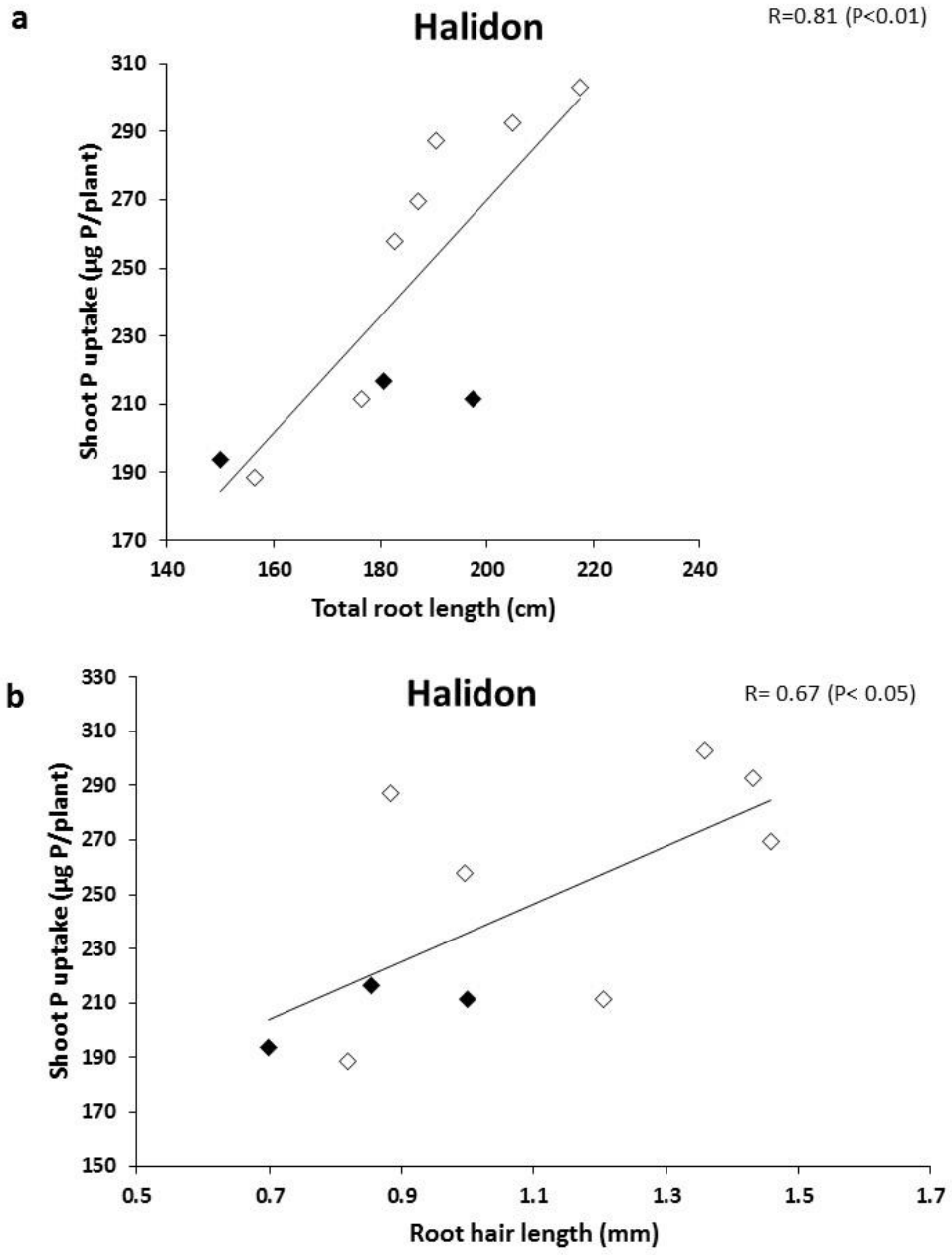

Figure 3.7. Correlation between total shoot P uptake and (a) total root length and (b) root hair length in Halidon soil. Each data point represents the mean of five replication of ten wheat varieties (♦ represents responsive group and ◊ represents nonresponsive group).

Table 3.4. Broad sense heritability of five root traits, shoot and root dry weight and shoot P uptake of ten wheat varieties

Root traits	Heritability (%)		
	Experiment 2	Experiment 3	Experiment 4
Crown root angle	75		-
Total root length	-	85	-
Average diameter	-	69	24
Root hair length	-	-	64
Rhizosheath size	-	89	76
Shoot DW	-	-	72
Root DW	-	75	72
Shoot P uptake	-	-	85

At low P, average root diameter was significantly correlated with rhizosheath size and root dry weight, and there was a significant positive correlation with root length and RDW at high P. Variation in SDM was not related to rhizosheath size in either P treatment.

Several positive correlations were observed in Experiment 4 (Table 3.6). Average root diameter was significantly correlated with RDW in both soils and with total root length in Mallala soil. In Halidon soil total root length was positively correlated with RDW and SDW. Shoot phosphorus uptake was correlated with RDW and SDW in Mallala soil but the correlation was more prominent for Halidon soil, where it was significantly correlated with rhizosheath size, RDW, SDW and root hair length (Table 3.5).

Table 3.5. Correlation among all the traits when grown in two different P levels. Correlations below the diagonal are for the low P treatment and above the diagonal for the high P treatment (* P<0.05; ** P<0.01 and *** P<0.001)

Root traits	Average diameter	Total root length	SDW	RDW	Rhizosheath size
Average diameter		0.072	-0.437	0.192	0.468
Total root length	0.092		0.011	0.835**	-0.621
SDW	- 0.077	-0.115		0.138	0.065
RDW	0.670*	0.513	-0.171		-0.385
Rhizosheath size	0.672*	-0.315	0.066	0.335	

Table 3.6. Correlation among all the traits when grown on two different soil types. Correlations below the diagonal are for the Halidon soil and above the diagonal for the Mallala soil (* P<0.05; ** P<0.01 and *** P<0.001)

Root traits	Ave. root diameter	Total root length	SDW	RDW	Rhizosheath size	RHL	Shoot P uptake	P conc.
Ave root diameter		0.671*	0.353	0.738**	0.041	0.229	0.385	-0.040
Total root length	0.229		0.568	0.592	-0.207	0.059	0.470	0.212
SDW	0.430	0.828**		0.753**	0.274	0.221	0.956***	0.338*
RDW	0.718*	0.680*	0.815**		0.301	0.356	0.731*	0.239
Rhizosheath size	0.492	0.356	0.663*	0.526		0.857**	0.251	0.063
RHL	0.003	0.708*	0.696*	0.322	0.472		0.109	-0.166
Shoot P uptake	0.393	0.807**	0.953***	0.696*	0.675*	0.670*		0.709***
P conc.	-0.096	0.206	0.058	-0.032	-0.157	0.055	0.548***	

Discussion

Correlation of root angle towards varietal P responsiveness

The importance of wide basal root angle for P acquisition is well established in maize and bean (Liao et al. 2004; Zhu et al. 2005), but the findings of this study suggest a wide seminal root angle is not important in wheat, at least among the 10 varieties used in this study. There is limited information on the importance of wheat seminal root angle for P efficiency but in this study P responsive wheat varieties had wide seminal root angles, which is the opposite trend observed in maize and beans. Liao et al (2004) found several QTLs associated with wide basal root angle of bean, but in addition to these there were also several QTL for P use efficiency that were not associated with root angle. This finding suggests that other root traits contribute to P efficiency of crops. In their study Liao et al (2004) concluded that a breeding programme that includes several traits can be more successful than selecting wide basal root angle alone. The findings from these experiments was that the crown root angle was associated with P responsiveness among wheat varieties rather that seminal root angle, with the non-responsive P group having a wider crown root angle than the responsive P group. In a field study Miller et al (2003) observed that efficient bean genotypes had greater adventitious rooting relative to basal root growth and those genotypes had greater growth and P uptake. Under low P conditions genetic mapping of adventitious rooting of bean identified several QTL (Ochoa et al. 2006), including a pair of QTL that contributed to 61% of the observed phenotypic variation, suggesting the possibility of selection of this trait for breeding programme. Adventitious roots (analogous to crown roots in wheat) obviously have some benefit to P uptake compared to seminal roots in terms of top soil foraging, as by nature they grow more horizontally and are concentrated in the surface soil layer. Crown

roots may have greater abundance of aerenchyma in the tissue compare to other root types that can reduce metabolic cost of soil exploration (Vartapetian and Jackson 1997). According to Miller et al (2003) adventitious roots can acquire more P than basal or tap roots and increased relative biomass of adventitious root was observed under P deficient conditions.

Considering the importance of crown root angle the non-responsive varieties of this study may have benefitted from the wider crown root angle. With plant development the seminal root goes deep into the soil but the crown root stays at the top layers of soil and can contribute to enhance P uptake. Some non-responsive varieties especially Trintecenco and Warigal producing more crown roots per tiller than responsive varieties, but crown root angle was not related to tiller number or to crown root number. It can be concluded that wide crown root angle might be one of the contributing factors explaining P response of the non-responsive varieties under field conditions.

Root morphology and P responsiveness

In this study genotypes differed significantly in their total root length in Experiment 3 but no effect of P treatment was observed. In Experiment 4 no significant difference for total root length was observed among varieties. Zhu et al (2005) observed that P-efficient maize genotypes showed greater total root length under low P conditions than inefficient genotypes, and no difference in high P conditions, which suggests that root length can be influenced by soil P availability. At low levels of available soil P, Manske et al (2000) observed that total root length was an important trait for improved P absorption and it was positively correlated with P use efficiency of wheat. The results of Experiment 4 found a similar result although the effect was only observed in the

coarse-textured Halidon soil. In a study with soybean, sunflower and maize Fernandez et al (2009) observed increased specific root length for all three species with decreasing P supply. Otani and Ae (1996) compared many plant species and concluded that in terms of P uptake, crops with longer root system are not necessarily more efficient.

The varieties tested in this study differed in their average root diameter and non-responsive P varieties had, on average, a greater root diameter compared to responsive varieties. In Mallala soil root diameter was greater than in Halidon soil suggesting soil type has a significant effect on root diameter. Woodfield and Caradus (1990) reported high heritability ($h^2 = 0.54$) of root diameter of white clover but the relationship of root diameter with P uptake is still not established because of lack of data (Gahoonia and Nielsen 2004). Different estimates of heritability for average root diameter were observed in the current experiments: in Experiment 3 moderate (69%) heritability was observed while a low value (24%) was observed in Experiment 4, presumably because of the influence of soil type on root diameter. In maize Zhu and Lynch (2004) observed the association of small root diameter with P efficiency. In contrast, this study found greater root diameter was related to the non-responsive wheat varieties, which were more P efficient. In a study with soybean, sunflower and maize Fernandez et al (2009) observed that maize had greater root diameter and was able to explore more soil per root length compare to other two species, although this did not result in greater P uptake. Although some previous work has suggested root diameter may be important to P efficiency (Fernandez et al 2009, Lynch and Zhu 2004) there is also considerable variation in the nature of the effect. In the current work, root diameter differed between the responsive and non-responsive groups, but there was no relationship between root

diameter and P uptake. The results on the importance of root dimeter to P efficiency are equivocal and may be influenced by the growing environment.

In this study root morphological traits such as total root length did not appear to be critical for P responsiveness of wheat varieties and a similar result was observed in maize (Liu et al. 2004). The wheat varieties here did not differ in their total root length, it suggesting that there are other mechanisms for P uptake which were responsible for the variation of P responsiveness of these varieties at field conditions, which is similar to the findings of Fernandez et al (2009).

Root hair length and rhizosheath size for varietal P responsiveness and P uptake

The findings of this study demonstrated that P-efficient genotypes had significantly longer root hairs, which agrees with the observation for other crop species showing that the root hairs are important for P acquisition (Brown et al. 2012; Yan et al. 2004). In *Arabidopsis thaliana*, significant effects of varying root hair length and density on P acquisition efficiency were observed (Ma et al. 2001). Genotypic variation in root hair length and density in maize and bean are controlled by several major QTL (Yan et al. 2004; Zhu et al. 2005) suggesting that these traits could be selected in breeding programs through marker-assisted selection and direct phenotypic screening. The findings of this chapter also support this as a moderately high heritability was observed for root hair length. Although wheat varieties produced shorter root hairs in Mallala soil compared to Halidon soil, the ranking of varieties was consistent between the two soils. In particular, the non-responsive wheat varieties Carazinho and RAC875 produced longer root hairs in both soils under low P conditions and have shown consistently low responses to P. Several studies with mutant barley genotypes lacking root hair have

shown reduced P uptake compared to the wild type plants at low P condition in soil culture and this was also associated with reduced biomass production (Gahoonia et al. 2001; Gahoonia and Nielsen, 2003; Brown et al. 2012; Haling et al. 2013). Variation in root hair length within species was correlated with improved PUE under P deficient conditions (Gahoonia and Nielsen, 1997; Wang et al. 2004; Zhu et al. 2010) and correlation with final grain yield was also observed by Gahoonia and Nielsen, (2004). Rhizosheath size and root hair length are strongly influenced by the environment because both were affected by soil type and by P availability. However, our findings especially for the non-responsive wheat variety Carazinho are consistent with the findings of Hailing et al (2010) as Carazinho showed a moderate to high rhizosheath size over both experiments and soil types. The values were greater than what was observed previously by Haling et al (2010), which is likely due to a longer growing period in this study. The non-responsive variety Trintecenco had a dramatic decrease in root hair length at Mallala. Genetic differences in rhizosheath size was observed in wheat germplasms comprised near-isogenic line when grown in acid soil (Delhaize et al.2012). James et al. (2016) observed association of improved PUE of wheat with large rhizosheath size in acid soil.

Several studies have shown the importance of root hair length for rhizosheath formation (Haling et al. 2010; Moreno-Espindola et al. 2007). In Mallala soil there was a positive correlation (r=0.85) between with root hair length and rhizosheath size (Figure 3.8) but not in Halidon soil. The findings of this thesis suggests the strong influence of soil type on the formation of rhizosheath. There are several contrasting results observed for the correlation between root hair length and rhizosheath size. Delhaize et al (2012) demonstrated a strong positive correlation between root hair length and rhizosheath size

in wheat growing in acid soil; a similar result was also observed by James et al. (2016). Similar to results from studies in acid soil, a strong positive correlation between root hair length and rhizosheath size of wheat was observed by Delhaize et al. (2015). George et al (2014) observed a partial relation of root hair length in the formation of rhizosheath and concluded that root hair length alone cannot explain rhizosheath size of barley. It is known that rhizosheath formation is not only strongly affected by root hair length but also by the soil environment such as soil pH, bulk density, soil moisture and texture (Haling et al. 2013; Watt et al. 1994).

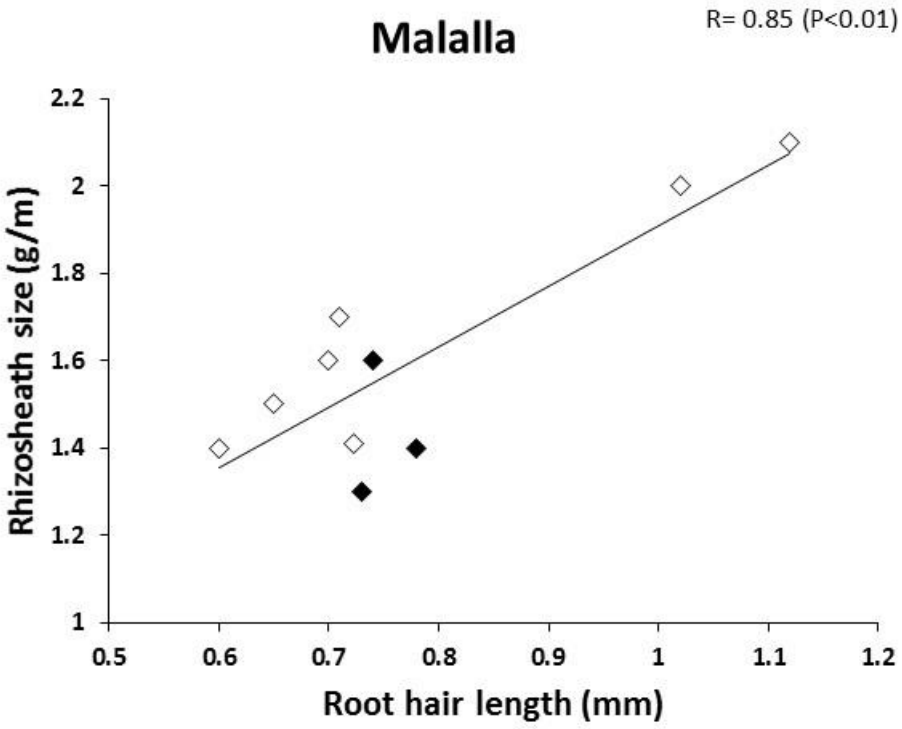

Figure 3.8. Correlation between root hair length and P uptake of ten wheat varieties grown in Halidon and Mallala soil. Each data point represents mean of five replications (♦ represents responsive group and ◊ represents nonresponsive group).

In this study non-responsive wheat varieties had larger crown root angle, longer root hairs and accumulated more shoot P than responsive varieties in Experiment 4. Our

findings are similar to those of Brown et al (2012) where they observed that the long root hair barley genotypes accumulated significantly more P under P deficient condition than genotypes with short root hair and genotypes which had no root hairs. In this study shoot P uptake was significantly correlated with total root length and root hair length in Halidon soil (Figure 3.7). In Mallala soil, shoot P concentration (Figure 3.6a) and P uptake (Figure 3.6b) were lower than in Halidon soil suggesting low P availability in Mallala soil, which reflects on the plant P status. In Halidon soil, SDW was significantly correlated with root hair length and is consistent with the findings of Brown et al (2012) who concluded that root hair length was critical for P accumulation and biomass production of barley. In this study, as a group the SDW of non-responsive varieties at high P level did not increase as much as the responsive varieties suggesting there is less dependency on additional P for improved growth. In Experiment 3 non-responsive varieties produced similar SDW at the low P and high P treatments. This finding is comparable to the previous field study by McDonald et al (2015), which was the basis of selection of varieties for this study. In their field study McDonald et al (2015) characterised varieties on the basis of how they utilise native soil P and the response to added P fertiliser and observed consistent genetic differences in growth and yield across environments when P fertiliser was not applied. For the current study, varieties which showed reasonably consistent genetic variation in terms of growth and yield at no added P were selected to identify the adaptive mechanism at low P environment. The findings of SDW of this study at low P treatment agrees with the categorisation of varieties by McDonald et al (2015) and demonstrates that the genetic difference is consistent not only at field condition but also in controlled environments. Moderate to high heritability of crown root angle, root hair length and rhizosheath size suggests high genetic control of these traits and potential for selection in future breeding programs.

Conclusion

In this study significant genetic variation for root traits among wheat genotypes was observed. Shallowness of crown root angle and the relationship with P responsiveness suggests the importance of the crown roots for P acquisition. To date there is very limited information available on crown root development and its relation with P acquisition in wheat. With a combination of small seminal root angle and a wide crown root angle, non-responsive wheat varieties may be able to acquire both greater water from deep soil layers and P from shallow soil layers. Wide crown root angle can assist plants to acquire more P at later growth stages when the demand for P increases. As root traits can be easily influenced by environmental conditions, it would be beneficial to select varieties that exhibit more than one adaptive mechanism to P deficient conditions. In this study, the wide crown root angle and long root hairs of non-responsive wheat varieties can explain their better performance under field condition.

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Chapter 4 : Contribution of mycorrhizal colonization in growth, phosphorus uptake and varietal difference of wheat

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Name of Principal Author (Candidate)	Kamrun Nahar		
Contribution to the Paper	Managed experiments and performed analysis on all samples, interpreted data, wrote manuscript.		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Overall percentage (%)	85%		
Signature		Date	17/5/2017

Name of Co-Author	William Bovill		
Contribution to the Paper	Helped to evaluate and edit the manuscript.		
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Contribution to the Paper	Supervised development of work, helped in data interpretation and manuscript evaluation and acted as a corresponding author		
Signature		Date	17/5/17.

Abstract

Arbuscular mycorrhizal fungi (AMF) are known to play an important role in plant P uptake, but there is limited and conflicting information on the influence of varietal differences on infection by AMF and its influence on P uptake in wheat. In this study the contribution of mycorrhizal colonization towards wheat growth and P uptake and how different wheat varieties differ in mycorrhizal colonization was investigated. Ten wheat varieties were selected on the basis of their grain yield response to P under field conditions. Several experiments were done in controlled environments with different P treatments and for one experiment there were two inoculation treatments. Plant samples were also collected from field plots to examine the genetic differences of AM fungal colonization under field condition. Substantial genetic variation was observed for AMF colonization, but it was not possible to relate this with varietal P responsiveness. The non-responsive varieties Carazinho and RAC875 showed consistently high colonization over all experiments. The mycorrhizal colonization was higher when there was no added P and with added P colonization declined in most varieties except in the non-responsive varieties Carazinho, Correll and Trintecenco which showed colonization similar to or greater than that measured at low P. The findings of the field study were not consistent with the controlled environment results. There was either no or a negative relation between mycorrhizal colonization and plant growth and shoot P uptake. Although some varieties showed a consistently high level of AMF colonisation across experiments, it was not possible to outline the contribution of mycorrhizal colonization for growth and P uptake. Inconsistency of the findings and lack of relationship with varietal P responsiveness suggests that under deficient condition AMF colonization may not be a useful trait for selection for improved P efficiency.

Introduction

Phosphorus (P) is an essential macronutrient for plant growth and development. Plants take up P from the soil as orthophosphate (Pi) but the concentration of Pi in soil solution is low and rarely exceeds 10 µM (Schachtman et al. 1998) and the diffusive movement of Pi in the soil solution is slow. Consequently, P availability in soil is an important nutritional constraint for crop production (Bates and Lynch 2000) and P fertiliser application has been required to increase and maintain productivity. After its application P can be taken up by the plant, retained by the soil, or lost through leaching (Bolland 2000). According to McLaughlin et al (1991) 50-80% of total P is fixed by the soil after application by reactions with soil minerals, which makes P poorly available to plants. This fixation has resulted in substantial banks of soil P being built up. Improving the ability of crops to access this bank has the potential to reduce dependence on P fertiliser and help improve the profitability of farming systems. To overcome the problem associated with low availability of soil P, improvements in the crop's ability to acquire P from the existing sources in the soil and utilize that P for growth and development is required (White and Brown 2010).

The symbiotic relationship between plants and arbuscular mycorrhizal fungi (AMF) may be one way of improving P uptake when soil P is low. In this symbiotic relationship the plant supplies C to the fungus and the fungus provides immobile nutrients such as P and other poorly available nutrients to the plant *via* an extensive hyphal network (Smith et al. 2003). It has also been suggested that AMF can also increase activity of phosphatase enzymes (Dodd et al. 1987; Tarafdar and Marschner 1994) and the solubilisation of rock phosphate or other forms of P (Omar 1998). However an increase in P concentration in AMF-infected plants is not always accompanied by increased

growth; sometimes it can be associated with growth depressions (Zhu and Smith 2001; Zhu et al. 2001) as AMF receive C from the plants than may otherwise contribute to plant growth. However, the cost of AMF will not be harmful if plant growth is not limited by C (Smith et al. 2011).

Plant responsiveness to AMF colonization is highly variable due to both environmental and genetic influences on colonisation. Infection and colonisation by AMF is sensitive to plant P status and to a number of soil and environmental factors (Graham and Abbott 2000b; Ryan and Angus 2003). There is also considerable variation between and within plant species in colonization (Baon et al. 1993; Hetrick et al. 1993; Koide et al. 1988). Mercy et al (1990) observed substantial genetic variation in colonization in cowpea. Variation in colonization among wheat varieties was also observed by Hetrick et al. (1992) and Zhu et al. (2001) and they concluded that modern wheat varieties had less colonization than old varieties (landraces). However, this may not be a general effect: the opposite was observed among oat and tomato varieties (Koide et al. 1988) and two earlier studies with wheat and barley showed no variation in colonization among different genotypes (Jakobsen and Erik Nielsen 1983, Kapulnik and Kushnir 1991). Contrasting information of genetic variation of colonization emphasises the need for more research to understand the variation of mycorrhizal colonization. Much of the previous work on genetic differences in colonization among wheat varieties focussed on examining the effects of the origin and the year of release (An et al. 2010, Zhu et al. 2001) rather than examining AMF infection among varieties that differed in P responsiveness.

The aim of this study was to understand the contribution of AMF to varietal differences in P use efficiency (PUE). Although the contribution of AMF on different plant species is well documented there is very little information on intra-species variation. Most of the reported work on AMF has either used a single variety of several mycorrhizal and non-mycorrhizal plant species (Smith et al. 2003) or has examined the functional diversity of the fungal species by selecting several fungal species (Graham and Abbott 2000a). Often when examining the importance of genetic variation in a trait to P efficiency, the approach is to characterise the response under controlled conditions and then evaluate the genotypes in the field. We took a different approach: varieties that showed differences in their response to P in the field were identified and then their responsiveness to AMF colonisation was examined. Therefore, the experiments were conducted to examine whether genotypes that showed differences in P responsiveness under field conditions also showed differences in AMF colonisation.

Methods and materials

Three experiments were conducted to examine the level of AM infection among varieties of bread wheat that showed differences in their response to P in the field. The plants were grown in a growth room at 20/18 °C and a 14/10 h photoperiod. Light intensity in the growth room was 300-400 µmole quanta/m^2/s.

Selection of varieties

Selection of varieties was based on their response to P in field trials in South Australia that were conducted at three sites per year over three years (McDonald et al 2015). The analysis of these data suggested there were some varieties that showed a generally

lower-than-average response to P and a number of varieties that showed a higher-than-average response to P across experiments. The non-P responsive group included Axe, Correll, Carazhino, Gladius, RAC 875, Trintecenco and Warigal, and the P responsive varieties were BT Schomburgk, Krichauff and Wyalkatchem.

Experimental details

Experiment 1 (preliminary experiment): Two wheat genotypes, Carazinho and Wyalkatchem, which had shown different response to phosphorus (P) in field experiments, were selected. Soil for this experiment was collected from one of the sites (Halidon, South Australia) used in the field evaluation. The soil was a loamy sand with available Colwell P of 8mg/kg and a pH (water) of 7.0. Seeds were sown in pots which were 17 cm high with a diameter of 17cm. For this experiment 2.5 kg of dry soil per pot was used. A basal nutrient solution was added to each pot which gave the final concentrations in soil of 918mg/kg Ca $(NO_3)_2.4H_2O$, 250 mg/kg K_2SO_4, 150 mg/kg $MgSO_4$, 26 mg/kg $ZnSO_4.7H_2O$, 9 mg/kg $CuSO_4.5H_20$, 17 mg/kg $FeSO_4.7H_20$, 5 mg/kg $MnSO_4$) and 0.1 mg/kg $Na_2MoO_4.2H_2O$ (). There were three P treatments, P0 without added phosphorus, low P (equivalent to 3 kg/ha) and high P (equivalent to 30 kg/ha). Calcium phosphate $Ca(H_2PO_4)_2.H_2O$ was used as the source of P. The P was added as liquid and mixed through the soil with the basal nutrient solution. Eight seeds of each variety were sown in each pot and these were later thinned to 5 plants per pot. The experiment relied on the natural levels of AMF inoculum in the soil to infect the roots. A completely randomized block design with three replications was followed for this experiment. Once the seedlings were established, the pots were watered regularly to 75% field capacity and seedlings were grown for 5 weeks. At harvest the number of tillers was counted. The roots were carefully washed from the soil and subsampled for

assessment of AMF infection (see below). Shoots were separated from roots and dried in an oven at 60°c to estimate shoot dry weight (SDW).

