Genotypic variation and mechanisms of phosphorus use efficiency in wheat (*Triticum aestivum* L.)

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Abbreviations

C_i	Intercellular CO_2
Cond	Stomatal conductance
DAS	Days after sowing
DM	Dry matter
F	Flag leaf
F-1	Leaf immediately below the flag leaf
F-2	Leaf immediately below the F-1 leaf
HI	Harvest Index
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
P	Phosphorus
PAE	Phosphorus Acquisition Efficiency
PHI	Phosphorus Harvest Index
P_n	Photosynthetic rate
PUE	Phosphorous Use Efficiency
PUtE	Phosphorus Utilization Efficiency
$PUtE_{GY}$	Phosphorus Utilization Efficiency (grain yield)
$PUtE_{SM}$	Phosphorus Utilization Efficiency (shoot matter)
RubisCO	Ribulose-1,5-biphosphate carboxylase
RuBP	Ribulose-1,5-biphosphate

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Abstract

Wheat (*Triticum aestivum* L.) is one of the world's most important food crops and the production of phosphorus (P) efficient wheat could contribute to enhanced crop yield on P-deficient soils. Improving our understanding of the mechanisms of P use efficiency (PUE) is essential for screening and further genetic manipulation, however the mechanisms of PUE are still not fully identified. It is hypothesized that P efficient wheat (low P tolerant) and P inefficient wheat (low P intolerant) differ in their efficiency mechanisms including root architectural and morphological traits and metabolic responses under P deprivation.

Six wheat genotypes including RAC875, Scout, Gladius, Mace, Correll and Wyalkatchem are known to differ in their PUE when analysed in field experiments, and in this thesis, were grown under controlled environment conditions to evaluate their PUE compared to field responses. Results reveal that their responses to P supply in growth room conditions generally correlate with their responses in field experiments. Plants produced greater biomass in the growth room than in greenhouse conditions and their responses to P were consistent between experiments. In the growth room, RAC875 showed greater shoot biomass and grain yield than Wyalkatchem under low P, while the results in the greenhouse were the opposite. To test why differences exist between growth room and greenhouse environments, the effect of light intensity on PUE was evaluated. A greater PUE was observed in RAC875 when compared to Wyalkatchem under both light conditions.

RAC875 possessed a smaller root dry matter but a higher root efficiency (mg shoot P uptake g^{-1} root dry matter) which appeared to be associated with greater PUE. To test

if RAC875 had more favourable root traits contributing to higher PUE, a simple soil-based cultivation was developed to assess root architectural traits related to PUE of RAC875 and Wyalkatchem. Although the majority of root architectural traits were not significantly different between RAC875 and Wyalkatchem, RAC875 showed higher relative ratios between low P and adequate P than in Wyalkatchem. RAC875 had greater root convex hull area (CHA) than Wyalkatchem under low P. Wyalkatchem had longer root hairs than RAC875 at both low and adequate P, while under low P, RAC875 produced more dense root hairs than Wyalkatchem, suggesting that CHA and denser root hairs may contribute to greater yield and shoot biomass in RAC875 during P starvation.

Variation in metabolite profiles between the two wheat genotypes was also investigated to explore metabolic mechanisms of greater PUE in RAC875. Through a metabolomics approach, 79 and 84 metabolites were measured from leaves and roots, respectively. A greater accumulation of raffinose in roots and aspartate, glutamine and β-alanine in leaves were associated with the P efficient phenotype in RAC875. Under P deprivation, the phosphorylated sugars, glucose-6-P and fructose-6-P were maintained in RAC875 and this would allow carbohydrate metabolism to be maintained and lead to greater shoot biomass. The mechanism of RAC875 would therefore be associated with the maintenance of carbon metabolism and transport to sink tissues.

Thesis Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Van Lam Nguyen

Date: 26 September 2016

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CHAPTER 1

Literature review

1.1 General introduction

The macronutrient, phosphorus (P) is important for plants since it is needed for building up cell structure and is essential for metabolic pathways such as photosynthesis, respiration and energy transfer (Shenoy and Kalagudi, 2005). Thus, P limitations affect plant growth and development and result in reduced yield (Lynch and Brown, 2001). For example, low P decreased yield in rice (Alam et al., 2009) and in wheat (Jones et al., 1989). Anthony et al. (2012) also identified a correlation between P application level and soybean yield.

While P is essential for plants, low P availability occurs in many agricultural lands since it often exists in less available forms within the soil (Balemi and Negisho, 2012). The application of P fertilizers is a routine occurrence for most farmers, however this increases production costs (Jones et al., 1989) and excess P fertilization can lead to environmental risks due to run-off (Liu, 2015; Cordell and White, 2013). Furthermore, P resources are non-renewable and are predicted to be depleted in the near future (Vance et al., 2003) and this can threaten global food supply (Cordell and White, 2013). Therefore, improving the phosphorus use efficiency (PUE) in crop plants is a potential strategy for crop improvement (Shenoy and Kalagudi, 2005) and genetic approaches seem to be advantageous to achieve this objective (Bovill et al., 2013).