Experiment 2a: Root box experiment: Ten wheat genotypes differing in their yield response to P were selected. A sandy soil was collected from an experimental field site Karoonda, which is low in available P (Colwell P =8mg/kg). Pre-germinated seeds were sown in a Perspex root box (23.5cm×23.5cm×1.5 cm) with 950 gm of dry soil. Soil was initially watered to 100% field capacity (15% w/w) with a basal nutrient solution to give final concentrations in the soil of 918mg/kg $Ca(NO_3)_2.4H_2O$, 250 mg/kg K_2SO_4, 150 mg/kg $MgSO_4$, 26 mg/kg $ZnSO_4.7H_2O$ (), 9 mg/kg $CuSO_4.5H_20$, 17 mg/kg $FeSO_4.7H_20$, 5 mg/kg $MnSO_4$ and 0.1 mg/kg $Na_2MoO_4.2H_2O$. The P was placed in a concentrated zone 5cm below the seed to simulate the banding of P in a commercial crop. There were three P treatments, equivalent to 0, 3 kg P/ha and 30 kg P/ha. A completely randomized block design with three replications was followed. Plants were grown in a growth room for 5 weeks (growing condition was same as mention before – see p 108). Infection of roots relied on naturally-occurring inoculum in the soil. At the end of the experiment the shoots were separated from the roots and oven dried at 60°C and weighed. The whole shoot sample was ground, the P concentration measured (see below) and P uptake estimated from the shoot dry weight and shoot P concentration. The proportion of the root system colonised by AMF was estimated after which the roots were oven dried and weighed.

Experiment 2b: Field assessment of AMF infection:

A field trial examining P response among wheat genotypes contained a number of the genotypes used in the pot experiments, which provided an opportunity to examine the

variation in infection in field-grown plants. The experiment was conducted at a low rainfall site near Karoonda, South Australia (map reference S 35.086, E 139.871; average annual rainfall = 342mm) on a sandy soil low on P (Colwell P = 8 mg/kg) and with pH (1:5 soil:water) of 7.1 in the top 20cm. The experiment compared the growth and yield of plants at nil applied P or with an application of 30 kg P/ha applied as triple superphosphate at sowing. The fertiliser was drilled with the seed at sowing and the seeds did not receive any fungicide seed dressing. Plants were sampled at the start of stem elongation (Zadoks growth stage 31-32; Zadoks *et al.* (1974) Of the 10 genotypes listed in Table 1, BT Schomburgk, Krichauff, Axe and Warigal were not included in the trial while the responsive genotype Scout was included in the sampling. A shovel was used to dig the soil and the sampling was done by digging the top 10 -15 cm of soil from three spots randomly selected within each plot. Care was been taken to remove the roots from the soil. The samples were then sealed in labelled plastic bags, transported in an insulated container and kept in a cold room at 4° C overnight. The next morning roots were washed carefully and three samples (with similar appearance) were randomly selected from each plot for mycorrhizal assessment. The mean value of three samples was taken to represent one replication.

Experiment 3: Pot trial

The aim of this experiment was to understand the contribution of mycorrhizal colonization towards varietal P efficiency. To achieve the aim of this experiment plants were grown with and without mycorrhizal fungi. The same 10 genotypes used in Experiment 2a were used in this experiment. Pots that were used for this experiment were 15 cm long x 10cm diameter with each was filled with 600 g of sterilised Halidon soil to which 10% soil filtrate was added to reintroduce general soil microorganisms

following the method described by Facelli et al. (2010). A basal nutrient solution consisting of 6.0mL Ca $(NO_3)_2.4H_2O$ (final concentration 918mg/kg), 6.0mL of K_2SO_4 (250 mg/kg) and $MgSO_4$ (150 mg/kg), 3.0mL solution of $ZnSO_4.7H_2O$ (26 mg/kg), $CuSO_4.5H_20$ (9 mg/kg), $FeSO_4.7H_20$ (17 mg/kg), $MnSO_4$ (5 mg/kg) and $Na_2MoO_4.2H_2O$ (0.1 mg/kg) was added to the soil in each pot and thoroughly mixed. There were two P treatments (0 kg P/ha and 30 kg P/ha). The P was mixed through the soil when the basal nutrient solutions were added. Commercial mycorrhizal inoculum (Start Up Super, Microbe Smart Pty Ltd) consisting of a mixture of *Glomus etunicatum, G. coronatum, G. intraradices, and G. mosseae* was used and there were two inoculum treatments (+ inoculum and – inoculum). Inoculum was applied at a density of 3000 propagules/g soil. There was a single plant per pot and three replications. In the previous experiments, based on natural populations of AMF, plants were grown for 5 weeks and good colonisation was observed. As this experiment used sterilised soil with the AMF introduced, it was decided to grow the plants for 7 weeks. The experimental design was a completely randomized design with 3 replicates.

Measurements

Mycorrhizal assessment: The root samples were collected at harvest time in each of the three experiments. After carefully cleaning the root samples of soil, roots were cut into small segments and kept in an appropriate cassette, as per the method described by Vierheiling et al (1998). The cassette with roots then transferred to a 10% KOH solution for 4-5 days for clearing. After clearing, root samples were rinsed in Milli-Q water and neutralized with 1N HCl. The cleared root samples were then immersed in a 5% ink and vinegar solution and placed in a water bath at 90° C for staining. After 5-10 minutes the roots were removed from the water bath and washed with cold Milli-Q water prior

to being stored in a glycerol:water (50:50) solution. A light microscope was used to calculate the percentage of colonization, using the method described by McGonigle et al (1990). Root samples were dried in an oven at 80°C for four days and the root dry weight (RDW) measured.

Shoot phosphorus uptake: Shoot samples were dried in an oven at 80°C for four days and weighted to get shoot dry weight (SDW). Coarsely milled 0.5g of dry shoot samples were used to determined total P uptake by shoot. Dry shoot samples were left overnight by adding 7 mL of HNO_3 and the next morning the digestion was done on a digestion block at 140°C for 4-5 hours. At the end of the digestion the volume of each digest was made up to 20 mL by adding 2% acidified water (998mL water and 2mL $HNO_{3)}$ and filtered by using filter paper. Samples were kept at room temperature for P determination. The P concentration was measured as absorbance at 390 nm in a plate reader after one hour incubation period following the addition of 265µL H_2O, 10µL of sample and 25µL of colour reagent. The colour reagent was made by adding 1L concentrated Nitric acid, 1L 0.25% ammonium vanadate (2.5g NH_4VO_3/L) and 1L 5.0% ammonium molybdate (50g $(NH_4)_6MO_7O_{24}$/L).

Data analysis

Data were analysed by analysis of variance (ANOVA) using the GenStat (11^{th} edition) statistical program. Differences between means were assessed by using a least significant difference (LSD). As well as assessing the effects of variety and the variety x P interactions in the ANOVA, orthogonal (or single degree of freedom) contrasts were used to test whether there were significant differences between the responsive and non-responsive groups of varieties. Relationships between variables were also explored by simple linear correlations.

Results

Experiment 1: Preliminary experiment

A significant variety x P interaction for root colonisation by AMF was observed (Figure 4.1a). At high P and nil P the two varieties had similar colonization, but at low P roots of Carazinho (21%) had twice the colonisation of Wyalkatchem (11%).

Tillering was increased by the addition of P and the response varied with the genotype. At 0 kg P/ha and 3 kg P/ha, the number of tillers did not differ significantly between Wyalkatchem and Carazinho (1.7 (Wyalkatchem) cf 1.7 (Carazinho) tillers/plant at 0kg P/ha and 3.2 cf 2.9 tillers/plant at 3 kg P/ha), but at the high P rate Wyalkatchem produced significantly more tillers than Carazinho (7.4 cf 4.8 tillers/plant). Adding P increased shoot dry weight and Carazinho produced a greater shoot dry weight than Wyalkatchem in all P treatments (Figure 4.1b). Both genotypes responded similarly to P. The two varieties did not differ in their root dry weight but the root dry weight increased with the increment of P from (0.22 g/plant at 0kg P/ha to 0.73g/plant at 30kg P/ha).

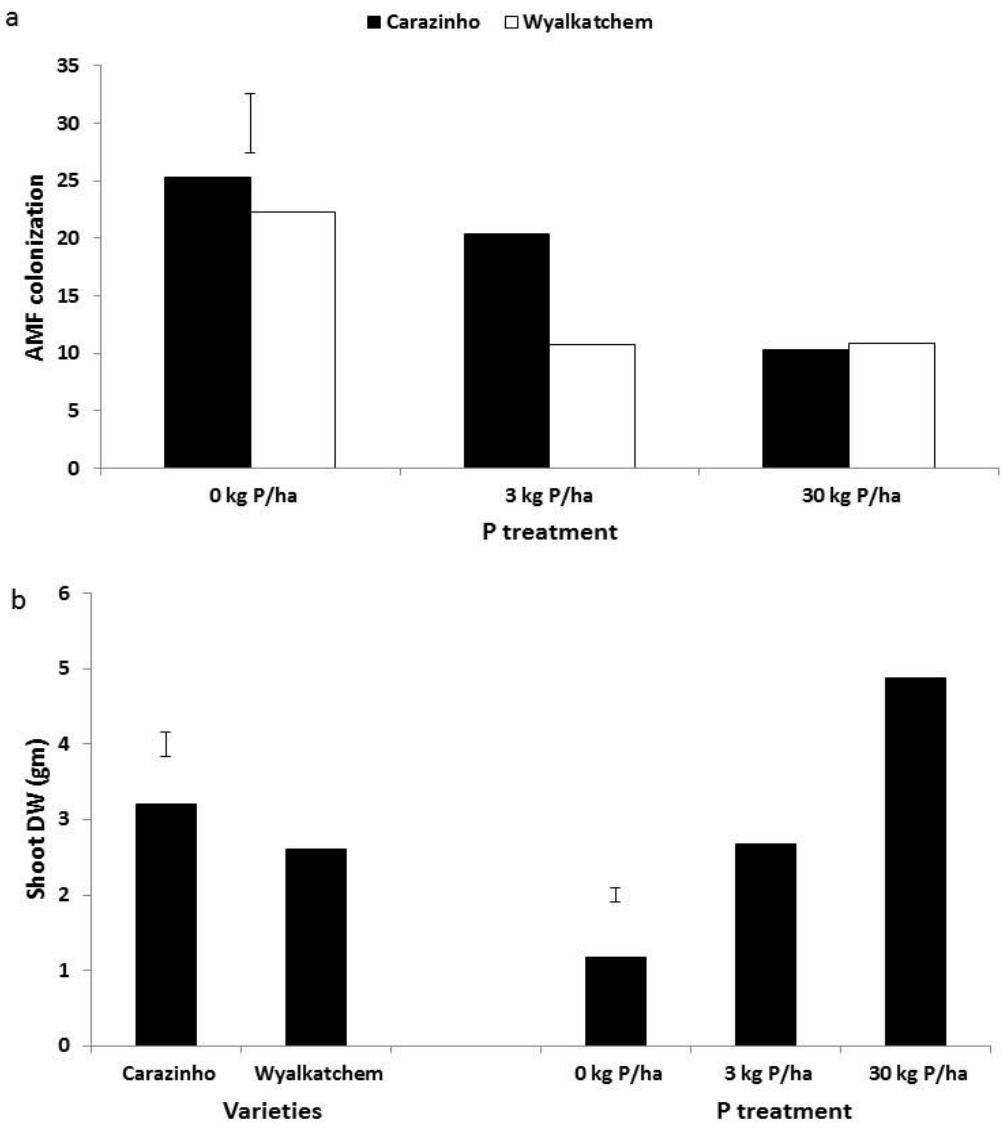

Figure 4.1. (a) Arbuscular mycorrhizal AMF colonization of two wheat varieties at three different P treatments. Error bar represents LSD value. (b) Shoot dry weight (five plants) of two wheat varieties and at three different P treatments. Error bar represents LSD for genotypes and for P treatment.

Experiment 2a: Root box

There were significant differences among the genotypes for all the traits measured and a significant variety x P treatment interaction was observed for AMF colonization, shoot

dry weight, crown root number, tiller number and P uptake (Table 4. 1). While there were significant genotype effects on AMF colonisation, the non-responsive and responsive groups of varieties did not differ significantly, nor did they show significant differences in their response to P (Table 4.1). Highest colonisation at nil P occurred with the nonresponsive varieties Trintecenco (31.0%), Carazinho (26.0%) and Axe (25.0%) and the lowest colonisation occurred with the non-responsive varieties Correll (11.5%) and Gladius (16.6%) (Figure 4.2). Colonisation decreased with increased P rate, except in Carazinho and Correll, where the highest infection occurred at 3 kg P/ha. For the remaining varieties, there was either no significant difference in infection between 0 kg P/ha and 3 kg P/ha or a significant decrease at the higher rate. The lowest colonisation occurred at the highest P rate with the responsive varieties BT Schomburgk and Krichauff showing a sharp decrease of colonization when P was added. Colonisation in Wyalkatchem did not differ among P treatments, although it had a relatively low colonization at 0 kg P/ha (17%).

A significant variety x P interaction was observed for shoot dry weight (Table 4. 1). All the varieties showed increased shoot dry weight with added P (Table 4.2). As a group the responsive genotypes differed significantly from the non-responsive genotypes (Table 4.1) in their shoot dry weight, but there was no difference in their response to P. Overall the non-responsive varieties had a higher shoot dry weight than the responsive varieties (the mean shoot dry weight of non-responsive group was 678 mg/plant and for responsive group was 608 mg/plant); the highest value was produced by the non-responsive variety Gladius (767 mg/plant) and lowest was produced by the responsive variety Wyalkatchem (504 mg/plant).

Table 4.1. Summary ANOVA of Experiment 2a, showing mean squares (m.s.) and degree of freedom (df).Significance is shown as: * - P<0.05; ** - P<0.01; *** P <0.001

	df	AMF (%)	SDW (mg/plant)	RDW (mg/plant)	Crown root no.	Tiller no.	Shoot P uptake (mg P/plant)	P conc. (mg P/g DM)
					m.s.			
Variety	9	249.8***	56923***	14403*	27.9***	4.8***	1.6***	0.87***
Nonresponsive vs Responsive	1	15.1_{NS}	94679**	39424*	11.0_{NS}	9.6***	1.1**	0.22_{NS}
P treatment	2	702.2***	6075613***	1050954***	1166.9***	171.2***	111.3***	30.02***
Variety*P treatment	18	32.7***	18496*	8469_{NS}	8.2*	0.9**	0.61***	0.16_{NS}
(Nonresponsive vs Responsive)*P	2	8.2_{NS}	27675_{NS}	26984*	0.08_{NS}	1.01_{NS}	0.68**	0.03_{NS}
Residual	58	9.1	8897	6698	3.9	0.4	0.11	0.09

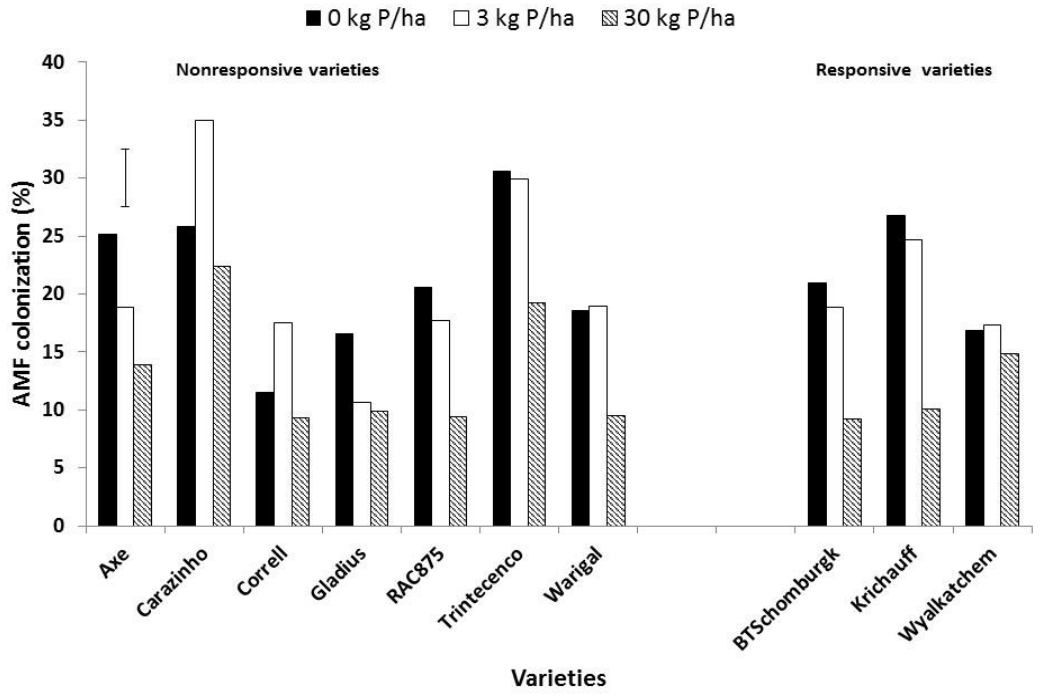

Figure 4.2. Mycorrhizal colonization of non-responsive and responsive wheat varieties from experiment 2a at three different P treatments. Error bar represents LSD value

There was significant variation among varieties for root dry weight and the nonresponsive and responsive varieties differed significantly in their response to P (Table 4.1). Root dry weight of the responsive genotypes responded more to the high P treatment than the non-responsive genotypes (Table 4.2). At the two lowest P rates, there was no significant difference between the two groups, but at 30 kg P/ha the responsive varieties produced more root growth than the non-responsive varieties.

Table 4.2. Experiment 2: Shoot dry weight and root dry weight of seedling of ten wheat varieties grown at three rates of P. The varieties were either considered to be non-responsive or responsive to P fertiliser based on yield responses in the field. Means for each group are shown as mean ± standard error of mean. (* P<0.05; ** P<0.01 and *** P<0.001, NS= non-significant)

	Shoot dry weight (mg/plant)			Root dry weight (mg/plant)		
	0_P	3 kg P/ha	30 kg P/ha	0_P	3 kg P/ha	30 kg P/ha
Nonresponsive						
Axe	250	560	1087	155	315	519
Carazinho	230	640	1133	114	268	403
Correll	170	685	1170	52	252	431
Gladius	239	812	1252	108	358	405
RAC875	188	682	1228	77	243	456
Trintecenco	250	633	913	146	426	454
Warigal	199	637	1290	84	204	456
Mean	218±12.1	664±29.2	1153±48.0	105±14.1	295±28.9	446±14.9
Responsive						
BTSchomburgk	300	712	1204	95	236	609
Krichauff	207	533	1000	139	346	560
Wyalkatchem	163	440	910	113	319	534
Mean	223±40.4	562±79.8	1038±87.0	116±12.8	300±33.1	568±22.0
LSD						
Variety		89.01***			77.23*	
Treatment		48.8***			42.3***	
Variety*Treatment		154.2*			133.76_{NS}	
CV (%)		14.4%			27.7%	

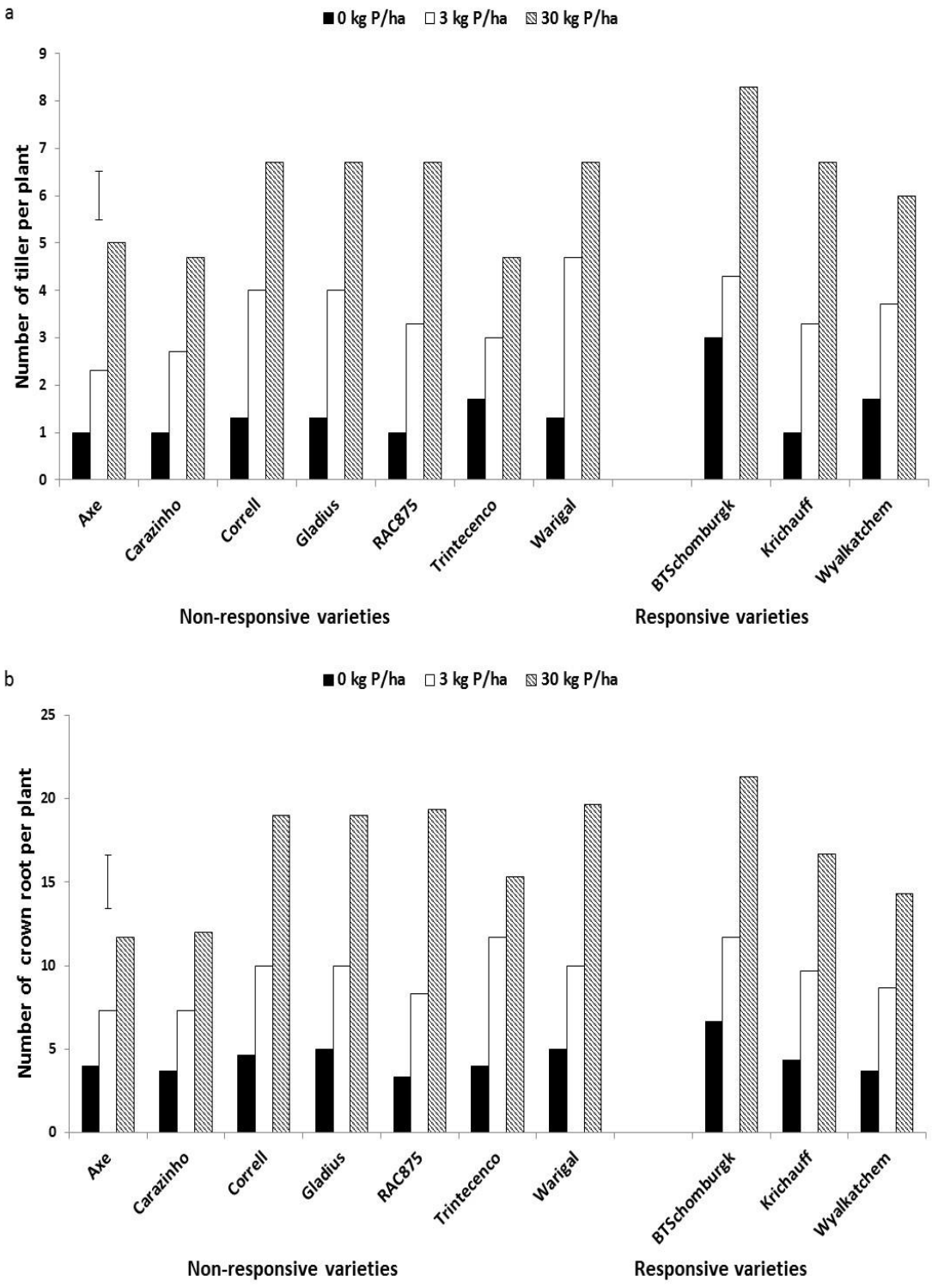

Figure 4.3. Experiment 2a: (a) Number of tillers of ten wheat varieties at harvest time from Experiment 2 at three different P treatments and (b) Crown root number per plant of ten wheat varieties at three P treatments from experiment 2. Error bar represents LSD value (P=0.05).

A significant variety x P interaction was observed for tiller number per plant but there was no consistent different between the responsive and non-responsive genotypes (Table 4.1). The severe P deficiency at 0kg P/ha resulted in no significant difference in tiller number among genotypes while in the low and high P treatments Axe, Carazinho and Trintecenco produced fewer tillers than the remaining genotypes (Figure 4.3a). A significant variety x P interaction was also observed for crown root number per plant (Table 4.1) and there was no consistent difference between the responsive and non-responsive genotypes. The response to P in crown roots number among the genotypes mirrored that observed for tiller number (Figure 4.3b).

Significant variety and P treatment effects were observed for shoot P concentration and variety x P interaction was observed for shoot P uptake (Table 4.1). Shoot phosphorus concentration increased with P rate and both the mean values for the responsive and non-responsive groups were the same. The responsive variety BT Schomburgk had the highest shoot P concentration. There was no difference in total shoot P uptake at 0kg P/ha and differences among varieties only became apparent as P rate increased (Table 4.3). The non-responsive group tended to accumulate more P than the P-responsive group as the P rate increased, but there was some variation in shoot P uptake among varieties within each group. Several correlations were observed among the AMF colonization and other traits for Experiment 2 but they differed with P rate (Table 4.4). At 0kg P/ha and 3 kg P/ha there was no association between AMF colonisation and growth, shoot P concentration and P uptake. A negative correlation of AM fungal colonization with shoot P uptake and P concentration was observed only at high P (30kg P/ha) treatment. Shoot P concentration and shoot dry weight were also positively correlated at nil P and 30 kg P/ha.

Table 4.3. Experiment 2: Shoot P concentration and P uptake of seedling of ten wheat varieties grown at three rates of P. The varieties were either considered to be non-responsive or responsive to P fertiliser based on yield responses in the field. Means for each group are shown as mean ± standard error of mean. (* P<0.05; ** P<0.01 and *** P<0.001, NS= non-significant)

	Shoot P con. (mg P/g DM)			Shoot P uptake(mg P/plant)		
	0 kg P/ha	3 kg P/ha	30 kg P/ha	0 kg P/ha	3 kg P/ha	30 kg P/ha
Nonresponsive						
Axe	1.8	2.2	3.3	0.4	1.3	3.6
Carazinho	1.6	2.3	3.1	0.4	1.4	3.5
Correll	1.8	2.4	4.0	0.4	1.7	4.6
Gladius	2.0	2.5	4.3	0.5	2.0	5.4
RAC875	1.5	2.5	4.0	0.3	1.7	4.8
Trintecenco	1.5	2.1	3.4	0.4	1.3	3.1
Warigal	2.0	2.8	4.2	0.4	1.8	5.4
Mean	1.7±0.08	2.4±0.09	3.8±0.18	0.4±0.02	1.6±0.10	4.3±0.36
Responsive						
BTSchomburgk	2.6	2.6	4.5	0.8	1.9	4.8
Krichauff	1.8	2.5	3.6	0.4	1.4	3.6
Wyalkatchem	1.3	2.5	3.3	0.2	1.1	3.0
Mean	1.9±0.38	2.5±0.03	3.8±0.36	0.5±0.18	1.5±0.23	3.8±0.53
LSD						
Variety	0.29***			0.31***		
Treatment	0.16***			0.17***		
Variety*Treatment	0.5_{NS}			0.5***		
CV(%)	11.5%			16.0%		

Table 4.4. Correlations among AMF colonization and other root traits in Experiment 2 at three different P treatments P (* P<0.05; ** P<0.01 and *** P<0.001).

Nil P							
Root trait	AMF	SDW	RDW	Crown root no	Tiller no	Shoot P uptake	Shoot P con.
AMF							
SDW	0.185						
RDW	0.541**	0.473**					
Crown root no	-0.169	0.695***	0.151				
Tiller no	-0.176	0.532**	-0.063	0.498**			
Shoot P uptake	0.014	0.871***	0.214	0.761***	0.647***		
Shoot P con.	-0.114	0.554**	-0.056	0.606***	0.533**	0.881***	
Low P							
AMF							
SDW	-0.177						
RDW	0.168	0.275					
Crown root no	-0.009	0.469**	0.396*				
Tiller no	-0.443*	0.458*	-0.053	0.563**			
Shoot P uptake	-0.297	0.846***	-0.017	0.388*	0.545**		
Shoot P con.	-0.202	-0.235	-0.530**	-0.139	0.199	0.309	
High P							
AMF							
SDW	-0.349						
RDW	-0.122	0.192					
Crown root no	-0.561**	0.496**	0.289				
Tiller no	-0.613***	0.499**	0.368*	0.716***			
Shoot P uptake	-0.584***	0.834***	0.160	0.606***	0.636***		
Shoot P con.	-0.632***	0.422*	0.067	0.531**	0.562**	0.851***	

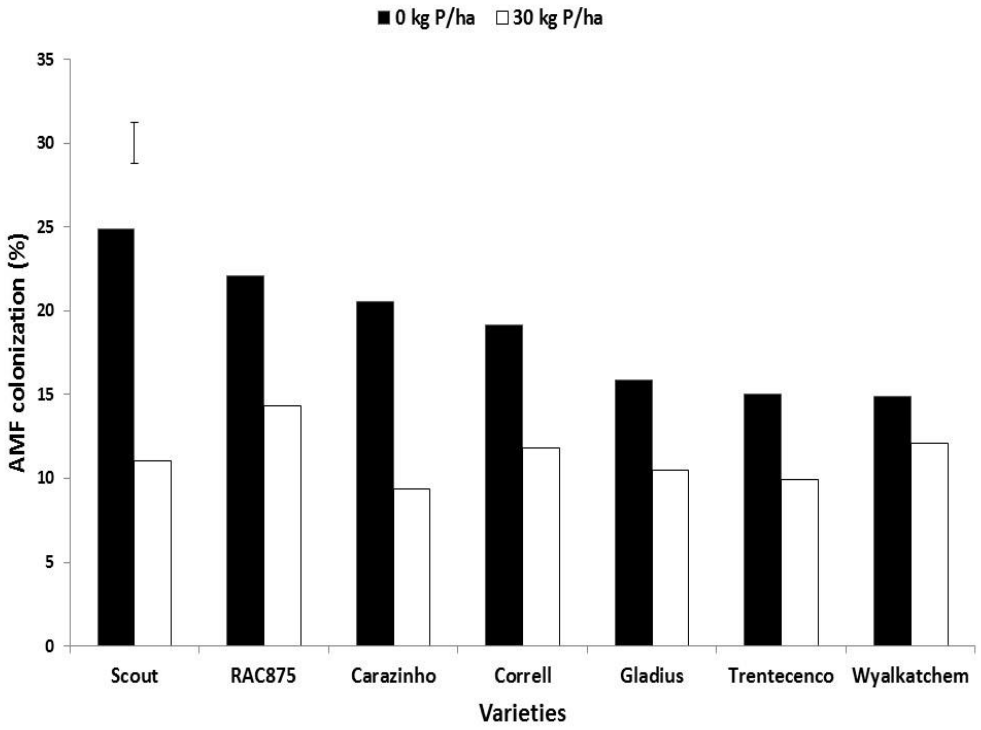

Figure 4.4. Mycorrhizal colonization of seven wheat varieties at two different P treatments from field. Error bar represents LSD value.

Experiment 2b: Field assessment

Significant variety x Phosphorus interaction was observed for AMF infection. Significant differences in colonization occurred when no P was applied (Figure 4.4). The P responsive variety Scout exhibited the highest (25%) colonization while the Brazilian variety Trintecenco and the variety Wyalkatchem had the lowest (15%). At 30 kg P/ha, colonisation was reduced and there was little difference in colonisation among varieties. Single degree of freedom contrasts suggested that there was no significant difference between the non-responsive and responsive varieties. There was no significant difference among genotypes for SDW/plant but seedling biomass

responded significantly to the P treatment and higher SDW was observed at high P (740 mg/plant) compared to no P (500 mg/plant).

The percentage of root colonization of plants from the field experiment decreased with P supply which was similar to the root box experiment. There was no significant relationship in the proportion of root colonisation between the root box and field experiments among the six varieties common to both experiments, in either the nil P treatment (correlation coefficient, r= 0.07) or the high P treatment (r= 0.70).

Experiment 3: Pot trial

Despite growing the seedlings under the same temperature and light conditions and for 2 weeks longer than the previous experiments the degree of colonization was much lower, with maximum colonisation of only 8.0%. However, significant treatment effects were evident (Table 4.5). Examination of the roots under the microscope showed that hyphae were present on the root surface but there was little penetration of the roots, suggesting the experiment was harvested too early to assess colonisation. Nevertheless there was still genotypic differences in infection that were consistent with results from the previous experiments and a significant variety x phosphorus interaction was observed for AMF colonization (Table 4.5, Figure 4.5a). The non-responsive variety Carazinho showed the highest (8.0%) colonization at nil P treatment while the non-responsive variety Warigal had the lowest (1.8%) and at high P treatment the non-responsive varieties Trintecenco had high colonization (6.7%), while the responsive varieties BT Schomburgk had lowest (0.4%). On average the non-responsive genotypes had a significantly higher level of colonisation than the responsive group (Figure 4.5b). Even at this early stage of infection AMF colonization was higher when plants were

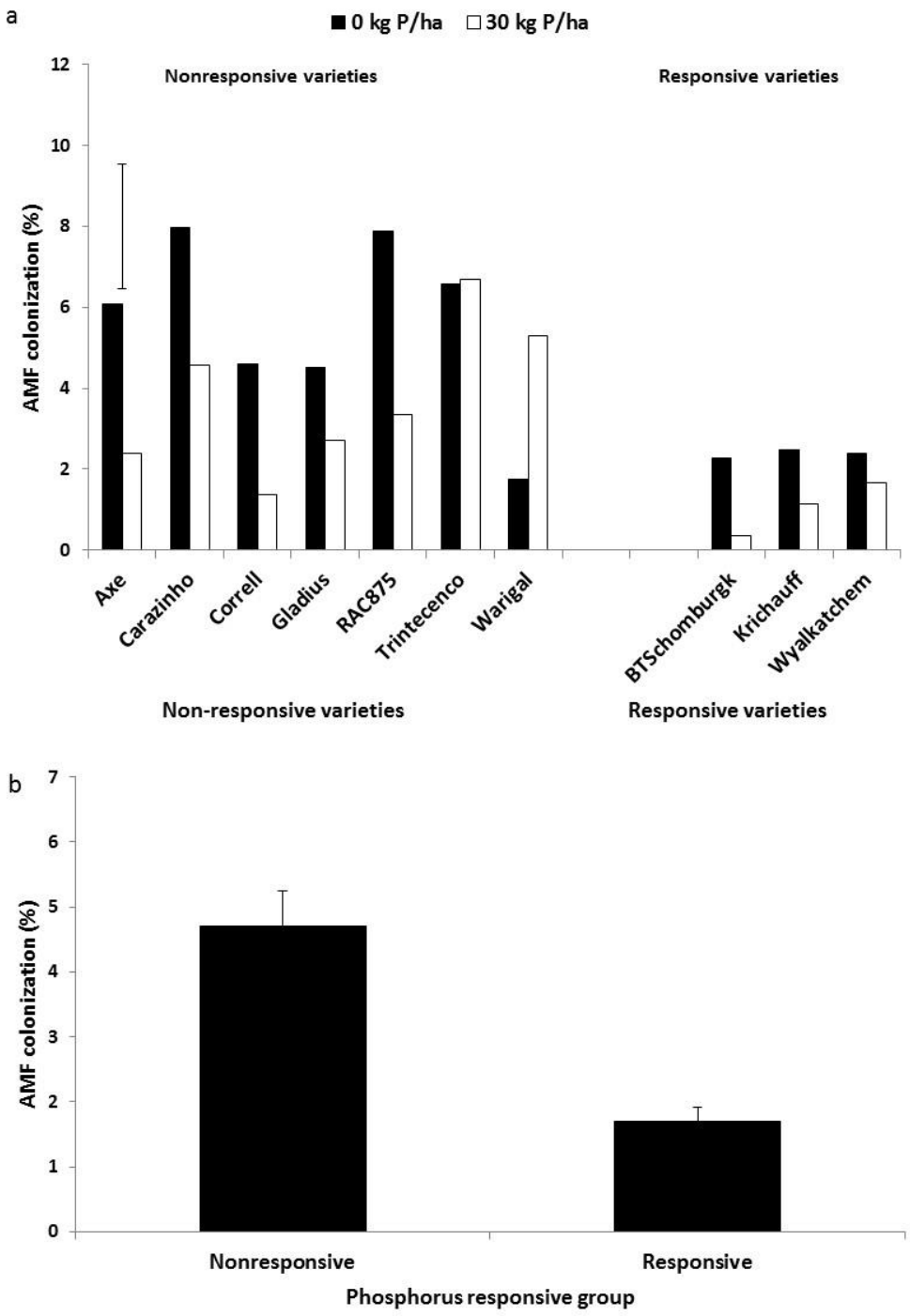

Figure 4.5. (a) Mycorrhizal colonization of ten wheat varieties at two different P treatments from experiment 4. Error bar represents the LSD value. (b) The difference of mycorrhizal colonization between the two groups of wheat varieties. Error bar represents the standard error of mean.

Table 4.5. Summary ANOVA of experiment 3 showing mean squares (m.s.) and degree of freedom (df).Significance is shown as: * - P<0.05; ** - P<0.01; *** P <0.001

	df	AMF	SDW	RDW	Crown root	Tiller no.	Shoot P uptake	Shoot P conc.
					m.s.			
Variety	9	21.14***	0.81***	0.08***	53.1***	33.9***	8.3***	1.5***
Nonresponsive vs Responsive	1	112.18***	3.3***	0.002 $_{NS}$	170.8***	86.2***	15.8***	2.4***
P treatment	1	42.77***	105.32***	12.6***	10868.03***	1526.5***	1647.8***	12.6***
VAM treatment	1		0.57**	0.08*	120.0***	9.6*	2.8 $_{NS}$	0.1 $_{NS}$
Variety*P treatment	9	8.18*	0.19**	0.04**	51.9***	18.6***	3.4**	0.2 $_{NS}$
(Nonresponsive vs Responsive)*P	1	0.95	0.11 $_{NS}$	0.04 $_{NS}$	144.8***	53.2***	2.6 $_{NS}$	0.5 $_{NS}$
Variety*VAM treatment	9		0.02 $_{NS}$	0.01 $_{NS}$	9.3 $_{NS}$	2.6$_{NS}$	0.8 $_{NS}$	0.3 $_{NS}$
(Nonresponsive vs Responsive)*VAM	1		0.04 $_{NS}$	0.02 $_{NS}$	3.2 $_{NS}$	0.4 $_{NS}$	0.11 $_{NS}$	0.5 $_{NS}$
P treatment*VAM treatment	1		0.6**	0.02 $_{NS}$	64.5 $_{NS}$	0.3 $_{NS}$	1.9 $_{NS}$	0.3 $_{NS}$
Variety*P treatment*VAM treatment	9		0.08 $_{NS}$	0.03 $_{NS}$	8.7 $_{NS}$	2.6 $_{NS}$	0.4 $_{NS}$	0.2 $_{NS}$
(Nonresponsive vs Responsive)*P *VAM	1		0.02 $_{NS}$	0.01 $_{NS}$	1.7 $_{NS}$	0.9 $_{NS}$	0.03 $_{NS}$	0.08 $_{NS}$
Residual	78	3.46	0.07	0.01	10.7	1.9	1.04	0.2

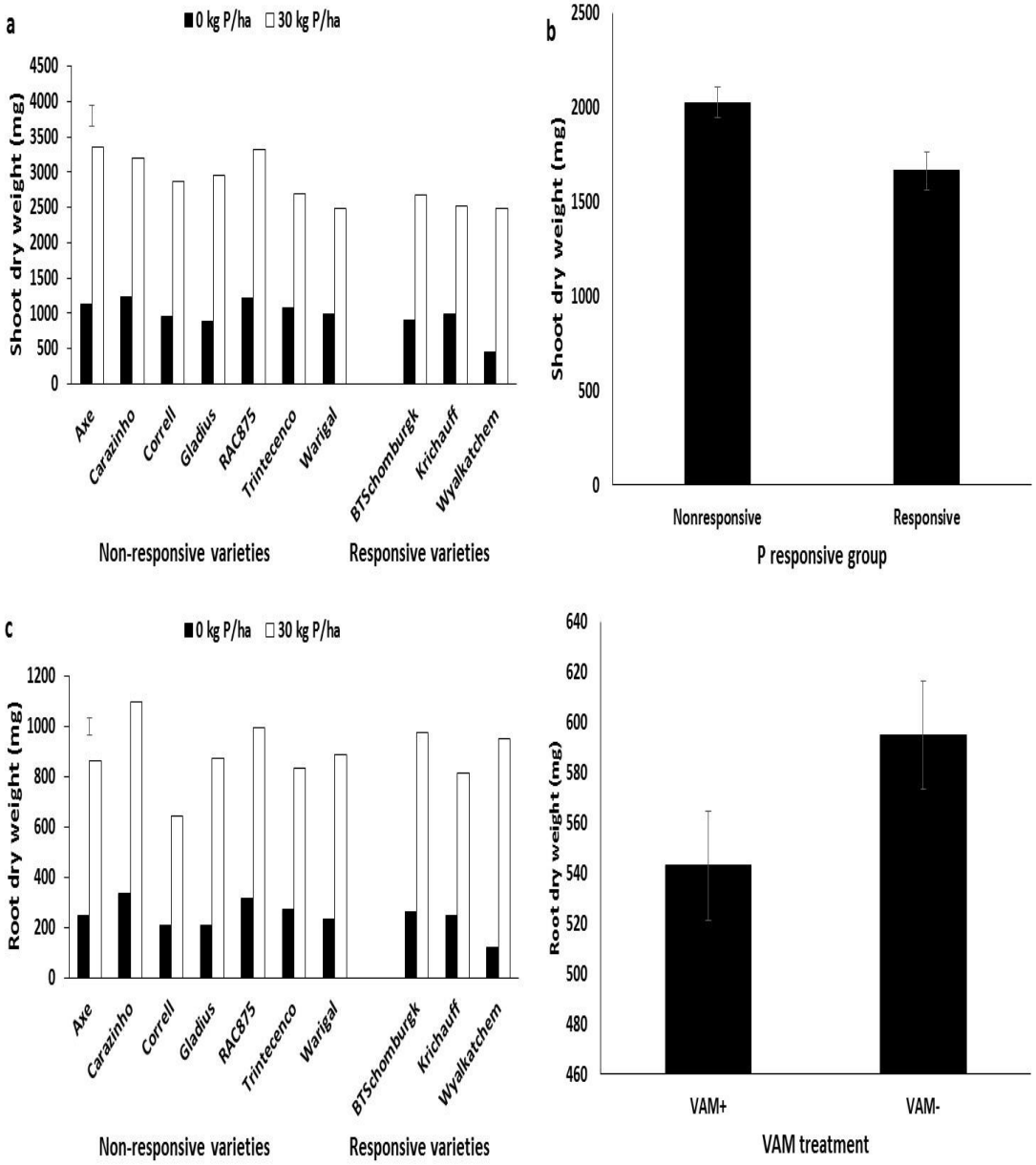

Figure 4.6. (a) Shoot dry weight of ten wheat varieties at two different P treatments from experiment 4. Error bar represents standard error of mean. (b) Shoot dry weight at two different inoculation treatments. Error bar represents LSD value. (c) Root dry weight of ten wheat varieties at two different P treatments from experiment 4. Error bar represents LSD value. (d) Root dry weight at two different inoculation treatments. Error bar represents LSD value.

grown without P compared to the high P treatment. No group × P treatment interaction was observed. The non-inoculated root samples were randomly checked and no colonization was observed in any sample. A significant variety ×phosphorus interaction was observed for SDW (Figure 4.6 a,b). The growth of the inoculated plants was slightly but significantly lower than the non-inoculated plants (1850 mg/plant cf 2000 mg/plant). Single degree of contrast results suggests that the non-responsive varieties produced more SDW (2025 mg/plant) than the responsive varieties (1665 mg/plant), but no group × P interaction was observed.

Significant variety × P interaction was also observed for RDW (Figure 4.6 c, d), but the non-responsive group did not differ from the responsive group. Plants grown without mycorrhizal inoculum produced significantly more (about 10%) RDW (595 mg) than inoculated plants (543 mg). A significant variety × P interaction was observed for tiller number (Figure 4.7 a, b) and the P-responsive group showed a larger increase in tiller number with P than the non-responsive group. The non-responsive variety Warigal produced the highest number of tillers per plant (10 tillers/plant) and the lowest number of tillers was produced by the non-responsive variety Trintecenco (4 tiller/plant). Tiller number was significantly higher (11 tillers/plant) in the high P treatment compared to 0P treatment (3 tillers/plant). A similar trend was observed in the number of crown roots. (Figure 4.7 c, d). Overall the responsive variety BT Schomburgk produced the highest number of crown roots per plant (25/plant) and the non-responsive variety Gladius produced the lowest number (19 /plant). At the high P treatment plants produced more crown roots (31 crown root/plant) compared to 0 P (12 crown root/plant).

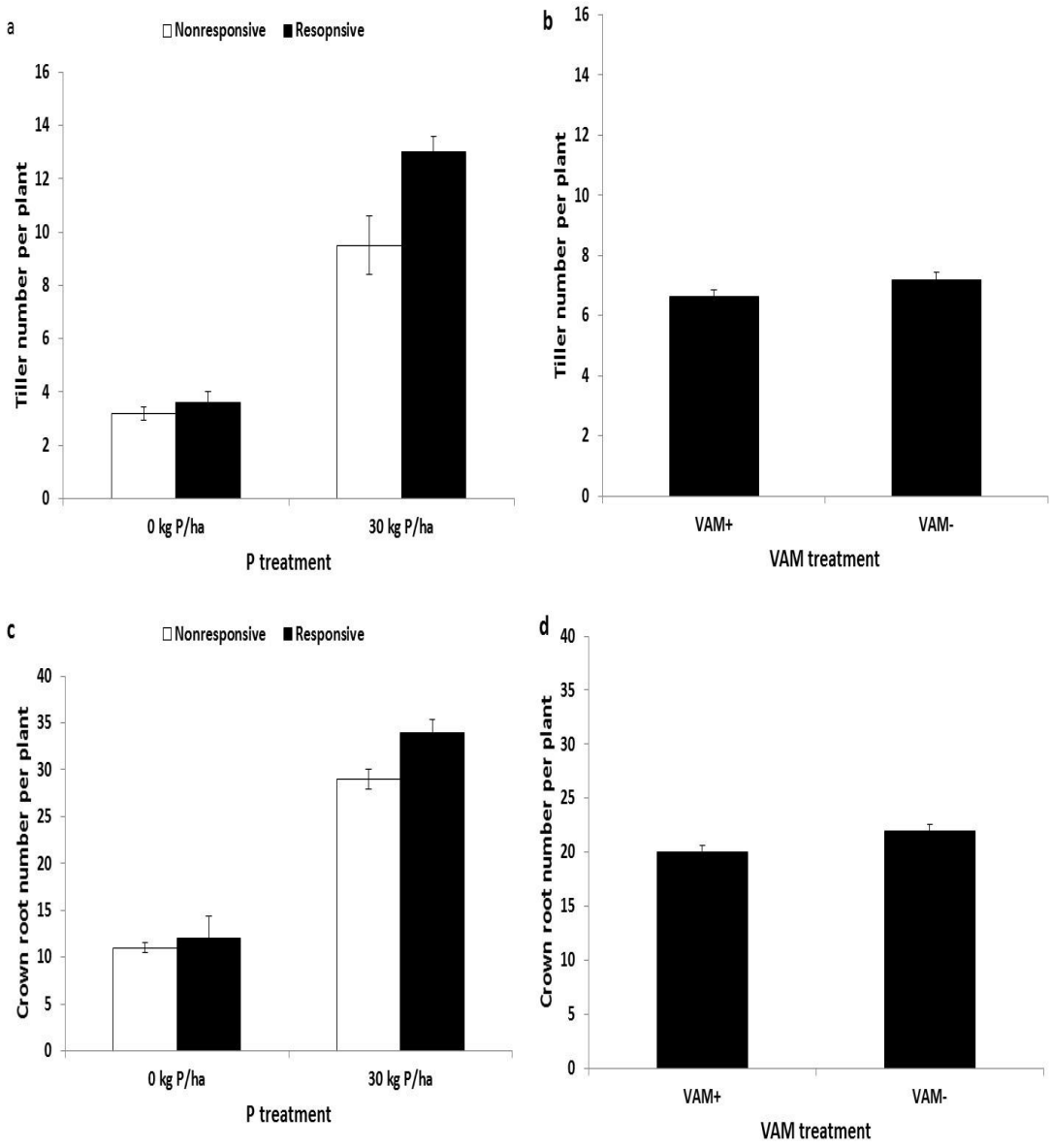

Figure 4.7. (a) Number of tillers of two groups of wheat varieties at two different P treatments. Error bar represents standard error of mean. (b) Number of tillers at two inoculation treatments. Error bar represents LSD value. (c) Number of crown root of two groups of wheat varieties at two different P treatments. Error bar represents standard error of mean. (d) Number of crown root at two inoculation treatments. Error bar represents LSD value.

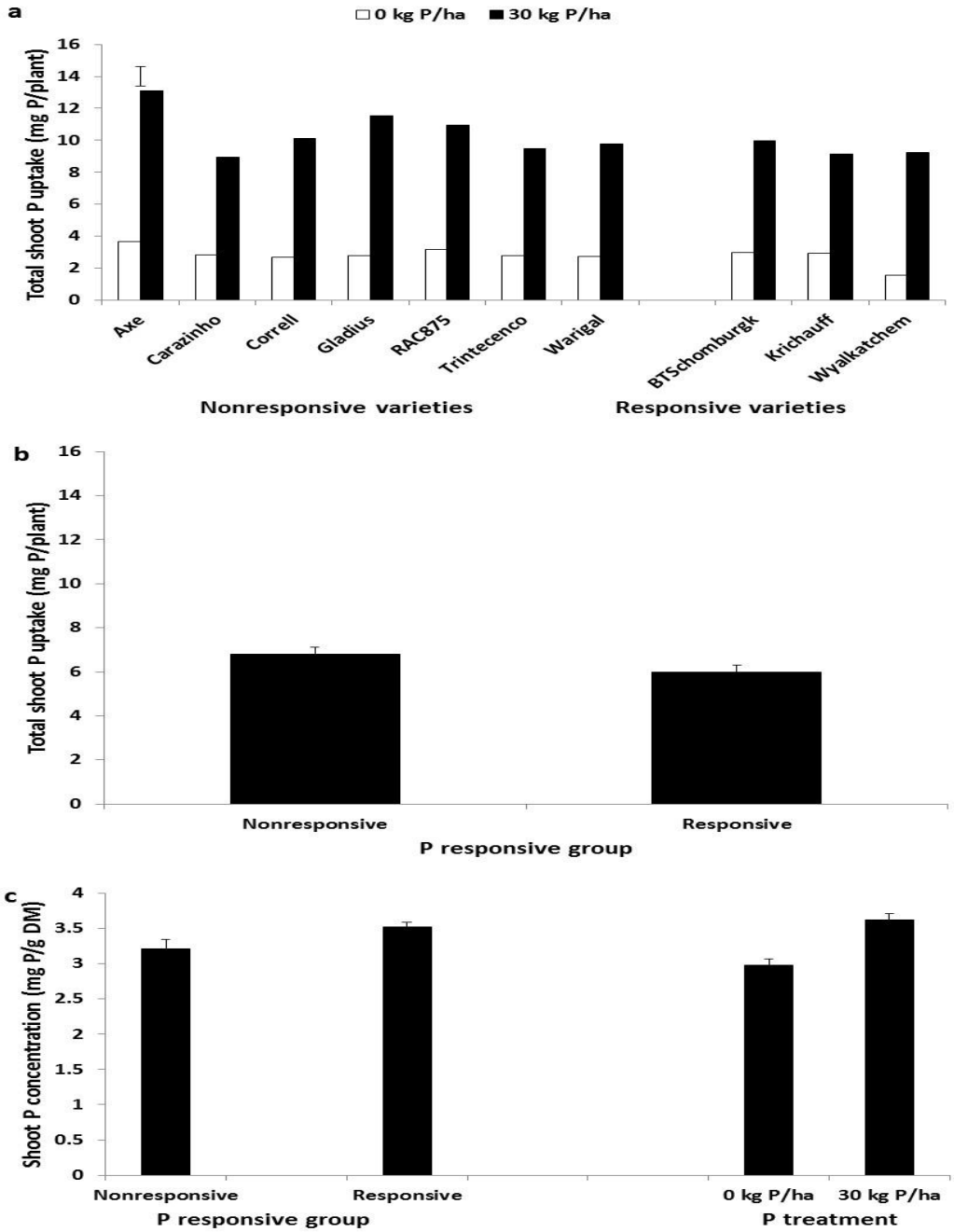

Figure 4.8. (a) Total shoot P uptake (mg P/plant) of ten wheat varieties at two different P treatments from experiment 4. Error bar represents LSD value. (b) The difference between two groups of wheat varieties in their total P uptake. Error bar represent represents standard error of mean. (c) Shoot phosphorus concentration of two groups of wheat varieties and at two different P treatments. Error bar represents standard error of mean for the group and LSD value for the P treatment.

Significant variety × P treatment effects were observed for total shoot P uptake (Figure 4.8a) and significant differences was observed for shoot P concentration between the two groups of varieties (Figure 4.8 c). Shoot phosphorus uptake of non-responsive varieties was significantly higher (6.8 mg P/plant) than the responsive varieties (6.0 mg P/plant), while mean P concentration of the responsive varieties were significantly higher (3.5 mg P/g DM) than that of the non-responsive varieties (3.2 mg P/g DM). No significant effect of inoculum treatment effect was observed.

Several positive correlations were observed in this experiment (Table 4.6) and the correlation table was arranged according to P treatment and the mycorrhizal treatment. Without additional P and without AMF, shoot P uptake was positively correlated with shoot dry weight but not P concentration. Phosphorus concentration was negatively correlated with shoot dry weight and root dry weight. At 30kg P/ha P uptake was positively correlated with shoot dry weight and with P concentration. Similar to nil P treatment P concentration was negatively correlated with shoot dry weight at 30 kg P/ha treatment.

When inoculated with AMF and with no added P, mycorrhizal colonization was positively correlated with shoot dry weight and negatively correlated with shoot P concentration. Shoot phosphorus uptake was not associated with P concentration, but only with shoot dry matter when no P was added. P uptake was correlated with both shoot dry matter and P concentration when plants were grown with AMF and added P. Shoot dry weight was negatively correlated with P concentration in both P treatments.

Table 4.6. Correlation among AM colonization and other root trait for Experiment 3. Below the diagonal is correlation at nil P treatment and above the diagonal is the correlation at high P (* P<0.05; ** P<0.01 and *** P<0.001).

VAM-						
Root trait	SDW	RDW	Crown root no	Tiller no	Shoot P uptake	Shoot P con.
SDW		0.264	-0.214	-0.309	0.429*	-0.477**
RDW	0.909***		-0.002	0.120	0.155	-0.099
Crown root no	0.726***	0.772***		0.567**	-0.134	0.091
Tiller no	0.289	0.427*	0.526**		-0.184	0.092
Shoot P uptake	0.882***	0.859***	0.793***	0.457**		0.575***
Shoot P con.	-0.548**	-0.396*	-0.131	0.187	-0.102	

VAM+							
Root trait	AMF	SDW	RDW	Crown root no	Tiller no	Shoot P uptake	Shoot P con.
AMF		0.057	0.229	-0.283	-0.302	-0.104	-0.186
SDW	0.517**		0.567**	-0.279	-0.355	0.617**	-0.458*
RDW	0.397*	0.837***		0.146	0.064	0.179	-0.467*
Crown root no	0.188	0.609***	0.654***		0.801***	-0.154	0.147
Tiller no	-0.466*	0.315	0.303	0.367*		-0.263	0.121
Shoot P uptake	0.220	0.752***	0.463*	0.391*	0.193		0.404*
Shoot P con.	-0.455*	-0.581***	-0.692***	-0.528**	-0.256	0.056	

Discussion

It is widely considered that the AMF colonization of wheat is generally low (10-30 %) (Li et al. 2005a; Mäder et al. 2000), but the degree of colonization can vary considerably. For example, Li et al (2005b) observed high colonization (up to 80%) of wheat from soil of Eyre Peninsula, South Australia where P availability was low. In the present study moderate colonization (10-35%) was observed from both controlled and field trials, which is consistent to the observed colonization of wheat from the literature. In the pilot experiment the non-responsive variety Carazinho showed significant greater colonisation than the responsive variety Wyalkatchem at a low rate of P fertiliser (3 kgP/ha), but not in the nil P treatment. It has been demonstrated by Grant et al (2005) that mycorrhizal colonization and spore germination can be restricted at very low available soil P. In a study with sunflower, very poor colonization was observed when plants were grown in a P-free sand medium and the colonization was increased with added P (Koide and Li 1990). Another study by Abbott et al (1984) recorded highest AMF colonization in the standard organic system in which recommended dose of P fertilizers was added. Prasad et al (2012) mentioned that some amount of P is required for growth of AM fungal strains. Based on the findings of this study and other studies from literature it can be concluded that at very deficient condition AMF will not be beneficial for plants growth.

Genetic variation for percentage of colonization was observed in Experiment 2a but it was not possible to relate that with P responsiveness of wheat varieties. The non-responsive varieties Carazinho and Trintecenco showed high colonization at all P treatments. In a study with three wheat genotypes with different P efficiency no difference in root colonization by mycorrhizal fungi was observed by Yao et al (2001). Another study with wheat also failed to identify varietal difference for AMF

colonization and also no clear relationship of AMF with nutrient concentration under low input system was observed (Hildermann et al. 2010). The results of field study also showed no consistent difference between P responsiveness and mycorrhizal colonization of wheat varieties of this present study. The non-responsive variety Trintecenco failed to maintained high colonization under field conditions. In contrast, the two non-responsive varieties Carazinho and RAC875 maintained high AMF colonization especially when no P was added and it was similar to Experiment 2a. There were no correlation between Experiment 2a and field study of AMF colonization at 0 kg P/ha treatment and at 30 kg P/ha treatment varieties which had higher colonization in controlled environment showed reduced colonization at field condition (Appendix 7). This suggest large environmental influence on AMF colonization and results were not reproducible.

In Experiment 3 a low mycorrhizal colonization was observed and our assumption for this result is the root samples were harvested early, despite allowing the plants to grow for another two weeks compared to previous experiments. The germination of spores and hyphal development into the root samples were investigated and plenty of spore germination was observed and it looked as if they did not have enough time to penetrate plant roots before plants were harvested. Some hyphal attachment to the root was also observed but no internal arbuscules were seen. This leads to the conclusion that for inoculated treatment, the fungi need more time to establish symbiosis than in experiments which relied on naturally-occurring fungi.

Although the degree of colonization in experiment 3 was low, as a group the nonresponsive varieties had higher colonization over responsive varieties and the variation was clear when no P was added. In this experiment non-responsive varieties Carazinho and Trintecenco maintained similar percentage of colonization over both P

treatment, which is consistent with the findings of Experiment 2a. With added P the difference between the two P responsive groups was not obvious at low P treatment and the negative correlation of shoot P concentration with AMF colonization suggests that plant and soil P status has reduced the percent colonization. A similar result was observed by Ryan et al (2000) for dairy pasture species and they concluded that soil P level and host plant P concentration will be the major determinants for level of colonization by AMF fungi. Decreased colonization was observed with added P by several previous studies on wheat (Baon et al. 1992; Li et al. 2006; Li et al. 2005b; Mohammad et al. 2004). When soil available P level is high enough (>50 mg/kg, extractable with 0.5 M $NaHCO_3$) for plants to acquire P by their root system, plants will be less dependent on AMF and reduced root colonization will be observed (Bolan et al.1984; Jansa et al. 2009). The results from Experiment 3 also suggest that genetic differences in AMF colonisation are established very early in the infection process because these differences were measured before there was substantial penetration of the roots by the fungus.

In this study a positive correlation between SDW and AMF colonization was observed in Experiment 3 only for nil P treatment. In Experiment 2 no correlation between SDW and AMF colonization was observed. The positive correlation of AM fungi with shoot dry weight only at nil P treatment suggest that for this study the symbiotic relationship made no contribution to growth at high P treatment. In their study, Li et al (2006) observed that wheat responded negatively to AMF colonization at the early growth stage (6 weeks) and no positive contribution of AMF was observed in terms of plant growth and P uptake. No correlation of AMF with shoot P uptake or P concentration was observed in Experiment 2 when plants were grown at 0 kg P/ha or 3 kg P/ha, but a negative correlation was observed at 30 kg P/ha. A negative correlation with P

concentration was also observed for Experiment 3 at nil P treatment. A negative correlation between AMF with shoot P uptake and concentration was observed by other workers (Lu et al. 1994; Menge et al. 1978; Valentine et al. 2001). It is increasingly appreciated that while mycorrhizal symbiosis can be beneficial for plant it does not necessarily increase P uptake sufficiently for better yield (Grant et al. 2005). In a study by Rayan and Angus (2003) in Australia, neither field peas nor autumn sown wheat showed a benefit in terms of P uptake or yield from enhanced mycorrhizal colonization. Ryan and Ash (1999; 2000) reported that despite enhanced mycorrhizal colonization the level of P in biodynamic pastures was lower that of conventional fertilized pastures and also concluded that the P deficiency may have restricted the yield of the biodynamic system.

In conclusion substantial genetic variations for mycorrhizal colonization among wheat varieties were observed and it was not possible to relate that with varietal P responsiveness. Some individual varieties such as Carazinho and RAC875 showed consistently high colonization both from the controlled environment and field study and another non-responsive variety Trintecenco showed high colonization at controlled environment, but failed to maintain this under field condition. One of the responsive variety Wyalkatchem had consistently low colonization across a range of environment. Inconsistency in findings among experiments and lack of relationship of AMF with shoot dry weight, P uptake and P concentration leads to the conclusion that under deficient condition AMF colonization is not a contributing trait towards varietal P responsiveness.

Acknowledgement

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Chapter 5 : Genetic variation of root traits and exudation of citric and malic acid in wheat varieties

Abstract

The release of organic acids, such as malic and citric acid, by plant roots has been shown to improve phosphorus (P) uptake under certain conditions, and it has been suggested to be a target for genetic improvement in P efficiency. Ten wheat varieties differing in their yield response to P were characterized for their ability to exude organic acids. The aim was to relate the difference in organic acid exudation and the varietal P responsiveness. The concentration of malic and citric acid from rhizosheath soil was measured along with rhizosheath pH, root hair length and shoot and root dry weight in plants grown under low and high concentrations of soil P under controlled conditions. The non-responsive varieties differed significantly from the responsive varieties in their root hair length and rhizosheath size. Genetic variation was also observed for malic acid and citric acid concentration and rhizosheath pH, but there was no clear relationship between organic acid exudation and P responsiveness among the varieties.

Introduction

Phosphorus (P) availability is the primary constraint for plant production in many agricultural soils around the world (Ramaekers et al. 2010). The reactive nature of P in soil makes it poorly available (Lynch and Brown 2008) and P deficiency poses significant challenges for agricultural productivity. Rock phosphate, from which P fertiliser is derived, is a limited natural resource and some have estimated current

reserves will be depleted within the next 60-80 years. Recovery of P in crop production and P use efficiency is low and developing plant species with the ability to use soil P more efficiently could reduce the demand for rock P (Liu et al. 2004). One approach may be to select for varieties of wheat that are able to modify their root environment by releasing organic acids to improve the recovery of P (Gahoonia et al. 2000, Dakora and Phillips 2002, Vance et al. 2003, Gahoonia and Nielsen 2004).

Plant roots release a wide range of carbon (C) containing components which are collectively known as rhizodeposits. According to Jones et al (2009) plants release about 11% net of their photosynthetically-fixed C as rhizodeposits. Recent evidence suggests that root exudates are involved in a range of functions that modulate nutrient availability (Cakmak et al. 1998, Wang et al. 2008), improve tolerance of heavy metals (Osawa and Kojima 2006), or attract rhizobacteria (Bais et al. 2004). Under P deficiency, plants release H^+ into the soil which can lower the rhizosphere pH and it is one of the induced mechanisms of P deficiency (Li et al. 2011). Changes in rhizospheric pH varies among crops and thus the uptake of P also varies (Hedley et al. 1982). It has been reported that when the rhizospheric pH decreases, P uptake by plant roots increases (Holford and Patrick 1979, Van Ray and Van Diest 1979). Gill et al (1994) observed higher P uptake by wheat cultivars was significantly related with the drop of root medium pH. Apart from releasing H^+ plant roots are known to secrete low molecular weight organic acids such as citric acid, malic acid, oxalic acid, malonic acid and tartaric acid (Hinsinger 2001), which are considered to be important for P uptake, especially in P-fixing soil (Li et al. 2011). Among the organic acids exuded in the rhizosphere, citric acid, malic acid and oxalic acids are the most efficient in solubilizing soil P (Maseko and Dakora 2013). Citrate secretion of P deficient common bean was found to be effective in mobilizing P from Al-P and Fe-P compounds (Shen et al. 2002) and citric

acid is also the dominant organic acid exudate produced by legume species (Neumann and Römheld 1999). Enhanced organic anion efflux from roots of P-deficient rice (Kirk et al. 1999), barley (Gahoonia et al. 2000) and maize (Gaume et al. 2001b) has been reported. Malate secretion from the root tip and its chelation of Al provides a mechanism for Al tolerant among wheat cultivars in acid soil (Delhaize et al. 1993).

The composition and quantities of root exudates depends a number of factors including plant age and health, environmental conditions such as pH (Meharg and Killham 1990), soil type (Van Veen et al. 1985), oxygen status (Wiedenroth and Poskuta 1981), light intensity and soil temperature (Graham et al. 1982), nutrient availability (Kraffczyk et al. 1984) and presence of microorganisms (Meharg and Killham 1991), and they are also known to differ between plant species and even cultivars (Mimmo et al. 2011). In a study with P deficient rapeseed (*Brassica napus*), citric and malic acid exudation was 14 and 44 nmol $h^{-1}m^{-1}$ respectively (Hoffland et al. 1989). In the presence of Al^{3+} rates of root exudation from the root tip of Al-tolerant maize was 30 nmol citrate h^{-1} m^{-1} (Pellet et al. 1995) and 1300 nmol malate h^{-1} m^{-1} from the root tip of wheat (Delhaize et al. 1993). Rates of citrate exudation from proteoid roots of white lupin (*Lupinus albus*) plants ranged from 610 nmol citrate h^{-1} m^{-1} root (Keerthisinghe et al. 1998) to 670 nmol citrate h^{-1} m^{-1} root (Neumann et al. 1999) and up to 1400 nmol citrate h^{-1} m^{-1} root (Watt and Evans 1999).

Genetic differences in root exudation are well documented but the relationship between these differences in root exudation and P efficiency appears to be inconsistent. Many plant species such as white lupin (Johnson et al. 1996; Neumann et al. 1999), rice (Kirk et al. 1999), maize (Jones and Darrah 1995), alfalfa (*Medicago sativa*) (Lipton et al. 1987), pigeon pea (*Cajanus cajun*) (Otani et al. 1996) and chickpeas (*Cicer arietnum*) (Ohwaki and Hirata 1992) are known to release different organic acids when grown

under low P stress. Under P deficiency a rice variety (JX17) non-sensitive to low P stress showed higher root exudation (such as organic acids, acid phosphatase and H^+ exudation) over a P-sensitive genotype (ZYQ8) (Ming et al. 2002). An increased excretion of organic acid (malic, citric and succinic acids) was observed in maize, especially by a low P tolerant genotype at P deficiency (Gaume et al. 2001a). In contrast, other work with maize found no significant difference between P sufficient and P deficient plants in organic acid exudation during the early stage of P deficiency (Carvalhais et al. 2011). Few studies on the importance of root exudates to P efficiency have been done in wheat. Most work in wheat on the exudation of citric and malic acid has been associated with studies on Al resistance (Ryan et al 2009). Recently Ryan et al (2014), in one of the few field-based studies on citrate exudation, found that growth and yield of lines with high citrate exudation did not differ from lines with low citrate exudation or show a difference in the response to P. The inconsistent results from the literature on the importance of organic acid exudation to P efficiency and the smaller amount of knowledge about genetic variation in organic acid exudation under P deficiency in wheat emphasizes that more research is needed.

Although some previous work on wheat has described differences in citrate and malate exudation from wheat genotypes, much of this work has been to look at tolerance to Al^{3+} toxicity; there is not much information on how root exudation differs under P deficiency in non-acidic soils and how genetic differences in citric and malic acid exudation is reflected in differences in P responsiveness. The aim of the experiment was to understand the influence of root traits on organic acid exudation and its relationship with the P responsiveness of the wheat variety. In this study wheat varieties with known differences in P responsiveness in grain yield were selected to test that

hypothesis that responsiveness to P is associated with a difference in the concentrations of citric and malic acid concentration in the rhizosheath soil.

Materials and methods

Soil and plant materials

The experiments used soil that was collected from the 0-15cm layer from a field site at Halidon, South Australia naturally low in P. The soil is a loamy sand, with a Colwell P of 8 mg P/kg and pH 7. The soil was air dried and passed through a 2mm sieve before being used for the experiment.

Ten bread wheat genotypes differing in their yield response to P from a recent multisite field study in South Australia (McDonald et al. 2015) were selected for this study. These varieties were grown with and without P over three years and up to 3 sites per year and while there were effects of site and seasons on the P responsiveness, the selected varieties showed consistent responses to P among the experiments (Chapter 3). The varieties were classified as relatively responsive to applied P (Wyalkatchem, Krichauff and BT Schomburgk) and non-responsive to P (Axe, Carazinho, Correll, Gladius, RAC875, Trintecenco and Warigal) based on the overall consistency of the responses.

Growth conditions and measurements

Three experiments were conducted to examine differences in pH and organic acid exudation among a range of bread wheat that differed in their P responsiveness. In Experiment 1 only the pH of the rhizosheath soil was measured while two subsequent experiments (Experiments 2 and 3) were conducted to measure exudation of organic

acids. In Experiment 2, two wheat varieties Carazinho (non P-responsive) and Wyalkatchem (P-responsive) were selected and only malic acid concentration was measured, in Experiment 3, the 10 wheat varieties used in Experiment 1 were selected and malic and citric acid concentrations in the rhizosheath were measured. In all experiments plants were grown under in a controlled environment room at 20°/18°C day/night temperature and a 14/10 h photoperiod at a light intensity of 300-400 μmole quanta/m^2/sec PAR.

The seedlings were grown in white plastic pots 10.5cm long and 7.0 cm in diameter which contained 355 g of dry soil. Basal nutrient solutions delivered macro- and micronutrients to give final concentrations of 918 mg/kg soil Ca $(NO_3)_2.4H_2O$, 250 mg/kg K_2SO_4 and 150 mg/kg $MgSO_4$, 26 mg/kg $ZnSO_4.7H_2O$, 9 mg/kg $CuSO_4.5H_20$, 17 mg/kg $FeSO_4.7H_20$, 5 mg/kg $MnSO_4$ and 0.1 mg/kg $Na_2MoO_4.2H_2O$ and all pots were watered to 75% field capacity (10% w/w). Phosphorus at the designated concentrations was added as Ca $(H_2PO_4)_2.H_2O$ to the soil as a liquid mixed with the basal nutrient solution. There were two P treatments for both experiments (nil P= no added P and high P, equivalent to 30kg P/ha). In each pot two seeds were planted and seedlings were grown until the plants were two weeks old. No additional water was added to the pots during the growing period. A completely randomized block design was followed with three replications.

Measurement of pH

The growing condition of plants to measure rhizosheath pH was similar as described above. To measure the bulk soil pH, dry soil was mixed with nutrient solution along with the different P treatments and oven dried at 40°C. A 1:5 soil: water extract was taken by adding 50 mL of RO water to 10 g dried soil and shaken for an hour and then

the solution was left to settle before measuring the bulk soil pH. To measure rhizosheath pH, the plants root with rhizosheath soil (see below) were washed in 20 mL of deionised water. The rhizosheath washing was then shaken for an hour and after settling the mixture was used to measure rhizosheath pH. To adjust for the effect of differences in soil:water ratio on pH, the pH in a series of independent samples with different soil:water ratios was measured. The relationship between pH and the soil:water ratio was then used to adjust the rhizosheath soil pH to an equivalent pH on 1:5 soil: water ratio. The rhizosheath pH was subtracted from the bulk soil pH. The pH was measured using a Denver instrument pH/mV/ISE conductivity meter (model 250). The water was then drained from the soil and the soil oven dried to measure the rhizosheath soil dry weight.

Root and shoot measurements

The rhizosheath size was measured using the method of Hailing et al (2010). The soil was removed from the pots and then the roots were separated carefully from the soil and shaken gently to remove excess soil. Root and shoot were separated and then the roots with the adhering soil, were transferred to a plastic tube containing 10mL of deionised water and shaken to remove the soil. The water was passed through a 0.45µM syringe filter into a sterilized plastic tube and the content was immediately frozen in liquid nitrogen to reduce the degradation of organic acid and stored at -80°C until the assessment for root exudates. The tubes with soil were then transferred to an oven (80°C) to dry and weighed to get the mass of rhizosheath soil. The shoots were dried at 80°C for 48 h to determine their dry weight.

Seedling root length was measured after the roots were washed gently to remove extra debris and then floated on water in a plastic Petri dish and scanned using an Epson Expression-10000 XL. Data was analysed by using WinRhizo (2005). Root samples

were then dried in an oven at 80°C for four days and the root dry weight measured. Rhizosheath size was estimated as the weight of dry soil per meter of root length.

A dissecting microscope fitted with an optical scale (x 2 eyepiece magnification) was used to measure root hair length at 3-5 cm from the root tip of the longest seminal root and the root hair. Ten measurements per sample was done to get the average for root hair length.

Measurements of citrate and malate

Malate and citrate concentrations of the rhizosheath solution were measured following the protocol described by Delhaize et al (1993). To measure malate concentration 100µL of sample solution was used by adding 10µL of buffer (pH 10.0) and 10µL of NAD^+/PVP. After 5 minutes the absorption at 340nm (the first A_{340}) was measured. After adding 2µL of malate dehydrogenase (MDH) with the reaction mixture, the production of NADH leads to an increase in A_{340}. The change of A_{340} before and after addition of MDH was used to calculate malate concentration. To estimate the concentration of citric acid, a 100µL sample was incubated with 50µL of buffer (pH 7.5), 20µL of NADH/PVP and 2µL of L-MDH/D-LDH (L-Malate dehydrogenase/D-lactate dehydrogenase). After 5 minutes the first A_{340} of the mixture was measured. After the addition of 2µL of citrate lyase with the reaction mixture the second measurement of A_{340} was taken; the reaction leads to the decrease of NADH, and the change in A_{340} was used to calculate the citric acid concentration. In the two assays a blank with no added solution and a standard of 0.15 mg/mL malic acid and 0.20 mg/mL citric acid respectively was used.

Data analysis

Data were analysed with general analysis of variance. The assumptions of the analysis of variance were checked during the analyses and no transformations of the data were necessary. Orthogonal contrasts (or single degree of freedom contrasts; Steele and Torrie 1960) were used to compare the measurements among the two groups of genotypes (responsive and non-responsive). Simple linear correlations were used to examine relationships between variables. All analyses were performed using GenStat 17th edition.

Results

Rhizosheath pH

The pH of the bulk soil was 7.93±0.02 for 0 kg P/ha and 7.85±0.02 for 30 kg P/ha treatment. By the time of harvest the pH of the rhizosheath soil was lower than the bulk soil and there was a significant variety × phosphorus interaction (Figure 5.1a, b) but it was not possible to relate this to the varietal P responsiveness. For most varieties the rhizosheath pH in the two P treatments were not significantly different and only the variety Gladius showed a significantly lower pH in the nil P treatment. A significant variety × phosphorus interaction (P = 0.004) was observed for pH difference of rhizosheath and the soil pH (Figure 5.1b). On average, the rhizosheath pH of the nonresponsive varieties was more acidic than that of the responsive varieties (difference between rhizosheath and bulk soil was 0.66 ± 0.10 compared with 0.50 ± 0.11) but there was considerable variation among the varieties. The greatest differences occurred with nonresponsive variety Gladius, Correll and RAC875 (Figure 5.1b).

Rhizosheath size, root length and root hair length

A significant variety x phosphorus interaction was observed for total root length (Table 5.1), but there was no significant difference between the non-responsive and responsive.

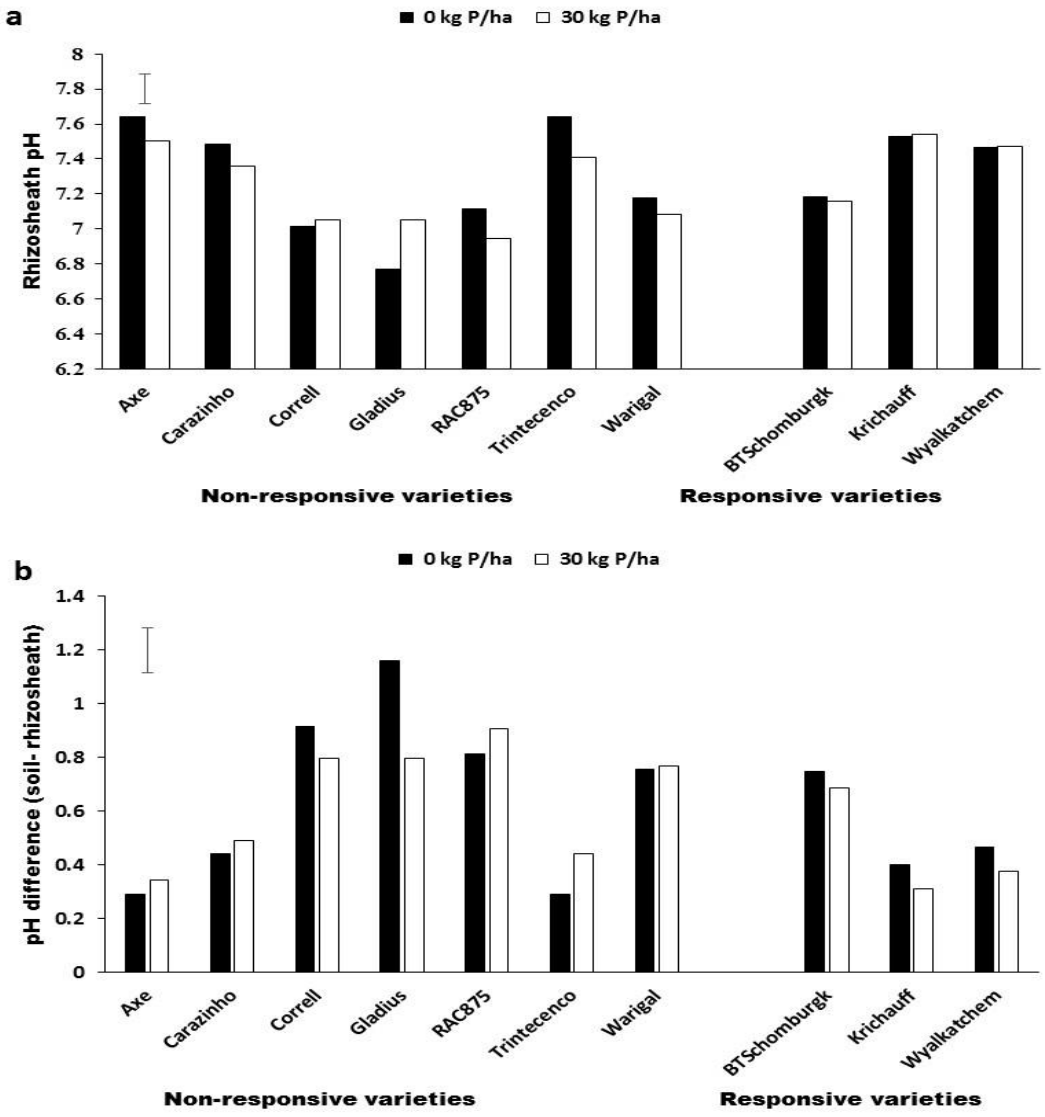

Figure 5.1. (a) Difference in rhizosheath pH of ten wheat varieties. The pH of the bulk soil was 7.93 (0 kg P/ha) and 7.85 (30 kg P/ha); (b) Difference in pH between the bulk soil and the rhizosheath soil in ten wheat varieties grown in Halidon soil that show differences in grain yield response to P. The error bar is the LSD (P=0.05) for the Variety × Phosphorus interaction.

Table 5.1. Total root length of ten wheat varieties grown in Halidon soil. Mean values for the P-responsive and non-responsive varieties are shown as mean ± standard error of mean (n = 3). The levels of significance are: * P<0.05; ** P<0.01 and *** P<0.001

Varieties	Total root length (cm)	
	0 kg P/ha	30 kg P/ha
Non Responsive		
Axe	107.5	127.8
Carazinho	89.2	102.0
Correll	102.2	123.3
Gladius	119.1	134.0
RAC875	117.7	132.5
Trintecenco	126.4	93.4
Warigal	114.9	113.2
Mean	111±4.7	118.03±5.93
Responsive		
BTSchomburgk	109.8	113.4
Krichauff	77.0	123.4
Wyalkatchem	113.5	106.4
Mean	100.1±11.6	114.4±4.93
LSD (P=0.05)		
Variety	16.36*	
Treatment	7.32*	
Variety x Treatment	23.14**	
CV (%)	12.4	

varieties. Compared to the other responsive varieties Krichauff had a dramatic decrease in root length at nil P treatment.

Varieties differed significantly in their rhizosheath size and the P treatment resulted in a significant reduction in rhizosheath size (Figure 5.2) but no variety x phosphorus interaction was observed. The non-responsive varieties had a significantly higher rhizosheath size than the responsive varieties and rhizosheath size was 30% lower when P was applied.

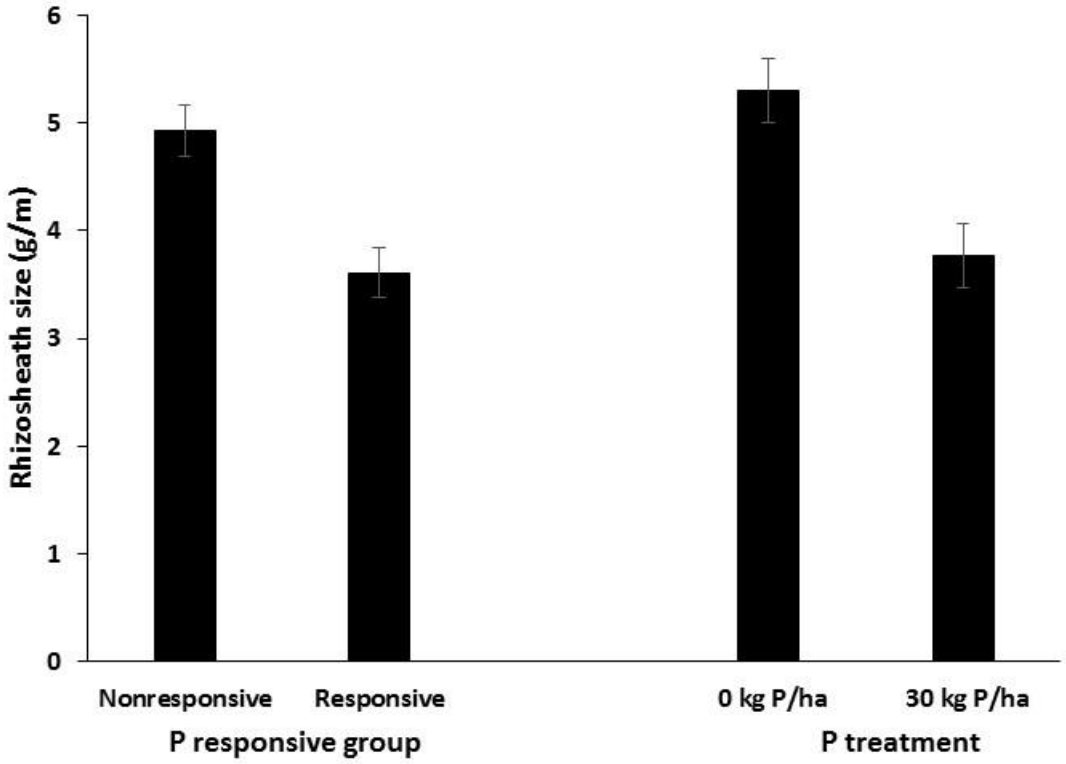

Figure 5.2. Rhizosheath size of nonresponsive and responsive wheat varieties grown at two P level. Error bars are the standard error of mean (n=3) for the varieties and lsd for the P treatment.

A significant group × phosphorus interaction was also observed for root hair length (Figure 5.3). The mean root hair length of the nonresponsive varieties was higher than that of responsive varieties at both P treatments and showed a larger response to P. Reduced root hair length at high P treatment was observed for all varieties, except for the variety Wyalkatchem which had similar root hair length in both P treatments. The nonresponsive variety Warigal had dramatic decrease in root hair length at high P treatment (Appendix 8).

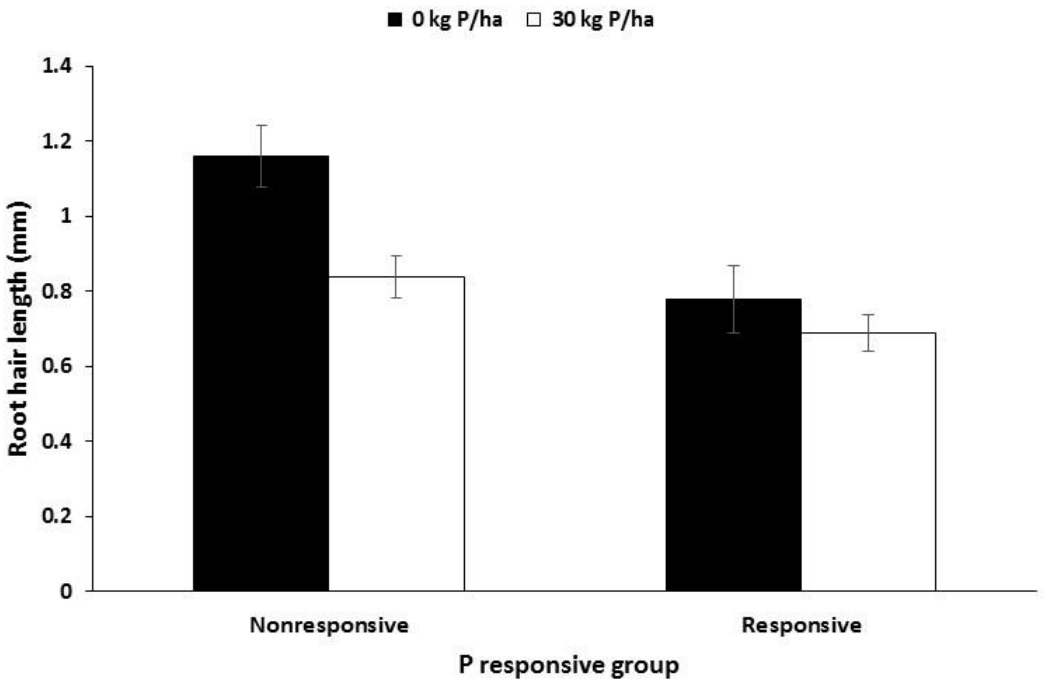

Figure 5.3. Root hair length of two groups of wheat varieties grown in Halidon soil. Responsive group represents mean of three varieties and nonresponsive group represents mean of seven varieties.

Dry matter production

A significant variety × phosphorus interaction was observed for both shoot dry weight and root dry weight (Table 5.2). The mean shoot dry weight of the nonresponsive varieties was higher at nil P treatment compared to the responsive varieties. The responsive varieties maintained similar shoot dry weight at both P treatments except Krichauff which had a dramatic increase at high P treatment.

Orthogonal contrasts suggest that root dry weight of the non-responsive group had higher root dry weight at both P treatments over responsive group. The root dry weight

Table 5.2. Shoot dry weight (SDW) and root dry weight (RDW) of ten wheat varieties grown in Halidon soil. Mean values for the P-responsive and non-responsive varieties are shown as mean ± standard error of mean. The levels of significance are: * P<0.05; **; P<0.01 and ***; P<0.001

Responsiveness group and Variety	SDW(mg)		RDW(mg)	
	0 kg P/ha	30 kg P/ha	0 kg P/ha	30 kg P/ha
Non Responsive				
Axe	20.0	25.7	19.7	17.7
Carazinho	22.0	21.3	13.0	13.7
Correll	21.3	25.7	17.7	16.7
Gladius	21.3	22.3	21.3	15.7
RAC875	22.0	28.3	18.0	17.0
Trintecenco	26.0	22.3	18.7	12.3
Warigal	17.0	21.8	13.3	13.3
Mean	21.4 ± 1.01	23.9 ±0.99	17.4±1.18	15.2±0.78
Responsive				
BTSchomburgk	17.0	17.7	14.0	12.3
Krichauff	14.6	21.1	11.3	14.3
Wyalkatchem	18.7	17.7	12.0	10.7
Mean	16.7 ± 1.18	18.82 ± 1.15	12.4 ± 0.82	12.4 ± 1.04
LSD (P=0.05)				
Variety	3.08***		2.48***	
Treatment	1.38***		1.11**	
Variety x Treat.	4.36*		3.51*	
CV (%)	12.4		14.0	

of non-responsive group was higher at nil P treatment (17.6 mg) compared to the high P treatment (15.0 mg).

Malate and citric acid measurement

Significant effects of variety (P<0.001) and P treatment (P=0.001) were observed for malic acid concentration in Experiment 2 (Figure 5.4). Carazinho produced more than

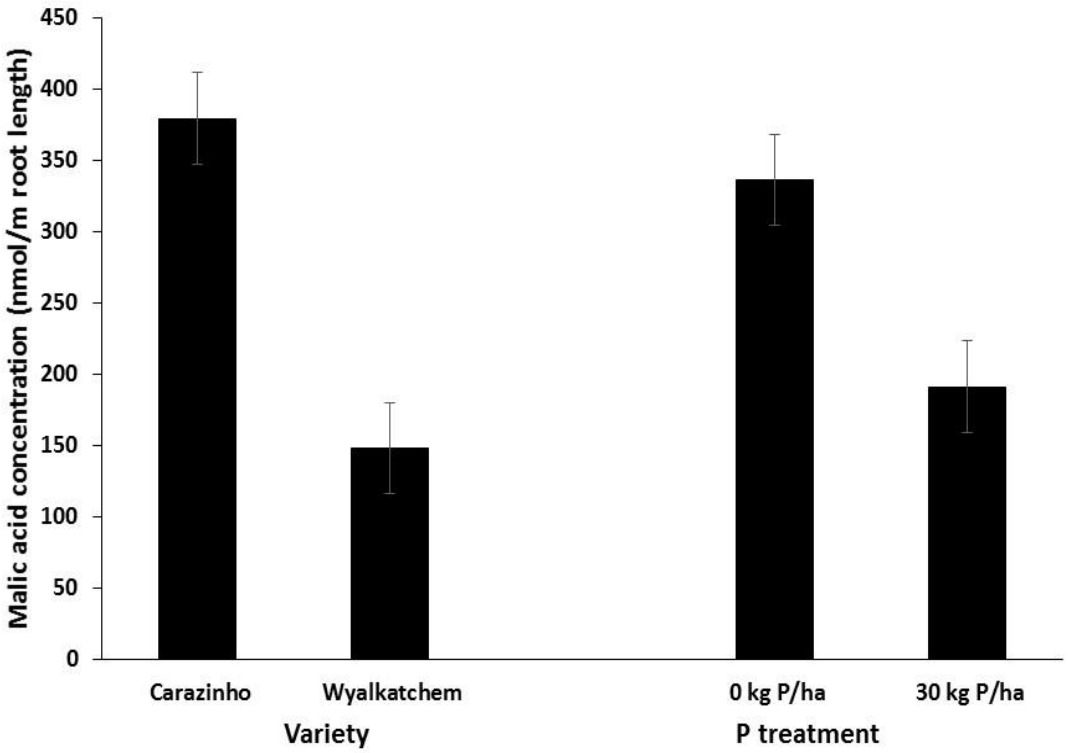

Figure 5.4. Malic acid concentration in rhizosheath soil of two wheat varieties at two different P treatment grown in Halidon soil. Error bar represents the LSD (P=0.05) for the variety x P treatment

twice as much malic acid as Wyalkatchem, and malic acid production under nil P was about 70% higher than when P was supplied.

A significant variety ×phosphorus interaction was observed for malic acid exudation in the rhizosheath soil (Experiment 3) (Figure 5.5a), but there was no significant difference between the nonresponsive and the responsive wheat varieties. The non-responsive varieties Correll, Gladius and Trintecenco had higher concentrations of malic acid in the nil P treatment whereas there was no effect of P in the remaining varieties. The highest malic acid concentration was observed in Trintecenco (214 nmol/m root length) and lowest was observed by the variety Correll (106 nmol/m root length).

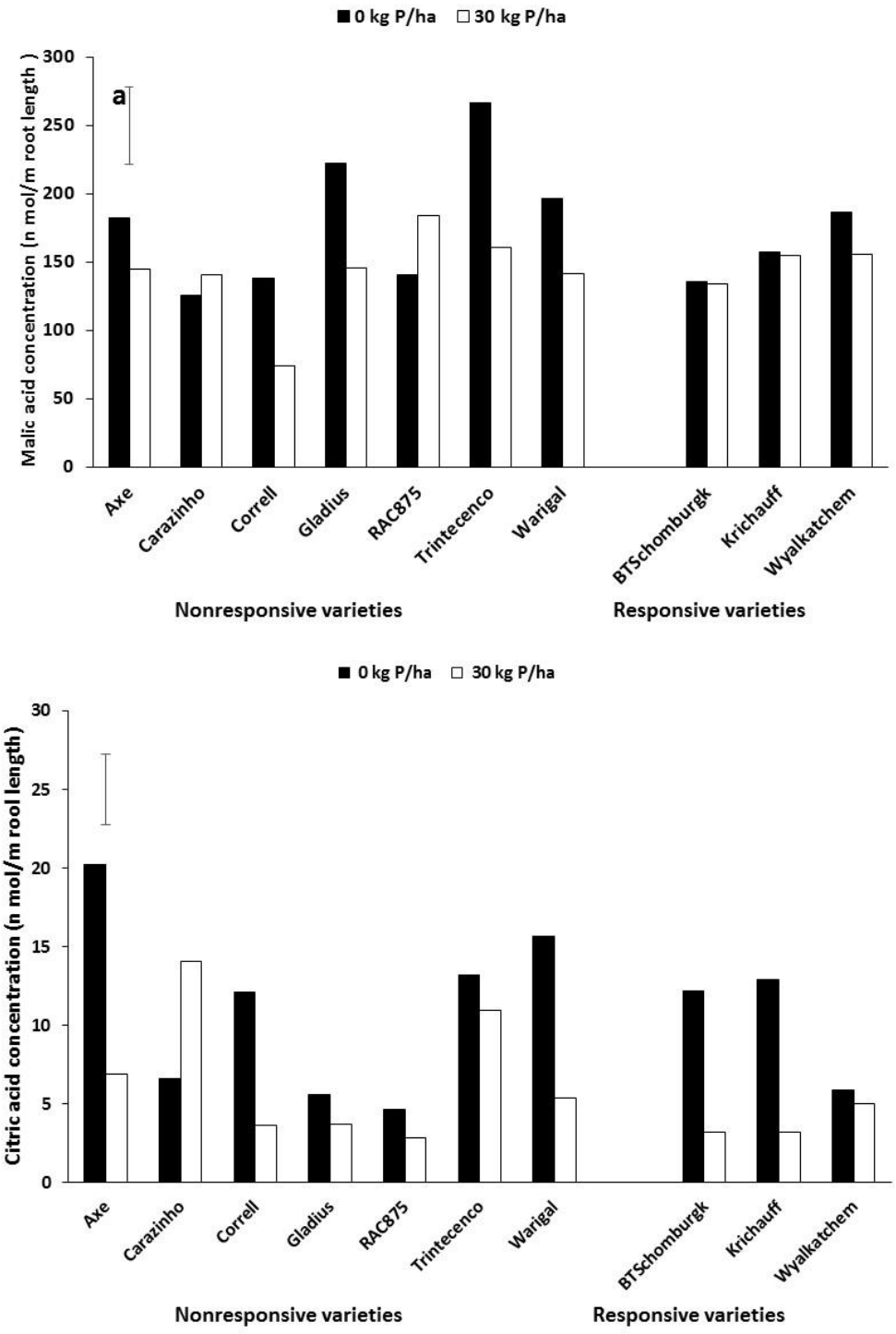

Figure 5.5. (a) Malic acid concentration in rhizosheath soil of ten wheat varieties at two different P treatment grown in Halidon soil. (b) Citric acid concentration in rhizosheath soil of ten wheat varieties grown in Halidon soil. Error bar represents the LSD (P=0.05) for the variety × phosphorus treatment.

A significant variety × phosphorus interaction was also observed for citric acid exudation and the non-responsive varieties differed significantly from the responsive varieties (Figure 5.5b). On average, the non-responsive varieties exuded significantly more citric acid (9.5 nmol/m root length) than the responsive varieties (7.1 nmol/m root length) and the exudation was twice as high at nil P treatment (11.6 nmol/m root length) than high P treatment (5.9 nmol/m root length). No Group × Phosphorus interaction was observed.

Correlation among root traits

In the nil P treatment malic acid concentration was correlated with root length and with rhizosheath size, but when P was supplied it was not correlated with any root trait (Table 5.3). In contrast citric acid concentrations were not related to any root trait under nil P but showed a positive correlation with root hair length when P was supplied.

Root length was positively correlated with rhizosheath size, shoot dry weight, root dry weight and malic acid concentration at nil P treatment. Rhizosheath size was positively correlated with most of the traits except root hair length and citric acid concentration in the nil P treatment. Root hair length was positively correlated with shoot dry weight at 0 P treatment and with citric acid concentration at high P treatment.

Table 5.3. Correlations among root traits and malic and citric acid concentration. Below the diagonal shows the correlation coefficients at nil P treatment and above the diagonal shows the correlation coefficients at high P treatment. The levels of significance are: * P<0.05; **; P<0.01 and ***; P<0.001 (n=29)

Root trait	Root length (cm)	Rhizosheath vol. (g/m)	RHL (mm)	SDW (mg)	RDW (mg)	Citric acid (n mol/m)	Malic acid (n mol/m)
Root length (cm)		0.449*	-0.152	0.510**	0.730***	-0.278	-0.227
Rhizosheath vol. (g/m)	0.484**		0.160	0.426*	0.402*	0.156	0.110
RHL(mm)	0.196	0.299		0.264	0.146	0.489**	0.207
SDW(mg)	0.440*	0.415*	0.472**		0.711***	-0.016	0.032
RDW(mg)	0.567**	0.523**	0.300	0.626***		-0.053	-0.199
Citric acid (n mol/m)	0.131	0.246	0.082	-0.138	0.240		0.077
Malic acid (n mol/m)	0.439*	0.469**	0.096	0.347	0.310	0.298	

Discussion

The findings of the experiments reported here confirmed substantial variation exist in rhizosheath pH, rhizosheath size, root hair length and malic and citric acid concentration in rhizosheath soil of wheat varieties grown in Halidon soil. In the following discussion the relationship of varietal P responsiveness with malate and citrate concentration and other related traits will be discuss.

In the experiments described in this chapter significant genetic variation was observed for malic and citric acid concentration from rhizosheath washing. In Experiment 2 the nonresponsive variety Carazinho had a higher malic acid concentration and the difference between Carazinho and Wyalkatchem was much greater compared to Experiment 3. High efflux of malic acid exudation by Carazinho was also observed by Delhaize et al (1993) and Ryan et al (2009) grown in acid soil but substantial variation between experiments was also reported by Ryan et al (2009). The results of this thesis suggests that it is difficult to replicate genetic differences among experiments and organic acid concentration may not be a reliable predictor of P uptake. Although care have been taken to reduce the post-harvest microbial degradation of organic acid, the microbial degradation of pre harvest was not considered. This could be a contributing factor for varietal inconsistency in the exudation of organic acid, as it is known that organic acid such as citrate degrades very fast in calcareous soil (Khademi et al. 2010). Strong influence of environmental conditions was also observed for citric acid efflux by Delhaize et al. (2003). Ryan et al (2014) concluded using non-transgenic lines that it was not the citric acid efflux but other attributes that explained the superior ability of Carazinho to perform well in P deficient soil.

Although significant genetic variation of malic acid concentration was observed, it was not possible to relate P responsiveness and malic acid releasing capacity. Some non-responsive varieties such as, Gladius, Trintecenco and Warigal had higher malic acid concentration than other varieties. In this study the concentration of malic acid in rhizosheath soil was ten times higher than the concentration of citric acid, which is consistent with the findings of Ryan et al (2009) in wheat and by Hoffland et al (1989) in P-deficient rapeseed. This appears to be a general characteristic of plants, with the exception of species that produce proteoid roots (Keerthisinghe et al 1998). Pears et al (2006) also observed that malic acid was the predominant form of carboxylates in the rhizosphere of wheat and accounted for over 85% of total carboxylates. In the literature there is not enough information on malic acid exudation and its relation to P is available in neutral and alkaline soils. The exudation of malic and citric acid was not induced at P deficiency for wheat (Delhaize et al. 1993) and buckwheat (Ma et al. 1998) but in this study there were significantly higher concentrations of malic acid under low P in most varieties . While significant differences in citric acid exudation were measured among the varieties, it was not possible to relate citric acid releasing capacity with varietal P responsiveness. This differs from a number of previous reports. Higher citric acid was related to greater uptake of P from strongly adsorbed soil P fraction between two barley varieties (Gahoonia et al. 2000). In common bean total exudation of organic acid was related to the P efficient variety (Yan et al 2004). In maize the concentration of citric acid in root and the exudation by a low P tolerant mutant was higher compare to wild type under both P sufficient and deficient condition (Chen et al. 2013). Based on the findings of this study and contrasting results from the literature, it is not possible to outline any potential benefit of malic and citric acid. The concentrations of malic and citric acid were higher at nil P treatment compared to the high P treatment, which is

consistent with previous studies (Gaume et al. 2001b, Gaume et al 2001a) but there was no strong association with the yield responsiveness to P under field conditions.

Varietal differences in rhizosheath pH were observed, which may assist in the acquisition of P by plants. On the basis of mean values from both P treatment there was no significant relation between pH and malic acid ($r = 0.23$, $df = 9$) among the 10 varieties, but there was a significant correlation with citric acid ($r = 0.60$, $df = 9$, $P < 0.05$). No correlation of total carboxylates release with pH was observed by Pears et al (2006) and no clear relationship of pH change with P treatment was observed. In this study although a significant variety × P treatment effect was observed, it was not possible to draw a clear relationship between varietal pH change and P treatment. The changes in rhizosphere pH by organic acid exudation also depends on plant species and the amount of exudation; for example, the acidification of rhizosphere by maize root exudation was negligible (not exceeding 0.2-0.3%), in contrast strong acidification was observed by the cluster root of white lupin as they are able to produce large amount of organic acid (Hinsinger et al. 2003). However, it is not only organic acid exudation that will influence the response; there are other factors such as cation-anion exchange, root exudation and respiration and redox-coupled processes that are involved in rhizosphere pH changes (Hinsinger et al. 2003).

In this study citric acid concentration was positively correlated with root hair length only in the high P treatment and there was no relation between malic acid concentration and root hair length in either P treatment. In an earlier study Yan et al (2004) observed a positive correlation between total organic acid exudation and basal root hair density and length in common bean (*Phaseolus vulgaris*). The results of the present study does

not support this relationship in wheat. Shoot dry weights of non-responsive varieties were higher at nil P treatment compared to responsive varieties. According to Akhtar et al (2008) shoot dry weight of seedlings is a good indicator for selection of nutrient efficient plant genotypes. The findings of this study agree with that as the non-responsive varieties had higher shoot dry weight at nil P treatment. In this study no correlation of citric and malic acid concentration with shoot dry weight was observed. Ryan et al (2014) concluded that the potential benefit of citrate to increase shoot biomass by mobilizing sufficient P may be limited. A positive correlation of shoot dry weight with total root length, rhizosheath size and root hair length was observed from the work described in this thesis, suggesting that all these root traits contributed towards varietal performance at P deficient condition. This was also confirmed from the Chapter 3 of this thesis. A strong positive correlation of root hair length and rhizosheath size of wheat was observed grown in both acid and non-acid soil (Delhaize et al. 2012; Delhaize et al. 2015; James et al. 2016). Association of large rhizosheath with improved PUE of wheat was observed by James et al. (2016).

In conclusion, from the present study varietal differences were observed for malic and citric acid concentration but there was not a consistent difference between the P-responsive and non-P responsive varieties. It is evident from this study that malic and citric acid concentration in rhizosheath soil is not explaining the varietal differences in growth under P deficient condition and variation in the measured concentration of malic acid between experiments also raised the question about the reliability of organic acid exudation to P efficiency. From this study it can be concluded that it is not the organic acid but other root traits such as root hair length and rhizosheath size that contributed towards varietal performance under P deficient condition.

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Chapter 6 : Assessing the relative importance of root traits towards varietal responsiveness to phosphorus

Introduction

Plant varieties are known to have several adaptive mechanisms to combat phosphorus (P) deficiency. The often low availability and uneven distribution of P in soil and its low rate of diffusion to the roots means that root traits play an important role in P uptake. There are several aspects of root growth that have been proposed as important for enhanced P uptake (van de Wiel et al. 2016), including root angle, root hair length and rhizosheath size, the ability to release organic acids from the roots, and to form symbiotic relationships with mycorrhizal fungi. However much of the previous work on the importance of root traits to P uptake and P efficiency has focused on one or two root traits (Gahoonia and Nielsen 1996; George et al. 2008; Lynch and Brown 2001) and there has been little assessment of the relative value of the various traits. The previous experimental chapters of the thesis examined the importance of a number of individual root traits to P responsiveness (Chapter 3, Chapter 4 and Chapter 5) among wheat varieties that had shown differences in their P responsiveness over a number of sites and seasons (McDonald et al. 2015). The purpose of this chapter is to integrate the results from the different controlled environment experiments to compare the contribution of the different root traits to P responsiveness in the field.

The analysis will be based on two methods: comparison of the rankings of the different traits using a rank correlation analysis and using cluster analysis to identify groupings of genotypes based on their root traits. The rationale for this approach is that rank

correlation will demonstrate the consistency of the different traits among the responsive and non-responsive groups of varieties, while cluster analysis can be used to identify groups of varieties based on the degree of similarity among the root of traits. Cluster analysis is a multivariate analysis that enables groups to be formed based on the degree of similarity among different variables. If the cluster analysis groups the genotypes according to their P responsiveness, the properties of the different groups can then be described and the importance of specific root traits inferred.

Methods

Source of data

All the data used for the statistical analysis for this chapter was taken from the mean values of root traits of Chapter 3, Chapter 4 and Chapter 5 of this thesis. The analysis will be focussed on root traits expressed at low P for two reasons: first, some traits such as root hair length and AM infection are greatly reduced at high P and often genetic variation at high P is lower compared to P deficiency; and second it is the goal to identify favourable traits that will contribute most to improvements in P uptake under limited P availability and will contribute to differences in P responsiveness.

Statistical methods for data analysis

Data analysis of this chapter was done by the statistical software GenStat 17th edition. Spearman's rank correlation among individual variates was done to obtain the correlation matrix. Among the 10 genotypes, the smallest value for a trait was given a rank of 1 and the largest was given a rank of 10. The consistency of ranking was then assessed by the correlations in rankings among the different traits using Spearman's

rank correlation. This approach was used because the variates show considerable differences in scale and the main interest of the analysis was to see how the relative rankings of genotypes, rather than the absolute values, changes among the different root traits.

Hierarchical cluster analysis was performed on the mean values of all the root traits at 0 kg P/ha and also from the two soil types (Halidon and Mallala soil). The similarity matrix was formed based on Euclidian distance and the different groups were identified by using he complete link method. To measure the genetic distance between individual (genotypes or population) by morphological data Euclidian distance is the most commonly-used measurement (Mohammadi and Prasanna 2003). The similarity matrix derives a value between two variable of between 0 (maximum difference between all variables) and 1 (identical for all values) which is then used to group the varieties at different levels of similarity. Hierarchical cluster analysis starts by assigning the n data objects or samples to n separate clusters, each containing one member. At each stage of the clustering, the two closest clusters are merged into one larger cluster, until finally all the units have been formed into a single cluster. This process can be represented by a hierarchical tree whose nodes indicate what merges have occurred. Complete link defines the similarity between two clusters as the minimum similarity between any two samples in those clusters. Genetic similarities of wheat varieties was further assessed by using pedigree analysis.

Results and discussion

A table of rankings was prepared (Table 6.1) based on the performance of each variety for the specific root trait. It is probably unreasonable to expect an individual root trait

will segregate completely between the two groups of genotypes because there is a range of characteristics that influence P uptake, P physiological efficiency and hence P responsiveness. Moreover, P responsiveness showed some variation among the field trails, and so the same degree of responsiveness will not be expressed in every case. Notwithstanding these qualifications, the approach taken in the analysis of the results is that if the majority of the varieties within a group express a trait, it suggests that there is an adaptive value of the particular trait to explain differences in P responsiveness among the 10 wheat varieties.

The importance of root traits

The ranking of seminal and crown root angles were not significantly correlated (Table 6.2) and therefore can be considered unrelated traits. Root angle has been suggested as a valuable trait on the basis that a wide root angle will promote exploration of the surface soil layers where much of the soil P is located (Walk et al. 2006), thereby improving growth and P uptake under limited supplies of P. However, there was little consistent relationship between the seminal root angle and the yield responsiveness of the variety. For example, among the non-responsive varieties, Gladius, Trintecenco and RAC 875 had narrow seminal root angles while Carazinho, Axe and Corell had wide seminal root angles and showed a similar ranking to the responsive varieties BT Schomburgk and Krichauff.

There appeared to be some greater consistently when crown root angles were compared, with the majority of the varieties showing rankings that were consistent with their P responsiveness. With the expectation of RAC 875, all the non-responsive varieties had intermediate to wide crown root angles while two of the three responsive varieties,

Krichauff and Wyalkatchem, had narrow crown root angles. BT Schomburgk, which had the widest crown root angle at low P was the exception among the responsive varieties. Crown roots develop later than seminal roots and their growth is shallower which may make them better able to acquire more P from the surface soil. A wider crown root angle may enhance this effect. The additional importance of adventitious root is that they require 42% less linear construction cost per unit of root length and have a lower metabolic demand than other root class, which according to Lynch (2015), is an important strategy for efficient acquisition of resources from soil.

There was evidence that root hair length was a trait which played a significant contribution towards varietal P responsiveness. The responsive group of varieties had low to intermediate root hair lengths and rhizosheath sizes, while generally the non-responsive groups showed consistently higher rankings (Table 6.1). The non-P responsive variety Carazinho had the longest root hair length and the non-responsive varieties such as RAC875, Trintecenco and Warigal also ranked more than average for their root hair length (Table 6.1). The major variation from the general trend was Correll, which was classified as non-responsive but showed short root hair length and small rhizosheath size across a number of experiments. The rank correlations indicated that varieties with long root also tended to have a long root hair length (Table 6.2). Previous studies have demonstrated that root hairs are an important characteristic for P acquisition efficiency of plants (Brown et al. 2012; Gahoonia and Nielsen 2003; Gahoonia et al. 1997; Gahoonia and Nielsen 2004; Gahoonia et al. 2001; Haling et al. 2013; Zhu et al. 2010). Root hairs are particularly important due to their capacity to increase absorption area and they are also known to assist in the dispersion of root exudates in rhizosphere (Zhu et al. 2005). Several QTLs controlling the genetic variation of root hair length and density of maize and bean have been detected (Yan et

al. 2004; Zhu et al. 2005), which suggest that this trait could be selected in breeding programs through marker-aided selection as well as through direct phenotypic screening. A positive correlation of root hair length and crown root angle was also observed (Table 6.2). As mention earlier, crown root angle was found to be related with the varietal P responsiveness, from this it can be concluded that root hair length and crown root angle were the traits were most strongly associated with the varietal performance from field.

Table 6.1. Ranking of varieties according to the values of various root traits from all the experiments, showing difference in P treatment (P0: 0 kg P/ha or P30: 30 kg P/ha) and also in soil types (Hal – Halidon; Mal – Mallala). The grand mean for each experiment is also shown. Ranking are fromfrom the smallest to the largest values.

Variety	Seminal root angle (°)		Crown root angle (°)		Total root length (cm)		Rhizosheath size (g/m)		Root hair length (mm)		Root hair length (mm)		Malic acid (nmol/m)		Citric acid (nmol/m)		AMF colonization (%)	
	1st pair	2nd pair	P0	P30	Hal	Mal	Hal	Mal	Hal	Mal	P0	P30	P0	P30	P0	P30	P0	P30
	Chapter 3		Chapter 3		Chapter 3		Chapter 3		Chapter 3		Chapter 5		Chapter 5		Chapter 5		Chapter 4	
Non-responsive																		
Axe	6	10	6	2	7	7	7	4	4	5	5	3	6	5	10	8	7	7
Carazinho	7	9	4	9	6	5	9	10	10	10	8	9	1	3	4	10	8	10
Correll	9	5	7	6	2	3	4	5	2	2	2	5	3	1	5	4	1	2
Gladius	1	4	8	10	5	9	10	6	5	3	6	7	9	6	2	5	2	5
RAC875	3	3	2	4	10	4	8	9	8	9	9	8	4	10	1	1	5	3
Trintecenco	2	2	9	8	9	6	6	8	9	4	10	10	10	9	8	9	10	9
Warigal	5	1	5	7	3	2	5	2	7	1	7	2	8	4	9	7	4	4
Responsive																		
BT Schomb	10	7	10	5	8	1	2	7	6	7	4	6	2	2	6	3	6	1
Krichauff	8	8	1	3	4	8	1	3	3	8	3	4	5	7	7	2	9	6
W'katchem	4	6	3	1	1	10	3	1	1	6	1	1	7	8	3	6	3	8
Grand mean	127.7	128.0	93		178.1		1.91		0.92		0.92		159.4		8.4		18.4	

Table 6.2. Rank correlation among all the root trait studied. Data for the rank correlation was taken from Table 1). Abbreviations in the table are: SRA1-first pair seminal root angle, SRA2-second pair seminal root angle, CRAplus-crown root angle , TRL-total root length, RV-rhizosheath size, RHL-root hair length, CA-citric acid, MA-malic acid, nil-0 kg P/ha, plus-30 kg P/ha, Hal-Halidon soil and Mal-Mallala soil (* P<0.05; ** P<0.01 and *** P<0.001).

	SRA1	SRA2	CRA nil	CRA plus	TRL Hal	TRL Mal	RV Hal	RV Mal	RHL Hal	RHL Mal	AMF nil	AMF plus	MA nil	MA plus	CA nil	CA plus	RHL2 nil	RHL2 plus
SRA1	1.00																	
SRA2	0.55	1.00																
CRAnil	0.02	-0.18	1.00															
CRAplus	-0.27	-0.38	0.47	1.00														
TRL_Hal	-0.20	-0.04	0.25	0.16	1.00													
TRL_Mal	-0.50	0.25	-0.33	-0.22	-0.27	1.00												
RV_Hal	-0.61*	-0.13	0.10	0.58*	0.37	0.14	1.00											
RV_Mal	-0.08	0.01	0.25	0.54	0.76**	-0.32	0.55	1.00										
RHL_Hal	-0.25	-0.26	0.16	0.61*	0.70**	-0.41	0.54	0.77**	1.00									
RHL_Mal	0.20	0.54	-0.48	-0.25	0.42	0.10	0.02	0.48	0.29	1.00								
AMF_nil	0.07	0.29	-0.09	-0.03	0.55	0.04	-0.09	0.33	0.50	0.50	1.00							
AMF_plus	-0.37	0.26	-0.24	0.08	-0.02	0.61*	0.30	0.07	0.24	0.26	0.53	1.00						
MA_nil	-0.77**	-0.56*	0.18	0.13	-0.08	0.47	0.15	-0.38	-0.07	-0.60*	-0.02	0.27	1.00					
MA_plus	-0.73**	-0.32	-0.41	-0.25	0.33	0.54	0.15	0.02	0.12	0.25	0.30	0.36	0.54	1.00				
CA_nil	0.30	0.13	0.24	-0.14	-0.01	-0.21	-0.33	-0.35	0.01	-0.37	0.46	0.13	0.22	-0.25	1.00			
CA_plus	-0.21	0.10	0.26	0.36	-0.04	0.14	0.39	0.09	0.37	-0.18	0.30	0.75**	0.25	-0.14	0.41	1.00		
RHL2_nil	-0.52	-0.42	0.13	0.56*	0.73**	-0.24	0.65**	0.69**	0.93***	0.13	0.46	0.26	0.21	0.36	0.02	0.33	1.00	
RHL2_plus	-0.24	-0.15	0.31	0.65**	0.71**	-0.18	0.52	0.94***	0.75**	0.33	0.39	0.16	-0.13	0.15	-0.30	0.13	0.72**	1.00

Rhizosheath size of the non-responsive varieties also ranked higher over the responsive varieties and rankings for rhizosheath size were generally consistent with root hair length (Table 6.2). The large rhizosheath size of the non-responsive variety Carazinho was comparable to the findings of Haling et al (2010). Measuring rhizosheath size is easy and associated with relatively high heritability, thus breeding cereal varieties for greater rhizosheath size is achievable and could contribute towards identifying nutrient efficient varieties (George et al. 2014). A strong correlation between root hair length and rhizosheath size of wheat was observed by Delhaize et al (2012), but in contrast George et al (2014) observed a partial relation of root hair length with rhizosheath size of barley and concluded that root hair length alone cannot explain rhizosheath size. The findings of this study especially the findings of Chapter 3 also support that and there were other attributes rather than root hair length alone controlling rhizosheath size. When the rank correlation was conducted, root hair length from Chapter 3 was strongly correlated with the root hair length at nil P treatment of Chapter 5 and the root hair length of Chapter 5 showed positive correlation with the rhizosheath size of chapter 3 (Table 6.2). It is known that rhizosheath formation is not only strongly affected by root hair length but also by the soil environment such as soil pH, bulk density, soil moisture and texture (Haling et al. 2013; Watt et al. 1994).

In some plant species P deficiency triggers the release of organic anions such as citrate and malate from roots (eg, wheat: Ryan et al. 2014) which can increase the soil P concentration by increasing the diffusion coefficient of orthophosphate (Dessureault-Rompré et al. 2007; Gerke 1994; Jones 1998; Khademi et al. 2010; Wei et al. 2010). Enhanced organic anion efflux from the roots of P deficient plants has been reported many crops including rice (Kirk et al. 1999), barley (Gahoonia et al. 2000) and maize (Gaume et al. 2001). Due to contrasting results direct evidence of organic anions release

and their importance to P nutrition is meagre (Ryan et al. 2014). The results of this study also could not demonstrated a strong relationship between varietal P responsiveness and the difference in organic acid releasing capacity. Non-responsive varieties ranked both highly (Trintecenco, Warigal) and poorly (RAC 875, Carazinho) for both malic and citric acid, when grown at low P (Table 6.1). Malic acid exudation at nil P treatment was negatively correlated with both first and second pair of seminal root angle and also with the root hair length from Mallala soil (Table 6.2). The exudation of citric acid was not correlated with any of the traits, except with AMF colonization when growing with added P.

In a study with rape plants negligible amounts of citrate and no malate exudation was observed by Ligaba et al (2004) and they also observed significant increase in malate and citrate exudation from both P sufficient and P deficient plants when 50µM Al was present. They concluded that presence of Al is prerequisite for organic acid exudation and similar result was observed in other studies (Delhaize et al. 1993; Yang et al. 2000). Delhaize et al (1993) observed that P deficiency did not induced the exudation of organic acid of wheat.

Root infection by AMF was greatly reduced by the addition of P, but even at low P there was not consistent relationship between the P responsiveness of a variety and the level of AMF infection. Although genetic variation was observed for AMF colonization, non P-responsive varieties showed both low (Correll, Gladius) and high (Carazinho, Trintecenco) levels of AMF infection. Work by Leiser et al (2016) concluded that due to low heritability and lack of positive relation with P uptake in sorghum, selection for AMF infection would not be promising. Several studies identified no potential benefit of AMF colonization of wheat at P limited condition (Hildermann et al. 2010; Ryan et al. 2002; Ryan and Angus 2003). Other than a positive correlation with citric acid

exudation, AMF colonization was only positively correlated with total root length when plants were grown in Mallala soil (Table 6.2). Due to the lack of consistency with the varietal P responsiveness and relation of AMF colonization with other traits it can be concluded that selection for AMF will not be beneficial for plant varieties.

Cluster analysis

The results for cluster analysis clearly shows four different group at 80% linkage distance. Cluster 1 consisted of three varieties (Axe, Correll and BT Schomburgk), Cluster 2 consisted of two varieties (Krichauff and Wyalkatchem), Cluster 3 had

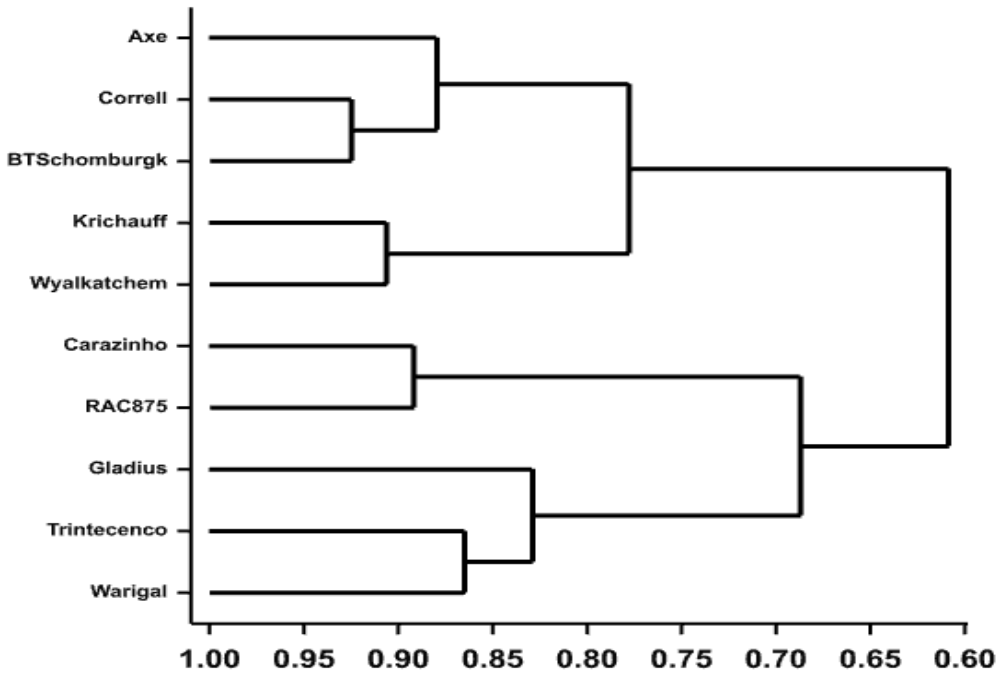

Figure 6.1. Varietal categorisation based on all the root traits studied (values were taken from plants grown at 0 kg P/ha and from Halidon and Mallala soil from Table 6.1).

two varieties (Carazinho and RAC875) and there were three varieties (Gladius, Trintecenco and Warigal) in Cluster 4. With two exceptions, the analysis largely separated the varieties based on their yield responsiveness in the field. Cluster 3 and Cluster 4 contained five of the seven non-responsive varieties and the two responsive varieties, Krichauff and Wyalkatchem, were grouped together in Cluster 2, which was as excepted as these two varieties showed consistent responsiveness at field condition evaluated by McDonald et al (2015). Cluster 1 contained a mix of responsive and non-responsive varieties; these showed expression of some traits that was not consistently related to the initial P-responsiveness classification. The coefficient of parentage matrix from the pedigree analysis (Appendix 9) suggested there was not a strong relationship among the varieties within each group. Within the responsive group the variety Wyalkatchem was different to the other two variety BT Schomburgk and Krichauff. The responsive varieties BT Schomburgk and Krichauff was somewhat related with the non-responsive variety Warigal.

The mean values of the traits for each cluster are shown in Table 6.3. Varieties in Cluster groups 3 and 4 had high rhizosheath sizes and long root hairs. The two groups differed in their seminal and crown root angles: varieties in Group 3 had wider seminal root angles and narrower crown root angles compared to Group 4. Cluster groups 3 and 4 contained the majority of the non-responsive varieties and the consistency of the results for rhizosheath size and root hair length suggests that these were traits which were most important in explaining varietal P responsiveness. In comparison, Group 2, consisting of Wyalkatchem and Krichauff which consistently showed a high response to P, had the shortest root hair length and rhizosheath size. This highlights the importance of root hair length and rhizosheath size in describing the P responsiveness

of the varieties in the field. These traits were more important than total root length of the seedlings because total root length did not change consistently with the P-responsiveness classification of the groups, but a close association of total root length with root hair length was observed (Table 6.2). It can be concluded that total root length can be a contributing trait for varietal P responsiveness. Seminal root angle was not associated

Table 6.3. Mean value of different root traits of each cluster group

Cluster group[A]	Seminal root angle (°)		Crown root angle (°)	Total root length (cm)		Rhizosheath size (g/m)		Root hair length (mm)		Root hair length (mm)	Malic acid (nmol/m)	Citric acid (nmol/m)	AMF colonization (%)
	1st pair	2nd pair		Halidon	Mallala	Halidon	Mallala	Halidon	Mallala				
1	143	141	86	181	154	2.19	1.49	0.90	0.71	0.90	152	14.8	19
2	134	141	73	166	202	1.89	1.32	0.78	0.76	0.73	172	9.4	22
3	126	127	78	202	167	2.58	2.03	1.41	1.07	1.33	133	5.6	23
4	110	106	87	188	173	2.33	1.52	1.21	0.66	1.21	229	11.5	22

[A] Group 1: Axe, Correll, BT Schomburgk

Group 2: Krichauff, Wyalkatchem;

Group 3: Carazinho, RAC 875;

Group 4: Gladius, Trinticenco, Warigal

with P responsiveness: varieties in Cluster 1 has the highest angles (first pair 143°and second pair 141°), but it consisted of varieties than showed different yield responses to P. Crown root angle was smallest in Cluster group 2 and highest in groups 4, suggesting it may contribute to the differences in responsiveness among the varieties, but not as consistently as root hair length. AMF colonization did not vary much among the different groups, suggesting this trait was not strongly related with the varietal P responsiveness.

Conclusion

Based on the results of the multivariate analysis it can be concluded that root hair length and rhizosheath size were the traits that were most important in explaining differences in varietal P responsiveness. Of the remaining traits, crown root angle may also be influential. From the findings of this study it is clear that total root length of seedlings cannot be a selection criteria alone, but can be supporting trait for P acquisition as the cluster 3 had longest root system in Halidon soil. The two varieties of the Cluster 3 had the greatest root hair length and rhizosheath size; as well these varieties produced the longest root system in Halidon soil, suggesting they may be especially useful in sandy soil types. Besides wider crown root angle and longer root hair and larger rhizosheath size the varieties of cluster 4 will sustain growth well under P stress due to their organic acid releasing capacity.

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Chapter 7 : QTL mapping for root hair length and rhizosheath size of a double haploid mapping population of wheat

Introduction

Previous studies of this thesis have identified root hair length and rhizosheath size as promising traits for improving varietal P responsiveness (Chapter 3, Chapter 6). It has been already established that root hair length is particularly important for P uptake (Gahoonia and Nielsen 1998; Gahoonia et al. 2001), contributing up to 80% of total plant P uptake (Jungk 2001). According to Brown et al (2013) genetic variation in root hair length of barley can be exploited in breeding programmes to improve P uptake and P utilization efficiency.

Measurement of root hair length on a large number of genotypes is time-consuming. Several sources (Delhaize et al. 2012; Delhaize et al. 2015) suggest that measurement of the rhizosheath (the soil that remains firmly attached with root system; (McCully 1999) can be indicative of root hair length. For example, rhizosheath size of wheat at the seedling stage was strongly correlated with root hair length when seedlings were grown in acid soil (Delhaize et al. 2012) and strong correlation was observed when grown in non-acidic soil (Delhaize et al.2015). Contrasting results have also been obtained however, with a poor relationship between rhizosheath size and root hair length observed in barley (George et al. 2014). Among the 10 varieties of wheat used in this study root hair length and rhizosheath size were significantly correlated in Mallala soil (Chapter 3).

Many root traits are complex and controlled by many genes (Ehdaie et al. 2001). The genetic loci that contribute to variation in complex traits are called quantitative trait loci (QTL) (Sharma et al. 2011). QTL affecting root hair length have been identified in a number of plant species including maize (Zhu et al. 2005) and common bean (Yan et al. 2004). George et al (2014) used genome-wide association analysis to identify QTL associated with rhizosheath size of barley. In wheat Delhaize et al (2015) identified several QTLs for rhizosheath size and based on the strong correlation of rhizosheath size with root hair they suggested that those QTLs were associated with the controlling of root hair length.

To investigate the genetic architecture of root hair length and rhizosheath size, in this chapter a QTL mapping approach has been employed using an RAC875/Kukri doubled haploid (DH) mapping population. The RAC875/Kukri DH population was extensively characterised particularly under water stress conditions for yield and key yield components in southern Australian environments (Bennett et al. 2012a; Bennett et al. 2012b), but no work has been done to evaluate root traits in this population. Evidence for potential segregation of root traits within the population is provided by work by Preuss et al (2011) who demonstrated that under P stress conditions RAC875 showed greater percent early vegetative cover than Kukri, and that Kukri showed greater response to added P compared to RAC875; and by McDonald et al (2015) who identified RAC875 as non-responsive to P. The aims of this chapter were (1) to gain further understanding of the relationship between root hair length and rhizosheath size and (2) to identify chromosomal regions associated with root hair length, rhizosheath size and other related traits within the RAC875/Kukri population.

Materials and methods

Plant material

The RAC875/Kukri DH population used in this study was kindly provided by Delphine Fleury (Australian Centre for Plant Functional Genomics). The population consists of 303 individuals but a subset of 200 lines, representing the genetic variation in the population, was selected to reduce work load and resources required for phenotyping.

Rhizosheath screening

Seedlings were grown in white plastic pots 10.5 cm long and 7.0 cm in diameter which contained 355 g of dry, sieved (2mm aperture) soil from Halidon. Each pot was watered with deionised water to 75% field capacity before two pre-germinated seeds with 3-6mm roots were transplanted into each pot. No additional water was added and plants were harvested after 4 days. The seedlings were grown in a controlled environment at 20°C/15°C day/night temperature with a 14h day length. The intensity of PAR in the growth room was 400 μmol quanta/m^2/s. The rhizosheath size was measured using the method of Hailing et al (2010) and one seedling was used and the other was discarded. Briefly, the soil was removed from the pots and the roots were separated carefully from the soil. Roots and shoots were separated and then the roots with the adhering soil were transferred to a plastic tube containing 10 mL deionised water and shaken to remove the soil. The roots were removed, excess water was wiped off by using paper towel and the roots were retained to measure fresh weight, root length and diameter. The tubes containing the soil were then left to settle before excess water was poured off. The tubes

were then transferred to an oven (80°C) for 48 h to dry and the soil weighed. The shoots were dried at 80°C for 48 h to determine their dry weights.

To measure seedling root length and related traits, the seedling roots were gently washed to remove any debris that still adhered to them and then floated on water in a plastic Petri dish and scanned using an A3 Epson Expression-10000 XL scanner. Images were analysed using WinRHIZO 2005 software to record total root length. Root samples were dried in an oven at 80°C for 4 days and the root dry weight measured. Rhizosheath size was estimated as the weight of dry soil per meter of root length.

To measure root hair length, three seeds were germinated on filter paper for three days and measurement was done on single seedling. A dissecting microscope was used to measure root hair length, with ten measurements taken on the primary seminal root per seedling (2× eyepiece magnification). Root hair length was reported in millimetres. A preliminary experiment with the two parent RAC875 and Kukri showed that the root hair length differ between soil grown plants and seedlings grown on filter paper. But the ranking of the parent was similar as there was a strong correlation (r=0.86) of root hair length in between filter paper and soil grown plants.

Statistical design and analysis

A completely randomized block design was followed with two replications. The replicates were grown consecutively because it was not possible to grow both replicates at the same time in the growth room. The statistical analysis were performed using GenStat 17th edition (Payne et al. 2009). Significant differences between genotypes were determined using analysis of variance (ANOVA), and the best linear unbiased estimates (BLUEs) were used for assessing phenotypic correlations and for QTL analysis. Broad sense heritability was also estimated using Genstat 17th edition.

QTL analysis

The map used for QTL analysis is reported in Shahinnia et al. (2016), and is composed of a 'base map' with a selection of 1345 markers that cover 2864 cM of 26 linkage groups assigned to the 21 wheat chromosomes. Composite interval mapping (CIM) was performed using Windows QTL Cartographer version 2.5 (Wang et al. 2012). Model 6, with a 10 cM window, five control markers and backwards regression was used for CIM. Significance thresholds for declaring presence of QTL for each trait were determined from 1000 permutations (Churchill and Doerge, 1994), and empirical QTL confidence intervals determined using a 1-LOD threshold. MapChart (Voorrips 2002) was used for graphical presentation of linkage groups and QTL.

Results

Phenotypic variation

The phenotypic values of the DH population and both parents (RAC875 and Kukri) are presented in Table 7.1. With the exception of root hair length, the mean of the population was between that of the parents for each trait. The largest broad sense heritability was observed for root hair length (0.71) and the smallest for rhizosheath size (0.29)

Transgressive segregation for all traits was observed, with the phenotypic values of the population exceeding that of the parents (Figure 7.1). The frequency distribution of all the measured traits showed continuous variation and were normally distributed (Figure

7.1). The parent RAC875 showed greater values for all traits compared to the parent Kukri.

Most of the traits showed positive correlation with each other, except total root length, which was negatively correlated with average diameter, rhizosheath size and root hair length (Table 7.2). In this study root hair length showed no relationship with any of the traits, with the exception of a weak positive relationship between root hair length and rhizosheath size. Rhizosheath dry weight was strongly correlated with all the traits studied here except for root hair length. A negative correlation of rhizosheath size and total root length was observed and no correlation between rhizosheath size and shoot dry weight was observed (Table 7.2).

Table 7.1. Phenotype of the parent and the DH population

Root traits	Parents		DH population			Heritability
	RAC875	Kukri	Mean ±SD	Minimum	Maximum	
Average diameter (mm)	0.67	0.59	0.60±0.10	0.41	0.92	0.44
Rhizosheath DW (g/plant)	2.00	1.45	1.57±0.56	0.10	3.31	0.34
Rhizosheath size (g/m root length)	5.18	4.11	4.49±1.84	0.58	10.08	0.29
Root DW (mg/plant)	8.00	5.00	6.0±0.002	10.00	20.00	0.54
Root FW (mg/plant)	250.00	110.00	169.00±0.07	20.00	400.00	0.32
Root hair length (mm)	1.25	0.70	1.42±0.39	0.57	2.50	0.71
Shoot DW (mg/plant)	10.00	5.00	7.0±0.002	10.00	13.00	0.35
Total root length (cm/plant)	38.66	35.42	37.53±12.21	7.9	73.05	0.30

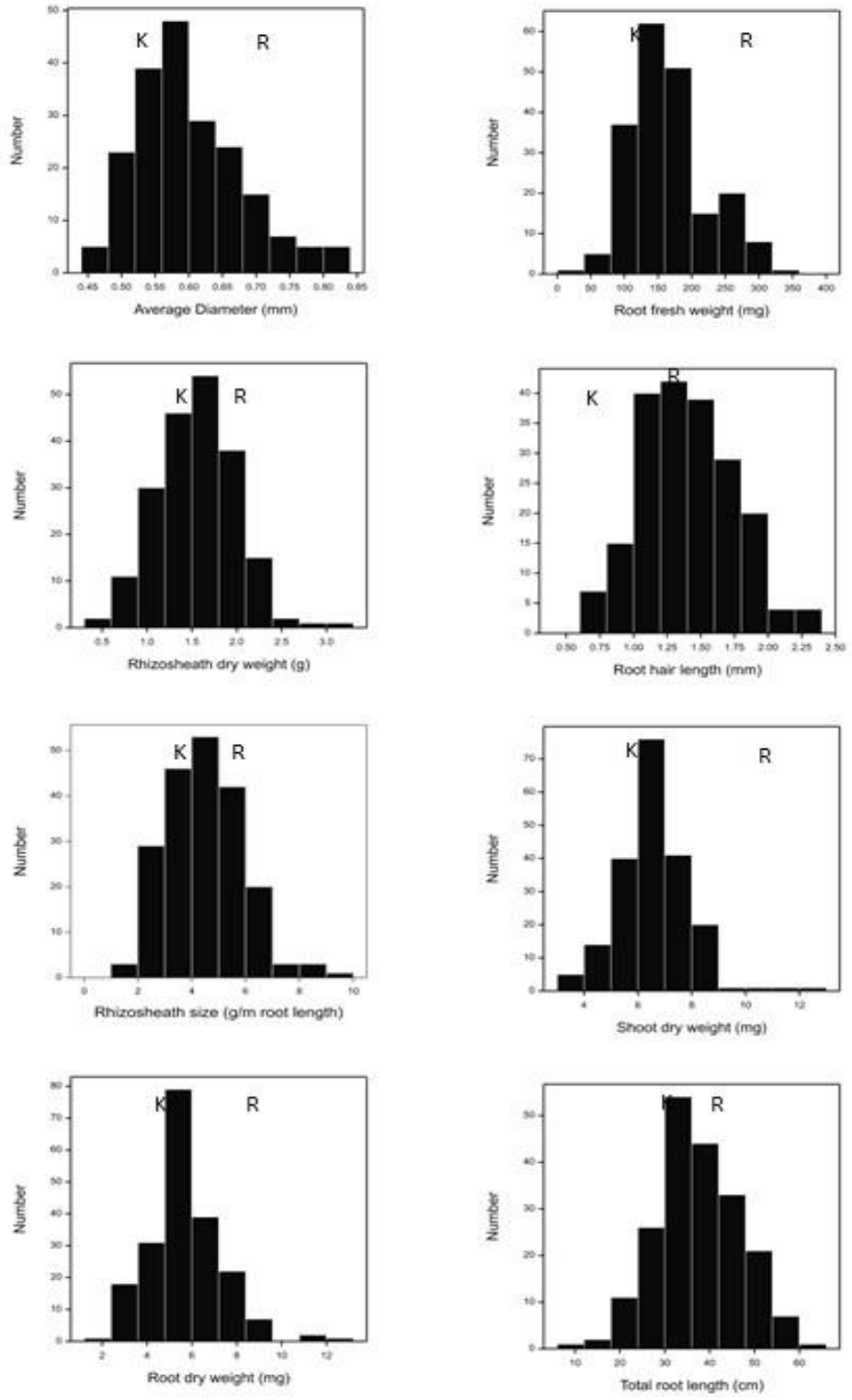

Figure 7.1. Histograms of frequency distribution of root traits. Data are the means of 200 lines (K= the parent Kukri and R= the parent RAC875)

Table 7.2. Correlation of rhizosheath size and root hair length and other traits for all double haploid lines (The levels of significance are: * P<0.05; **; P<0.01 and ***; P<0.001)

Root trait	Average diameter (mm)	Rhizosheath DW (g/plant)	Rhizosheath size (g/m root length)	Root DW (mg/plant)	Root FW (mg/plant)	Root hair length (mm)	Shoot DW (mg/plant)	Total root length (cm/plant)
Average diameter (mm)								
Rhizosheath DW (g/plant)	0.005							
Rhizosheath size (g/m root length)	0.58***	0.57***						
Root DW (mg/plant)	0.37***	0.46***	0.23**					
Root FW (mg/plant)	0.17*	0.48***	0.19**	0.58***				
Root hair length (mm)	0.06	0.02	0.15*	-0.12	-0.09			
Shoot DW (mg/plant)	0.18*	0.34***	0.10	0.49***	0.45***	-0.02		
Total root length (cm/plant)	-0.62***	0.35***	-0.50***	0.19**	0.24***	-0.15*	0.23***	

QTL detection

A total of 26 QTLs were identified on fifteen chromosomes; 1B, 1D-2, 2A, 2B, 2D, 3A-2, 3B, 4A, 4B, 5A, 5B, 6A, 6B, 6D and 7A-1 (Table 7.3). The QTL accounted for between 3.8 and 9.7% of the phenotypic variation, with the LRS (likelihood ratio statistic) ranging from 9.29 to 23.36. Five QTL were detected for each of rhizosheath dry weight (Rhizo DW), rhizosheath size (RhizoVol) and root fresh weight (RFW); three QTL for shoot dry weight (SDW), root dry weight (RDW) and root hair length (RHL); and one QTL for both average diameter (AvgDiam) and total root length (TRL)

The major QTL for rhizosheath dry weight was located on chromosome 5A explaining 9.7% variation and it was co-localized with a QTLs for root fresh and dry weight and shoot dry weight, with positive allele inherited from RAC875. A second QTL on chromosome 4B explaining 9.6% of the variation for rhizosheath dry weight was detected and the source of the positive allele was the parent Kukri. Co-localization of two other QTLs controlling root and shoot dry weight on the chromosome 4B was also detected and positive allele controlling both traits came from Kukri. Two other QTLs for rhizosheath dry weight was detected on chromosome 2B and 6B, explaining 9.3% variation altogether where the Kukri allele resulted in higher rhizosheath dry weight.

The major QTL for rhizosheath size was detected on the chromosome 7A-1 at 2.75 cM which explained 8.2% of the variation (positive allele from RAC875); on the same chromosome another QTL for rhizosheath size was detected at 83.5 cM which explained 3.9% variation (positive allele from Kukri). In total, five QTLs contributing to variation in rhizosheath size were detected. Two QTL explaining 14.6% variation were inherited from the parent RAC875, whereas the other three QTL, explaining a combined 16.9% of the variation were inherited from Kukri.

Table 7.3. List of all the QTLs and peak marker detected for this study. LRS = likelihood ratio statistic. A positive additive effect indicates that an RAC875 allele is increasing trait values, whereas a negative additive effect indicates that a Kukri allele is increasing trait values

Trait	Chromosomal location	Peak marker	Distance (cM)	LRS	% variation	Additive effect
Average diameter (mm)	6A	wsnp_Ex_c6604_11441257	16.09	9.78	4.6	-0.017
Rhizosheath DW (g/plant)	2B	wsnp_JD_c23434_20022750	13.37	10.21	4.1	-0.090
	4B	Tplb0061a20_153	67.44	22.23	9.6	-0.139
	5A	BS00111119_51	24.74	23.36	9.7	0.139
	5B	BS00009335_51	102.96	13.65	5.5	0.106
	6B	Kukri_c66478_299	122.81	12.76	5.2	-0.104
Rhizosheath size (g/m root length)	2D	RAC875_c24201_984	39.51	14.00	6.4	0.355
	3A2	wsnp_Ex_c21950_31124594	85.98	13.01	5.8	-0.338
	5B	Ku_c12603_639	49.24	17.46	7.2	-0.622
	7A1	Tdurum_contig42694_1450	2.75	16.51	8.2	0.413
	7A1	CAP8_c3496_118	83.51	9.29	3.9	-0.278
Root DW (g/plant)	4A	D_GCE8AKX01B34J2_144	68.48	10.89	4.8	0.001
	4B	wsnp_Ex_c5187_9195120	69.38	17.15	8.1	-0.001
	5A	BS00079189_51	20.14	19.05	8.5	0.001
Root FW (g/plant)	1D2	BS00000717_51	12.64	11.43	4.7	-0.012
	2A	BS00092550_51	96.88	9.59	4.4	-0.012
	3B	nw2711	28.48	14.88	6.6	-0.015
	5A	D_F5MV3MU01AYYIX_194	24.28	11.41	5.0	0.013
	6D	RAC875_c7178_404	0.00	11.99	5.0	0.013
Root hair length (mm)	1B	BS00072289_51	122.07	10.01	4.6	0.077
	7A1	Tdurum_contig42694_1450	2.75	12.85	6.1	-0.091
	7A1	BobWhite_c15497_199	84.43	16.84	7.9	-0.102
Shoot DW (g/plant)	2A	BobWhite_c19822_818	94.59	11.90	5.3	-0.001
	4B	IACX5640	96.00	12.49	5.5	-0.001
	5A	wsnp_Ku_c9559_16000086	10.03	12.06	5.3	0.001
Total root length (cm/plant)	3B	wsnp_Ex_c3907_7088011	82.17	10.19	4.6	-1.939

On chromosome 7A-1, two QTLs for root hair length were detected and one of these was co-localized with the QTL for rhizosheath size. An allele inherited from the parent Kukri was associated with increased root hair length. Another QTL on chromosome 1B explaining 4.6% variation was detected and in this case the parent RAC875 was associated with increased root hair length.

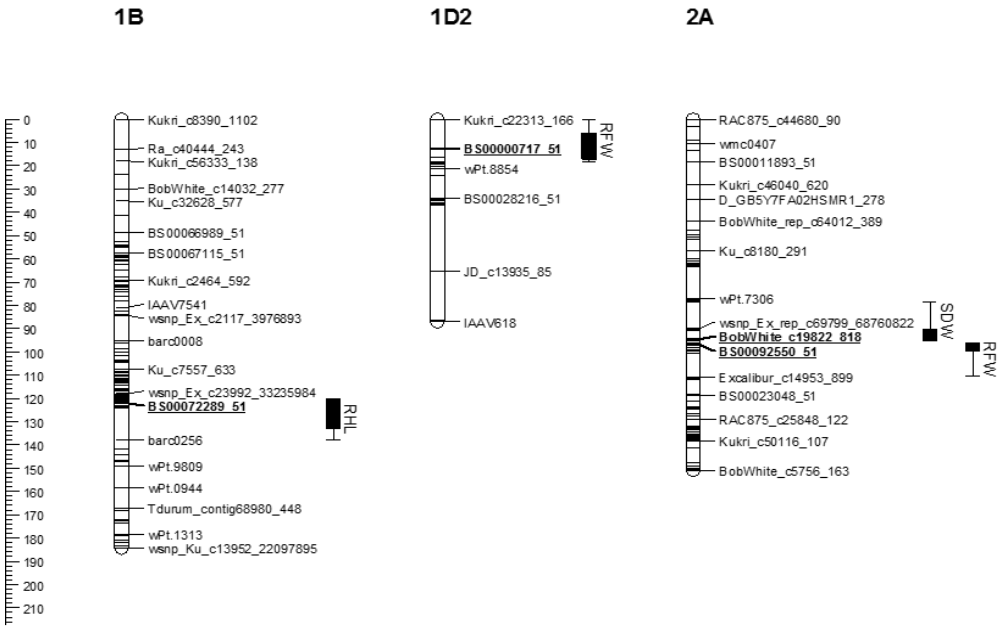

Figure 7.2. QTL detected for rhizosheath size (RhizoVol), root hair length (RHL), root dry weight (RDW), root fresh weight (RFW), rhizosheath dry weight (RhizoDW), shoot dry weight (SDW), total root length (TRL) and average diameter (AvgDiam) of RAC875× Kukri population. Peak markers for each of the traits are highlighted, bold and underlined.

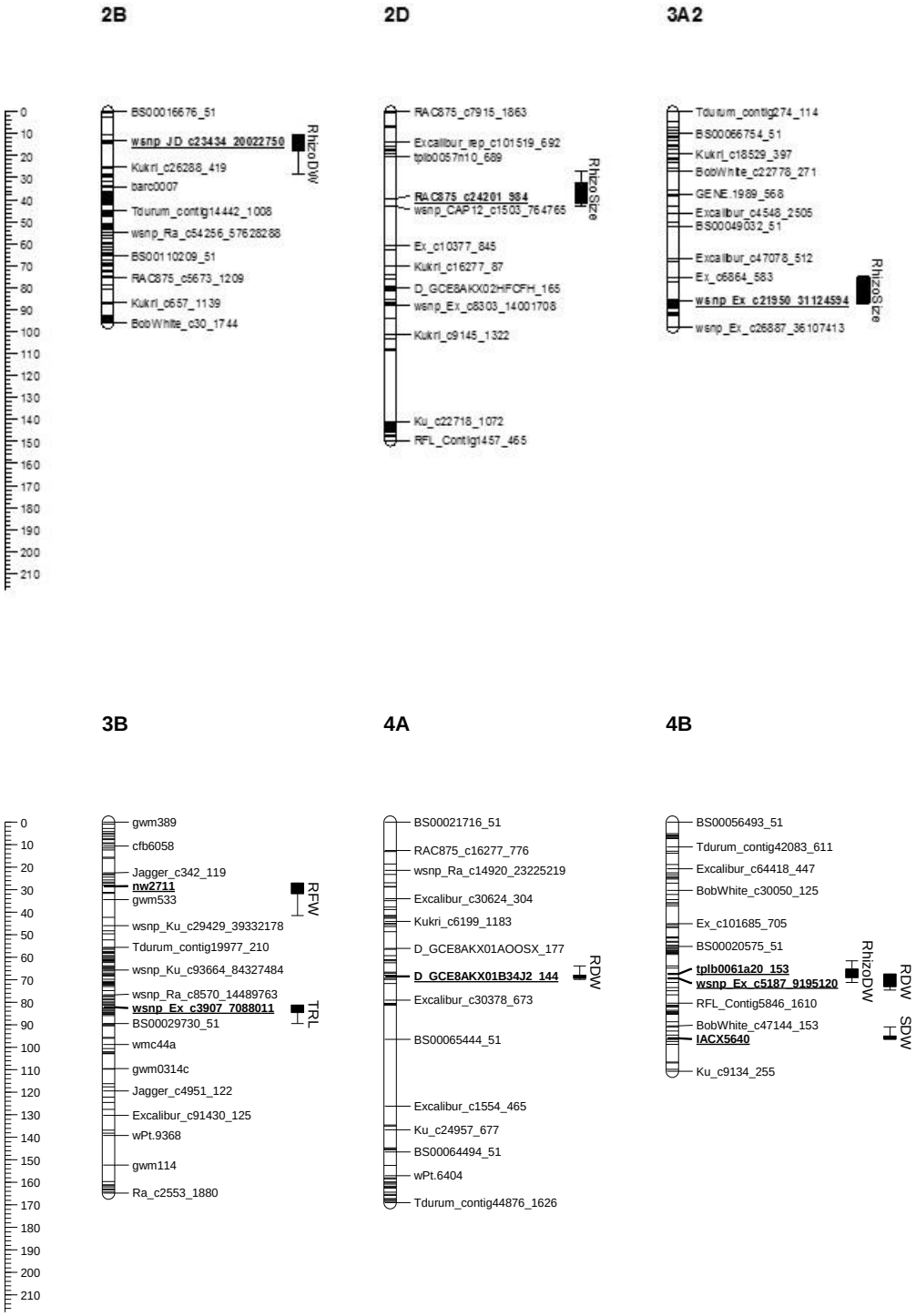

Figure 7. 2. Continued

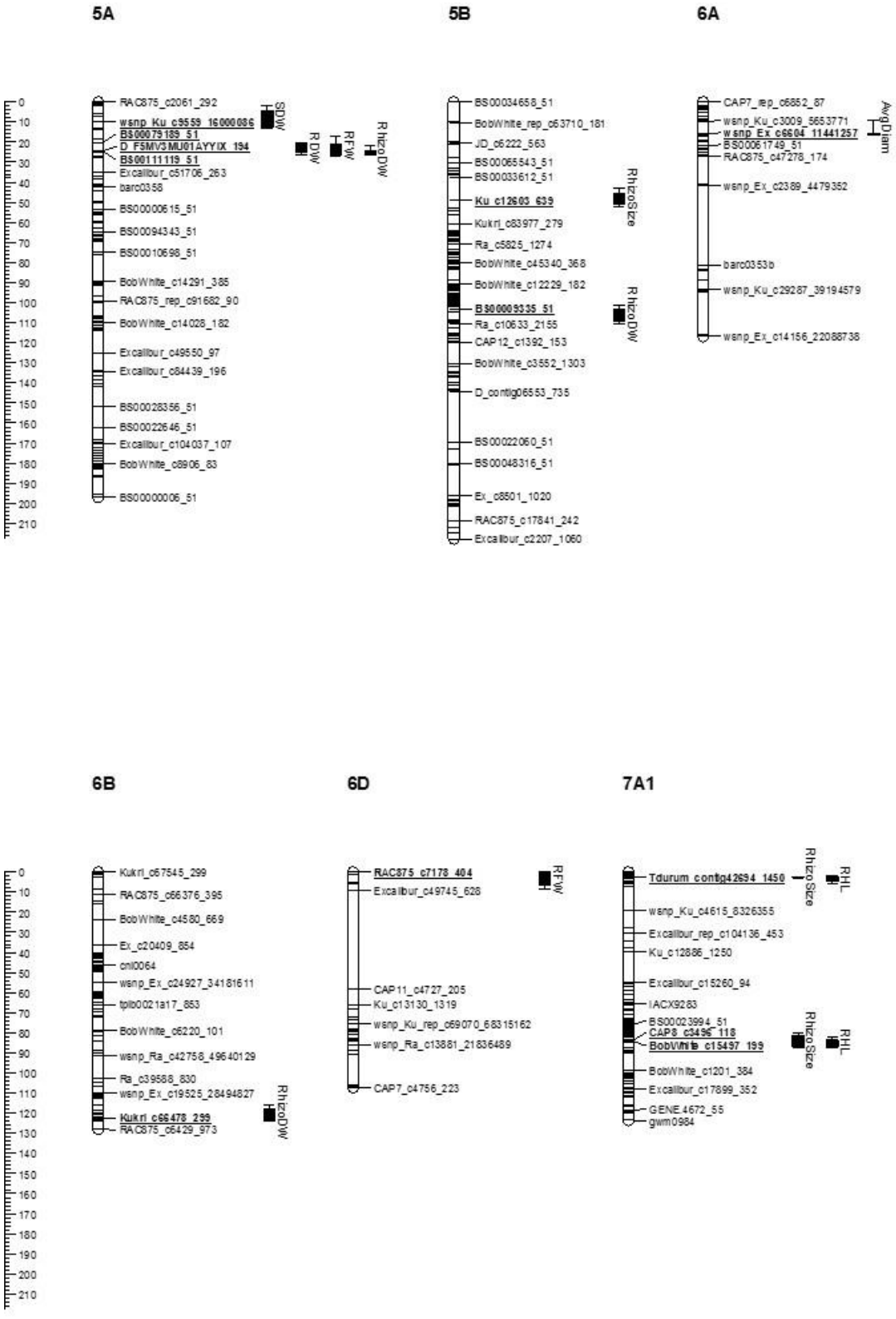

Figure 7. 2. Continued

Discussion

In the previous chapters of this thesis, root hair length and rhizosheath size were identified as promising traits for improving P uptake. In the current chapter, a QTL mapping approach was used to screen a subset of the RAC875/Kukri wheat DH mapping population to identify loci that contribute to variation in these important root traits. The population was grown under controlled environments, and traits including average diameter, rhizosheath dry weight, rhizosheath size, root hair length, root fresh and dry weight, shoot dry weight and total root length were measured.

Transgressive segregation for all the measured traits were observed, indicating that each of parents possess alleles which contribute to variation in the measured traits. The parent RAC875 showed greater value for all the traits studied here compared to the parent Kukri, which may reflect the P responsiveness of this variety. The performance of the parent RAC875 is consistent with the findings of other studied of this thesis (Chapter 3 and Chapter 5).

Five QTL (located on chromosomes 2D, 3A_2, 5B and two QTL on 7A_1) accounted for 31.5% of the variation in rhizosheath size. Of these, the QTL on chromosome 7A-1 at 84.43 cM, explaining 8.2 % of the phenotypic variation is of most interest. This QTL is located in the same region as reported by Delhaize et al (2015), who found a major QTL for rhizosheath size on this chromosomes which explained 9.2 % of the genetic variation in a multi-parent advanced generation intercross (MAGIC) wheat population. Further, in the current study this rhizosheath size QTL co-located with a QTL for root hair length and in both cases the Kukri allele resulted in an increase in root hair length and rhizosheath size. The detection of this QTL using different soil types and with different populations suggests that it may be considered as a robust QTL,

and that the markers that flank this QTL could be used by breeding programs in marker-assisted selection to improve rhizosheath size. There is no evidence from the literature for the four additional QTL that contribute to rhizosheath size that were identified in this study, suggesting that these may be novel QTL for rhizosheath size.

In this study, two alternative approaches were used for measuring rhizosheath size: one based upon rhizosheath dry weight (g per plant) and the other based upon rhizosheath size (g per m root length). The relationship between the two measures was significant ($r = 0.57$; Table 7.2) but QTL for the two measures did not co-locate. Interestingly, the QTL for rhizosheath dry weight on 5B co-locates with a QTL for root hair length that Delhaize et al. (2012) identified in their study. The lack of common QTL for the two measures of the rhizosheath are not uncommon; for example, George et al (2014) identified a locus on barley chromosome 2H (LOD 4.47) that was detected for rhizosheath weight (total soil adhering with the root system) but not associated with specific rhizosheath weight (g of soil g^{-1} of root). Together, these results suggests that alternate methods of determination of rhizosheath size and volume may provide additional evidence for the value of particular rhizosheath QTL, and their potential relationship with root hair length.

Three QTL were detected for root hair length, located on chromosomes 1B and 7A_1 (two QTL). The 1B QTL did not co-locate with rhizosheath size or dry weight, whereas each of the 7A_1 QTL did co-locate with QTL for rhizosheath size. As previously mentioned, Kukri contributes to the increase in root hair length and rhizosheath size for the 7A_1 QTL at 84.43 cM. In contrast, for the QTL on chromosome 7A_1 at 2.75 cM each parent contributes contrasting effects, with an RAC875 allele associated with an increase in rhizosheath size, but a Kukri allele associated with an increase in root hair length. These findings are consistent with the weak phenotypic correlation between

root hair length and rhizosheath size (r = 0.15) that was found in the current study. Delhaize et al (2012) observed a strong correlation of rhizosheath size with root hair length in wheat, and concluded that rhizosheath size can be a reliable surrogate for root hair length when wheat seedlings are grown in an acid soil. In contrast, a study by George et al ((2014)) observed a weak relationship of root hair length with rhizosheath size in barley, and concluded that rhizosheath size will not be good indicator, if the aim is to select for root hair length. The findings of this study are in agreement with the findings of George et al (2014). Numerous QTL were detected for the other traits that were measured but generally, there was little co-location of QTL. The exceptions are QTL for rhizosheath size and root hair length on chromosome 7A_1 (as previously discussed); the QTL for shoot dry weight and root fresh weight on chromosome 2A; the QTL for root dry weight and rhizosheath dry weight on chromosome 4B; and the QTL for rhizosheath dry weight, root fresh weight, root dry weight, and shoot dry weight on chromosome 5A. For the 2A and 4B QTL, alleles from the Kukri parent are associated with increased trait values, whereas for the 5A QTL alleles from the RAC875 parent are associated with increased trait values.

Under P deficiency two QTL were detected on chromosome 5A for shoot dry weight and a positive linkage with P uptake efficiency was observed on the same chromosome for wheat at the seedling stage (Su et al. 2006). A QTL controlling shoot dry weight on chromosome 5A was also detected on a DH wheat population by Bai et al (2013). Two QTLs controlling thousand grain weight of Chinese winter wheat was detected by Wang et al (2009) on chromosome 5A on the similar region of this study. Targeting the chromosomal region of 5A will be useful for further breeding programme and for marker aided selection. QTLs related to biomass were detected on the chromosome 4B and co-localization with yield trait was observed by Xie et al (2016) and from this study

on the same region of chromosome 4B a QTL for shoot dry weight was detected. This findings suggests the stability of the QTL and the region of the chromosome could be targeted for selection to improve biomass and yield production. On chromosome 4B co-localization of rhizosheath dry weight and root dry weight was also observed, on the same chromosome another QTL for shoot dry weight was also detected with an interval from the QTLs for rhizosheath dry weight and root dry weight, Xie et al (2016) also observed co-localization of biomass related traits on this same region. So this region of the chromosome 4B will be useful for selection to improve biomass production and yield. In this study two co-localized QTLs for shoot dry weight and root fresh weight were detected on chromosome 2A, the flanking marker region is coincides with the region for grain yield and yield component detected by Bennett et al (2012b). The QTLs detected from this study on chromosome 2A, 4B and 5A and the association of the chromosomal region with yield and yield related trait from previous studies from literature warrants further physiological and genetic dissection for breeding programs to improve yield through marker aided selection.

The majority of markers used to produce the genetic linkage map in this study are from the 90K iSelect gene-associated SNP platform (Wang et al. 2014). Few studies that have focussed upon root traits have been conducted using this high-density marker platform, and hence direct comparisons between QTL detected here and with QTL from the literature are difficult. However, several studies on other traits in wheat have been conducted using this platform. QTL for ear emergence time were also detected on chromosome 7A-1(at 79.7 and 95.2 cM) (Bennett et al. 2012a), in this study the position of the peak marker of the two QTLs controlling rhizosheath size and root hair length is in between the position of marker for ear emergence identified by Bennett et al (2012b).

On the chromosome 7A_1 a QTL controlling yield under field condition was detected and co-localization of stomatal trait was also observed by Shahinnia et al (2016), the marker that flanked the QTL is the same peak marker that was detected for root hair length of this study. As it is known that root hair length contributes towards P uptake of wheat these QTLs may facilitate the design root architecture for optimum P uptake. Identification of more than one QTL on the same chromosome will allow selection for multiple traits at a time. Another QTL controlling heading date was detected on the chromosome 7A-1 and the closest marker was *wsnp_Ku_c6065_10682531* (at 71.07cM) (Mahjourimajd et al. 2016). The work by Mahjourimajd et al (2016) also identified QTLs for relative maturity on the chromosomes 2A, 2B and 4A. This study also detected QTLs on chromosome 2A, 2B and 4A and it was on the same region as QTLs identified by Mahjourimajd et al (2016). A major locus controlling heat stress was detected on the short arm of chromosome 3B (marker interval *wsnp_Ra_c41135_48426638* to *wsnp_BE497169B_Ta_2_1*) by Shirdelmoghanloo et al (2016), which is closely associated with the peak marker identified for root fresh weight of this study.

In summary, QTL for rhizosheath characteristics (per plant and per m root length) and root hair length have been mapped using the RAC875/Kukri population, and the relationship between QTL for these and associated traits explored. Despite the weak phenotypic correlation between root hair length and rhizosheath characteristics, co-located QTL were detected on chromosome 7A, and literature supported the effect of this. Co-localization of QTLs on chromosome 2A, 4B and 5A were observed and information from the literature also support that the region on these chromosomes are important for yield and yield related traits. Phenotypic distributions confirmed that root

characteristics such as these are complex in nature. However, the assays that were conducted are high-throughput in nature with repeatability that would warrant selection for further marker aided breeding program.

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Chapter 8 : General discussion

Introduction

The main objective of the thesis was to assess the importance of root traits for P uptake in low P environments among 10 varieties of wheat and to estimate their contribution towards differences in P responsiveness among the varieties. The hypothesis was that root traits of the non-responsive varieties will be different than those of the responsive varieties. The previous chapters (Chapter 3-5) examined the variation in selected root traits that have been identified as being responsive to P and which often have been suggested as important for genetic differences in P efficiency. Chapter 6 undertook a comparative analysis of all the root traits studied for this thesis and Chapter 7 provided information on the genetic control of some the root traits identified in Chapter 6 that appeared to be most consistently associated with P responsiveness. The aim of this chapter is to discuss the key findings of this thesis. In this chapter varietal selection will be discussed along with some important direction for future work.

Trait dissection for P responsiveness

A common approach to assessing the importance of a trait to P efficiency is to examine genetic variation in a particular trait under controlled conditions among genotypes, genetic populations or in near isogenic lines. Promising lines can then be tested in the field for their P responsiveness and P efficiency. This approach has been used successfully in common bean (Liao et al 2004) and maize (Zhu et al 2005) to assess the value of root angle, but frequently root traits are assessed without a critical evaluation of their value to P efficiency under field conditions. There are also a number of studies

where there has been little or no validation of the importance of the root trait in field conditions (Bates and Lynch 2001; Liao et al 2001; Walk et al 2006; Zhu and Lynch 2004). Moreover, there are a number of potential root traits that can contribute to improvements in P efficiency and many are listed in reviews on the need to breed for more P efficiency varieties without any assessment of the relative merits of the different traits. The approach taken in the current study differs from many previous studies in that it assumes no prior knowledge of the merits of different root traits. Instead, it is based on firstly identifying varieties that differ in their P response and then examining the variation in a number of different root traits to see if there are consistent differences between the P-responsive and non-responsive groups. The approach assumes that those traits that show the most consistent differences will, potentially, be the most valuable for selection for improvement in P efficiency. However, selection of appropriate germplasm to examine differences is critical to this approach. It is also recognised that variation in yield at low P and the response to P will be due to characteristics that were not considered in this study. However, the main objective was to investigate the importance of different root traits to PUE.

Selection of varieties

The analyses presented in the thesis were based on the yield response to P of a large number of varieties in the field that was described by McDonald et al (2015). Varieties that differed in their P responsiveness in grain yield in these field trials were chosen for the study and the evaluation of root traits was performed at the seedling stage in controlled environments. A reason for this was to examine how well assessing seedling response to P can help explain differences observed at maturity. Plant responses to P at

the seedling stage can be quite different than at maturity. According to Wang et al. (2010) plant genotypes with a high P uptake capacity at the seedling stage may not necessarily have a similar high ability to take up and utilise efficiently P in later growth. Apart from the high P uptake during the initial vegetative phase of growth, wheat yield depends on generative growth such as spikelet number and grain development (Wang et al. 2010). The P required for grain development comes from two different sources, post anthesis uptake which goes directly to the grain and from the remobilization of stored P in the vegetative plant parts before anthesis (Wang et al. 2010). According to P availability, climate and plant genotype the amount of remobilization of P to grain varies considerably and can range from 11 to 100% (Batten et al. 1986; Papakosta 1994), which plays a key role towards varietal P efficiency at maturity.

The selection of varieties was based on their grain yield response to P rather than their vegetative response in field trials and so any disparity in the interpretation between the field and the controlled environment experiments may be associated with comparing early vegetative responses with yield responses. To assess whether this may had an effect on the interpretation of the results and the classification of varieties, cluster analysis (Chapter 6) of the data from the controlled environment experiments was used to identify different groups of varieties. When varieties were analysed using cluster analysis based on their shoot dry weight at 0 kg P/ha at Halidon and Mallala soil across all the experiments two major groupings were evident which corresponded to the yield responsiveness of varieties (Figure 8.1). The three responsive varieties, BT Schomburgk, Krichauff and Wyalkatchem, formed separate groups to the seven non-responsive varieties, with BT Schomburgk separating from Krichauff and Wyalkatchem. Most of the non-responsive varieties clustered together (cluster 1) and the two responsive varieties Krichauff and Wyalkatchem clustered together (cluster 3).

Comparison between cluster 1 and cluster 3 demonstrate that varieties of cluster 1 produce more shoot dry weight over the varieties in cluster 3 (Table 8.1). The varietal categorization from the experiments based on seedling responses was similar to that of grain yield with little variation (except BT Schomburgk). This was also demonstrated in Chapter 3 where the findings of shoot dry weight and varietal response to P was comparable to the observed field result conducted by McDonald et al (2015).

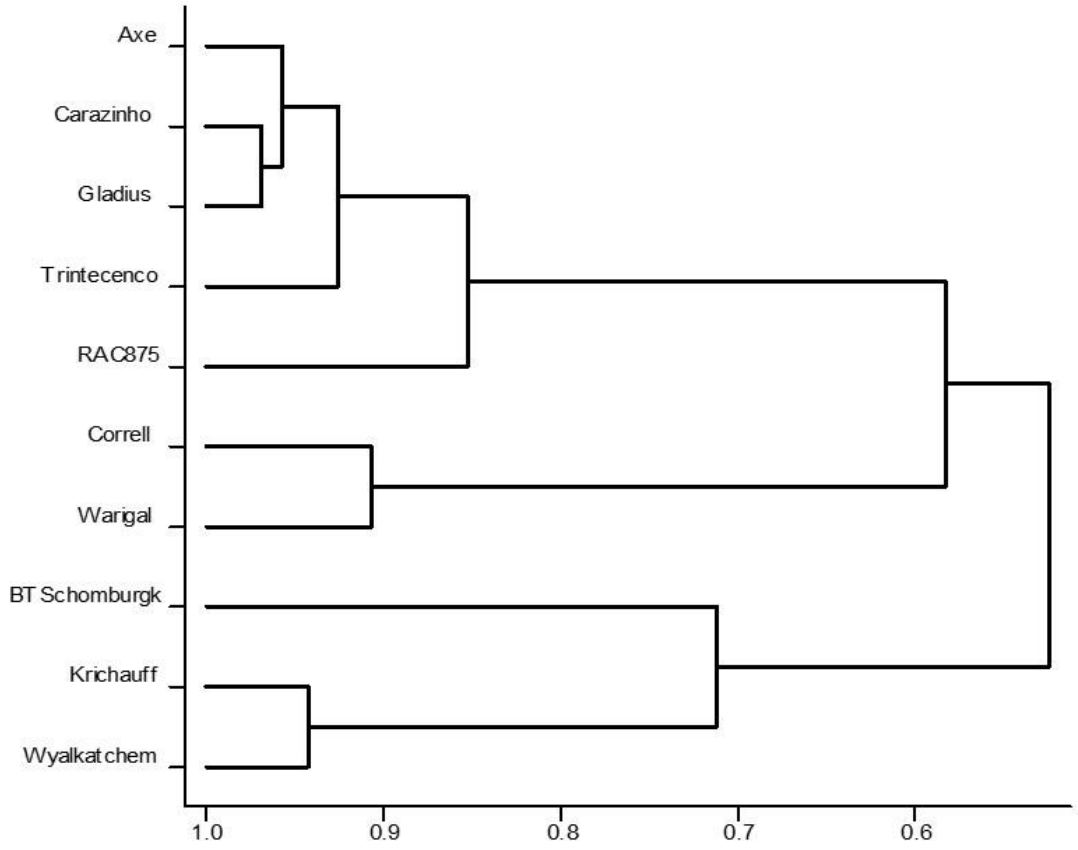

Figure 8.1. Clustering of varieties according to their shoot dry weight at 0 kg P/ha and also from two different soil type (values were taken from Experiment 3 & 4 from Chapter 3; Experiment 2a from Chapter 4 and from Chapter 5)

Table 8.1. Mean shoot dry weight (mg/plant) at 0 kg P/ha showing four different cluster group (data was taken from some previous experiment conducted for this thesis)

Cluster group	Chapter 3			Chapter 4	Chapter 5
	Experiment 3	Experiment 4		Experiment 2a	
Cluster1	28.3	48.6	38.0	231.3	22.3
Cluster2	38.2	36.5	30.2	184.7	19.2
Cluster3	25.6	34.6	30.7	185.0	16.6
Cluster4	28.1	37.4	20.6	300.0	17.0

[A] Group 1: Axe, Carazinho, Gladius, Trintecenco, RAC 875; Group 2: Correll, Warigal; Group 3: Krichauff, Wyalkatchem; Group 4: BT Schomburgk

Key findings

The main focus of this study was to understand the contribution of several root traits to varietal differences in P responsiveness. The major findings of this thesis are presented and discussed below.

The importance of root hair length

Root hair length was found to be the trait that was most consistently associated with differences in P responsiveness between the two groups of varieties. Average root hair length of non-responsive varieties was consistently longer than that of responsive varieties across experiments (Chapter 3 and Chapter 5) and soil type, which showed high genetic control of this trait. It also demonstrated that the longer root hairs of the non-responsive varieties contributed to their superior performance under field condition. Modification in root hair growth can be an important root trait that can be achieved with very low carbon cost (van de Wiel et al. 2016). Increased root hair length

and densities were related to increased P uptake capacity (Wang et al. 2004; Yan et al. 2004). The findings of Chapter 3 outlined a positive correlation between root hair length and shoot dry weight in Halidon soil, which demonstrates the importance of root hair length to seedling growth in this sandy soil. The same relationship was not found in the heavier-textured Mallala soil, which was due in part to the shorter root hairs in Mallala soil and the smaller amount of variation among the varieties. Overall the ranking of varieties based on their root hair length was similar across the different soil type. Hailing et al (2014) reported that root hair length was less sensitive to high soil strength compared to root length and will be sufficiently long to benefit P acquisition in denser soil.

Shoot P uptake was positively correlated with total root length and root hair length, as both these root trait can significantly increase nutrient absorption area by root system. The consistency and the relationship of longer root hair with varietal P responsiveness warrants that selection of this trait for crop improvement is possible. Three QTLs controlling root hair length were detected on chromosomes 1B and 7A-1(two QTLs) and one of them was co-localized with rhizosheath size. There was weak phenotypic correlation of root hair length with rhizosheath size but high heritability was observed for root hair length suggesting that the trait could be a reliable selection criteria for improving P responsiveness of wheat varieties.

Rhizosheath size

Rhizosheath sizes varied significantly among the 10 varieties and there were differences between the two groups of varieties. Along with root hair length, differences in rhizosheath size were most consistently associated with the differences in P responsiveness between the two groups of genotypes. The rhizosheath sizes of the non-

responsive varieties were higher than those of the responsive group of varieties when grown in two different soil and also grown at two different P level (Chapter 3). The non-responsive variety Carazinho maintained consistently higher rhizosheath size which was comparable to the findings of Haling et al (2010), which was conducted on acid soils. In Halidon soil rhizosheath size was positively correlated with shoot P uptake. Rhizosheath size in Mallala soil was strongly correlated with root hair length, but surprisingly not in Halidon soil. This could be due to the fact reported by Haling et al (2014) that soil particle of denser soil remains tightly attached with the root hair and will reduce disintegration during mechanical handling.

Although some studies claimed a strong positive correlation of root hair length and rhizosheath size (Moreno-Espindola et al. 2007, Haling et al. 2010, Delhaize et al. 2012), contrasting results also exist (George et al 2014). Other than root hair length, many factors such as plant and microbial mucilage production, soil texture, pH, bulk density and soil moisture content are known to influence rhizosheath formation (Haling et al. 2014; Watt et al. 1993; Watt et al. 1994). The heritability for rhizosheath size was found to be high, which suggest high genetic control and potential for selection for rhizosheath size. High heritability was observed for rhizosheath size of barley (George et al. 2014). Measuring rhizosheath is relatively easy and on the basis of strong correlation of rhizosheath size and root hair length of wheat on acid soil Delhaize et al (2012) reported that rhizosheath size can be a reliable surrogate for root hair length, this was later confirmed by James et al. (2016). Not only on acid soil but also in non-acid soil a strong correlation of rhizosheath size with root hair length of wheat was observed by Delhaize et al (2015). Both root hair length and rhizosheath size will differ with soil type and other environmental factors and Delhaize et al (2015) argued that in case of longer root hair (longer than 1mm) rhizosheath size may not strongly correlate with root

hair length. In the QTL analysis (Chapter 7) despite a weak phenotypic correlation of rhizosheath size with root hair length, co-localized QTLs were detected on chromosome 7A, and on the same region a QTL controlling rhizosheath size was also detected by Delhaize et al (2015). Four novel QTLs controlling rhizosheath size were also detected from this study. Co-localization of other QTLs on chromosome 2A, 4B and 5A was also observed and information from available literature suggests that those chromosomal regions are important for yield and yield related components. On chromosome 4B and 5A rhizosheath dry weight was co-located with other QTLs suggesting that targeting those chromosomal regions could improve rhizosheath characteristics such as rhizosheath mass and rhizosheath size.

Seminal and crown root angle

It has often been suggested that root angle is important for improved P uptake efficiency and there are a number of studies, largely with dicotyledonous species (Ho et al. 2005; Liao et al. 2001; Lynch and Brown 2001), that have supported this argument. The results of this study are equivocal about the value of root angle: seminal root angle showed a different relationship to P responsiveness than crown root angle. The argument in previous studies has been that a wide root angle promotes greater exploration of the P-rich surface layers of soil, improving yields at low P and, by inference, reducing the need for additional P fertiliser. This study demonstrated that the crown root angle was more strongly related with varietal P responsiveness than seminal root angle (Chapter 3), with the non-responsive varieties generally having wider crown root angles. Adventitious, or crown roots of wheat, grow mainly in the upper soil layers and the number of adventitious roots are positively correlated with the tillering ability of plants (Chapter 3, Manske et al. 2001), but there was no association between crown root angle and tiller number, suggesting they are independent traits. Adventitious roots

have many advantages over other root classes such as, shallow growth angle, increased top soil foraging and reduced inter-root competition (Lynch 2011; Lynch and Brown 2001). It is evident from this study that the shallow crown root angle of the non-responsive varieties was associated with their low P responsiveness in the field.

Total root length

It has been observed that the yield stability of oat and barley was related to their total root length (Leon and Schwarz 1992) and total root length of winter wheat was positively correlated with grain yield (Barraclough 1984). While genetic variation in total root length was found among the 10 varieties there was no significant difference between the two groups of varieties in their total root length (Chapter 3 and Chapter 5). This suggests that early seedling root elongation was not an important trait explaining the differences in P responsiveness. Though there was no relationship between varietal P responsiveness and total root length, it cannot be completely ignored as an indicator for P uptake because it determines the soil absorption area. The work was conducted over a short period of time in small volumes of soil, so any effects of genetic differences in total root length may have been masked. However, from the results of this study it can be concluded that total root length of seedlings alone cannot be a selection criteria for evaluating P efficiency.

AMF colonization

Infection of roots by AM fungi can enhance P uptake when soil P availability is low (Hetrick et al. 1992; Kaeppler et al. 2000; Koide et al. 1988) and genetic differences in AM fungal infection of roots have been reported previously (An et al. 2010; Baon et al. 1993; Hildermann et al. 2010; Kaeppler et al. 2000; Smith et al. 2009). This study also

demonstrated significant differences in infection by AM fungi, however it was not possible to relate the degree of infection to P responsiveness in the field trials. Nevertheless, there was evidence that some varieties, such as RAC 875 and Carazinho, were able to maintain high AMF infection consistently over a range of conditions. Interestingly, these varieties were non P-responsive varieties. The results from the field for AMF colonization showed that Carazinho and RAC875 maintained high colonization, while Carazinho and Trintecenco also maintained high colonization regardless of the P treatment in the controlled environment experiments. A positive correlation of shoot dry weight with AMF colonization was observed only at nil P treatment suggesting that selection for greater colonisation by AMF could contribute to growth only under very deficient conditions. No or negative correlation of AMF with P uptake and P concentration was also observed. The benefit of the symbiosis will depend on the soil P status and the carbon drain by the AMF fungi. A study Li et al (2005) observed that grain yield of non-mycorrhizal wheat was greater that of the mycorrhizal plants when soil P was limited and this was due to the carbon drain by the fungi. Based on the results of this study it can be concluded that selection for higher AMF colonization will not be beneficial in terms of an adaptive mechanism at P deficient condition.

Organic acid exudation by roots

Significant genetic variation in organic acid releasing capacity and changes in rhizosphere pH were observed in this study (Chapter 5), but it was not possible to demonstrate the relationship between organic acid releasing capacity and varietal P responsiveness. Delhaize et al (1993) observed that P deficiency did not induced the exudation of organic acid of wheat. The non-responsive variety Carazinho is known to secrete citrate constitutively from its root apices (Ryan et al. 2014) but it ranked below

average (Chapter 6) for citrate and poorly ranked for malate release into the rhizosheath soil. One possible reason for the non-responsive variety Carazinho not to be consistent in citrate secretion could be due to the microbial degradation. As very fast degradation of citrate was observed by Khademi et al (2010). Ryan et al (2014) concluded that Carazinho had other attributes other than citrate efflux for its better performance under P deficient condition. The results of this study also were similar to findings of Ryan et al (2014) as there were no relationship between shoot dry weight and the organic acid releasing capacity in a series of field trials.

Conclusion

Varietal selection based on root traits is complex and to get the maximum benefit from selected root traits in terms of P responsiveness will depend on which environment the plant genotypes are growing. Lynch (2015) outlined several merits of trait-based selection including, traits are more robust and stable than yield and trait based selection will facilitate selection of suitable varieties that can grow well in specific growing environment. However, ultimately the trait needs to show a benefit in yield as this is what drive the economic return to the farmer. Based on the results of this study it can be concluded that most likely crown root angle, root hair length and rhizosheath size are the traits that can best explain differences in P responsiveness between the two groups of varieties used in this study and will contribute towards their performance in a P deficient environment. Novel QTLs for rhizosheath size were detected from this study and on chromosome 7A a QTL for rhizosheath size was co-localized with the QTL for root hair length. Co-localized QTLs were also detected on other chromosomes and with references from literature it can be concluded that the chromosomal region identified from this study can be selected for gaining further understanding on the

genetic control of those traits and could be targeted for marker aided selection to improve wheat varieties.

Future direction

Although this study has addressed several questions, it encountered some difficulties and some questions remains unsolved. Some steps to follow for future research to clarify those unsolved questions are described below.

- Harvesting at different stage of life cycle and using different P rates

This study was done mainly on seedling stage with two level of P treatments. To understand the effect of P deficiency at different stages of the plant's life cycle plants needs to be grown till maturity and harvested at different stages. Differences among varieties may also be sensitive to the severity of P stress and to understand at which level of P deficiency root traits contributes most towards P uptake, different rates of P fertilizer can be used. For this study the selected P rates were mainly either severely deficient or luxury amount of available P for plant growth. Most soil conditions are not like that and for this reason it is necessary to introduce different rates of P.

- Different forms of P fertilizer

In this study mainly calcium phosphate was used as a source of P. It is necessary to introduce different forms of P fertilizer to understand the effect of different source of P on root traits. Also soil testing after harvesting on bulk soil is necessary. This will allow to understand the effect of root traits on depletion of different P pool.

- Field trial on similar soil

While the initial selection of varieties was based on their responses to P in the field, most of the work for this study was done in controlled environments with very limited

field study. It is important to select some representative varieties from this study to evaluate in the field to validate the results. Field evaluation will help to understand the consistency of the contribution of root traits towards varietal P responsiveness.

- More work on mycorrhiza and different harvesting time

In this study difficulties with the mycorrhizal work in the main experiment were due to the timing of the harvest. More work can be done by selecting some varieties which showed high colonization for longer growing period. To measure and quantify the contribution mycorrhizal contribution towards varietal P uptake and responsiveness more work with radioactive P is necessary.

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Chapter 9 Appendices

Appendix 1. Summary ANOVA of experiment 1a

Source of variation	df	Mean square	
		First pair root angle	Second pair root angle
Variety	9	2001.5***	2353.5*
Nonresp. Vs Resp.	1	2837.1***	4426.4*
Residual	43	217.8	857.4

Appendix 2. Summary ANOVA of Experiment 2

Source of variation	Crown root angle	
	df	m.s.
Variety	9	578.9***
Nonresp. vs Resp.	1	2537.3***
P treatment	2	3325.8***
Variety*P treatment	18	147.6_{NS}
(Nonresp. vs Resp.)*P treatment	2	550.7*
Residual	57	159.6

Appendix 3. Summary ANOVA of Experiment 3

Source of variation	df	Mean squares					
		Root length	Average diameter	Rhizosheath size	SDW	RDW	Root to shoot ratio
Variety	9	4966.8***	0.006**	0.67***	51.65***	19.13***	0.026***
Nonresp. vs Resp.	1	194.0$_{NS}$	0.009*	1.92***	9.93$_{NS}$	0.28$_{NS}$	0.001$_{NS}$
P treatment	1	673.5$_{NS}$	0.002$_{NS}$	3.98***	11.76$_{NS}$	42.53***	0.01$_{NS}$
Variety*P treatment	9	722.0$_{NS}$	0.002$_{NS}$	0.08$_{NS}$	228.03***	4.85$_{NS}$	0.05***
(Nonresp. vs Resp.)*P treatment	1	2352.3$_{NS}$	0.003$_{NS}$	0.12$_{NS}$	333.7***	9.58$_{NS}$	0.02$_{NS}$
Residual	71	772.3	0.002	0.09	13.49	3.47	0.007

Appendix 4. Total root length Experiment 3

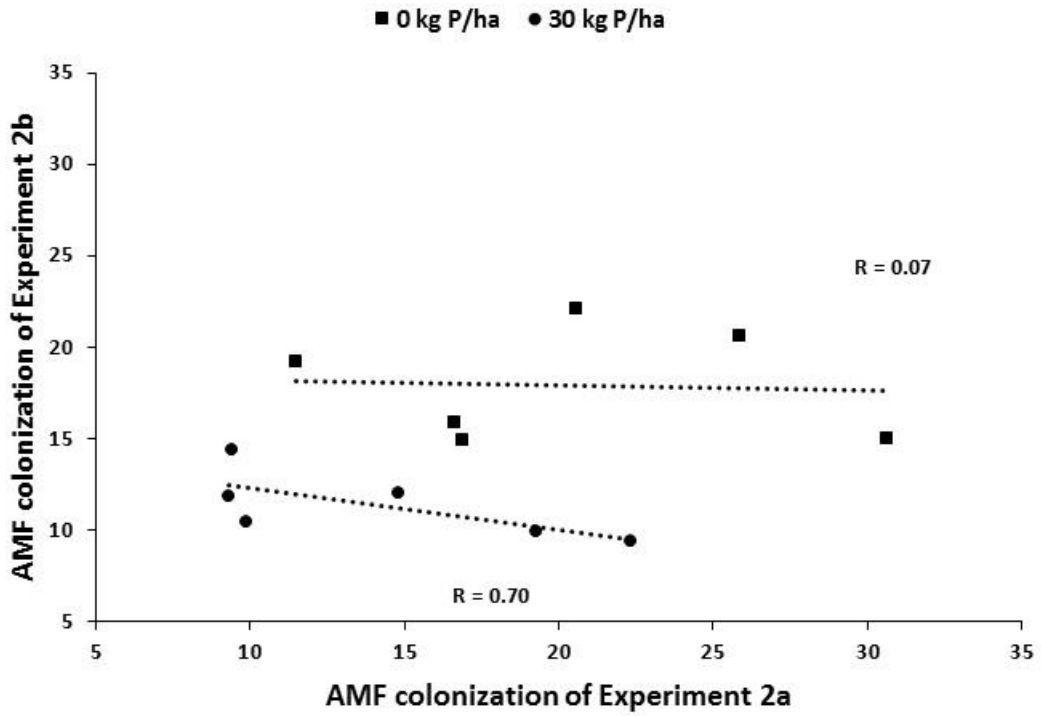
Responsiveness and Variety	Total root length (cm)	
	Low P	High P
Non Responsive		
Axe	176	188
Carazinho	189	166
Correll	147	148
Gladius	162	146
RAC875	143	119
Trintecenco	212	208
Warigal	193	166
Mean	175±9.7	163±11.0
Responsive		
BTSchomburgk	161	157
Krichauff	172	183
Wyalkatchem	169	191
Mean	167±3.2	177±10.2
LSD (P=0.05)		
Variety	24.8***	
Treatment	11.1_{NS}	
Variety*Treatment	35.1_{NS}	
CV(%)	16.4	

Appendix 5. Summary ANOVA of Experiment 4

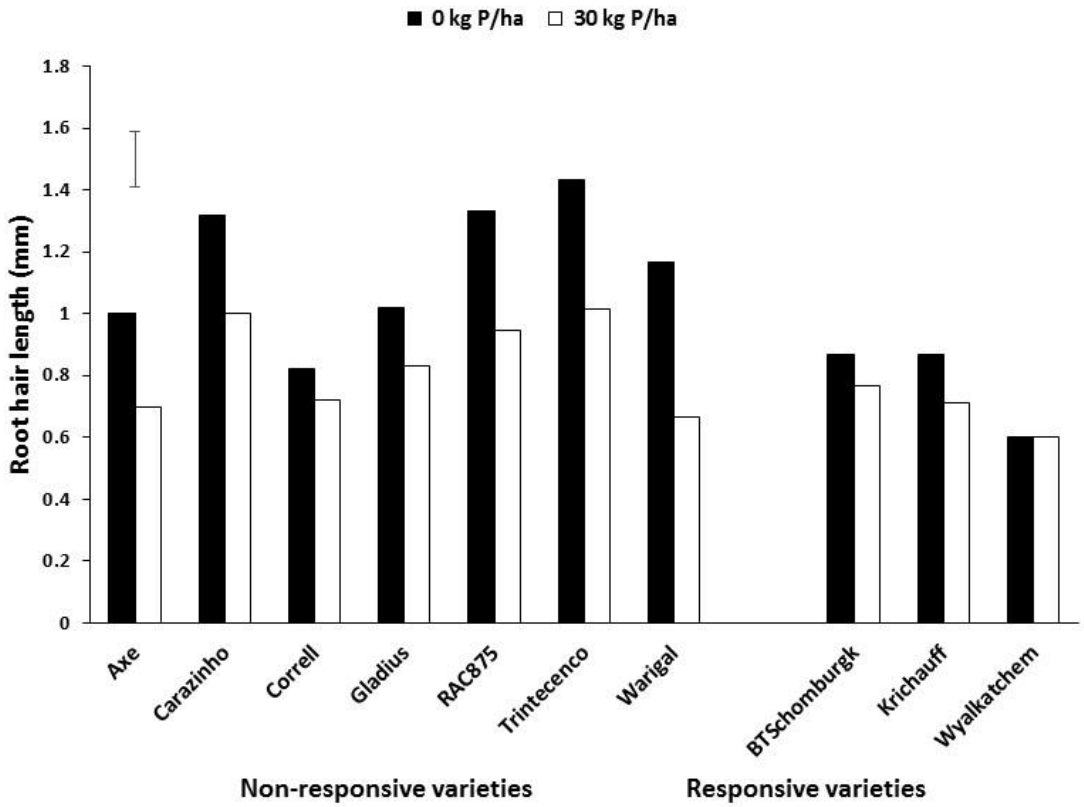
	df	Mean squares								
		Root length	Average diameter	Root hair length	Rhizosheath size	SDW	RDW	Root to shoot ratio	P concentration	P uptake
Variety	9	2042.0 $_{NS}$	0.006***	0.38***	0.67***	394.43***	36.57***	0.27*	1402488*	15534***
Nonresp. vs Resp.	1	58.0 $_{NS}$	0.0003$_{NS}$	0.64***	2.5***	1704.6***	17.32 $_{NS}$	0.06*	4650261**	80058***
Soil treatment	1	3873.0 $_{NS}$	0.0128**	2.19***	11.57***	2043.04***	67.08**	0.05*	35883258***	211118***
Variety*Soil treatment	9	3339.0 $_{NS}$	0.0045***	0.14***	0.16 $_{NS}$	111.77 $_{NS}$	10.41 $_{NS}$	0.008 $_{NS}$	972273 $_{NS}$	2649 $_{NS}$
(Nonresp. vs Resp.)*Soil treatment	1	2216.0 $_{NS}$	0.0056*	0.41***	0.28 $_{NS}$	7.56 $_{NS}$	0.39 $_{NS}$	0.001 $_{NS}$	5779686**	727 $_{NS}$
Residual	76	1701.0	0.00127	0.03	0.14	72.68	8.54	0.012	565205	2601

Appendix 6. Root hair length of ten wheat varieties grown in Halidon and Mallala soil

Responsiveness and Variety	Root hair length (mm)		
	Halidon	Mallala	Mean
Non Responsive			
Axe	0.884	0.723	0.804
Carazinho	1.458	1.116	1.287
Correll	0.819	0.652	0.736
Gladius	0.995	0.695	0.845
RAC875	1.359	1.020	1.190
Trintecenco	1.432	0.706	1.069
Warigal	1.205	0.585	0.895
Mean	1.170±0.1005	0.79±0.0757	
Responsive			
BTSchomburgk	1.000	0.740	0.870
Krichauff	0.854	0.781	0.818
Wyalkatchem	0.698	0.729	0.714
Mean	0.850±0.0872	0.75±0.0158	
Mean	1.070	0.775	
LSD (P=0.05)			
Variety		0.143***	
Treatment		0.064***	
Variety*Treatment		0.203***	
CV(%)		17.5	



Appendix 7. Correlation of AMF colonization between controlled environment experiment (Experiment 2a) and field study (Experiment 2b) grown with two different P treatments.



Appendix 8. Root hair length of ten wheat varieties.

Appendix 9. Pedigree analysis of wheat varieties showing the coefficient of parentage matrix

Varieties	BTSchomburgk	Krichauff	Wyalkatchem	Correll	Gladius	Axe	Warigal
BTSchomburgk		0.3598	0.0494	0.0839	0.2084	0.1718	0.5163
Krichauff			0.0486	0.0804	0.3060	0.2032	0.3008
Wyalkatchem				0.1156	0.0911	0.1043	0.0491
Correll					0.0930	0.0983	0.0891
Gladius						0.3995	0.1994
Axe							0.1730
Warigal							