Wheat (*Triticum aestivum* L.) is one of the most important crop staples in the world (Shewry, 2009) and to improve crop productivity and meet increasing global food demand, it is essential to develop wheat that is tolerant to abiotic stress, such as P deficiency. In order to produce P efficient wheat, it is vital to understand the mechanisms of PUE. In response to P deficiency, plants may employ a variety of adaptive mechanisms such as modifications to root architecture and morphology (Ao et al., 2010; da Silva et al., 2016), exudation of organic acids from roots to improve P acquisition (Gaume et al., 2001), alternation of metabolites (Huang et al., 2008; Ganie et al., 2015) or activation of alternative pathways (Duff et al., 1989).

Roots are important for water and nutrient acquisition and can be targeted for improvements of plant productivity (Paez-Garcia et al., 2015). Changes in root system architecture (RSA) and morphology have been observed in response to low P (Heppell et al., 2015; Zhang et al., 2013; Bates and Lynch, 2000a). Observations included a stimulation of root growth, enhancement of root branching and increase in root hair length and density. Although a number of root phenotyping platforms have been developed, genetic studies on root traits are still hampered due to root complexity and their underground location (Kuijken et al., 2015), therefore, understanding unexplored root traits relating to P efficiency in wheat may yield strategies to enhance its productivity.

In terms of phenotyping root traits, approaches to obtain roots for analysis has its limitations. For instance, root images can be captured from gel-based cultivation in glass tubes (Iyer-Pascuzzi et al., 2010), but root behaviour in gel may be different from that in soils. This limitation can be overcome by using X-ray (Mooney et al., 2012) or magnetic resonance imaging (MRI) technologies (van Dusschoten et al., 2016) that

can take root images from soil grown plants, but these technologies are expensive. Glass bead-based cultivation in designed rhizoboxes is another approach to retain two-dimensional (2-D) RSA in rice (Courtois et al., 2013). This method appears to be close to replicating a soil medium since glass beads are physically more similar to soil colloids than are gels. However, the system is still a hydroponic environment in which the roots are growing and therefore it does not take into consideration the interaction of nutrient on soil colloid or the rhizosphere interactions that are present. An improved system is required for simulating field conditions to understand how root traits affect P uptake in soil. One aim of this thesis is to look at developing such a method.

Plants also modify metabolic pathways to adapt to P limitations (Plaxton and Tran, 2011). A study in barley showed that P deficiency resulted in an increase of di- and trisaccharides, including sucrose, maltose and raffinose, and a decrease in phosphorylated intermediates (glucose-6-P, fructose-6-P, inositol-1-P and glycerol-3-P) in shoots and roots (Huang et al., 2008). An improved level of sugar in tissues and a decline in levels of phosphate esters were found in rice under low P (Nanamori et al., 2004). The enhanced accumulation of di- and trisaccharides in plants under P starvation can reduce inorganic P (P_i) consumption in phosphorylation of sugar metabolites. Under P shortage, the increased accumulation of di- and trisaccharides and the reduction in P-containing metabolites occurred in maize (Ganie et al., 2015). However, Muller et al. (2015) reported that both sugars and phosphorylated metabolites declined in lupin under P deficiency, therefore modification of metabolites under low P may be different between plant species. Although alterations of metabolites under P deficiency have been observed in many plant species, whether this occurs in wheat is unknown. Furthermore, the majority of reported studies have

investigated metabolic responses to low P rather than elucidate differences in metabolic responses to low P between P efficient and P inefficient plants, which could provide a better understanding of the mechanisms of PUE.

This review will cover the nature of P in plants and soils, general information on PUE, mechanisms of P efficiency and root trait measurements. It will also provide aims and a framework for the research.

1.2 Nature of P in plants and soils

1.2.1 P in plants and its functions

Phosphorus in plants occurs in two forms, the free inorganic orthophosphate (P_i) and organic phosphate esters (Veneklaas et al., 2012). P_i is directly absorbed by plant roots with the support of P_i transport systems (Shen et al., 2011), therefore, the P_i concentration of tissues generally reflects available P_i concentration in the soil solution (Grant et al., 2005). Physiologically, tissue P_i is divided into two major pools, (1) the cytoplasmic P_i pool and (2) the vacuolar P_i pool (Veneklaas et al., 2012). The cytoplasmic P_i is considered as the metabolically active pool, while the vacuolar P_i is the stored pool. When there is an excess of cellular P_i, P_i is stored in the vacuole and is used to buffer the P_i requirements of the cytoplasm (Rebeille et al., 1983). For example, when barley (*Hordeum vulgare*) was grown under P deficient conditions, the vacuolar P_i concentration was very low, whereas the cytoplasmic P_i content remained relatively constant (Foyer and Spencer, 1986). A similar study in maize showed that under P starvation, the vacuolar Pi content declined rapidly, while the cytoplasmic P_i content initially remained stable and only began to decline when P starvation became severe (Lee et al., 1990). Pratt et al. (2009) also reported a rapid decline in the cytosolic

P_i concentration following the onset of P deficiency. Therefore, the P_i in the cytoplasm appears to be the initial source that responds to P stress since P_i efflux from the vacuole was insufficient to compensate for a rapid decrease of external P under P starvation.

Organic phosphate esters are diverse and present in macromolecules including nucleic acids, phospholipids, phosphorylated water-soluble low relative molecular mass metabolites (referred to as P esters) and phosphorylated proteins (Veneklaas et al., 2012). The nucleic acid P pool is a major organic pool in the plant and it varies depending on species, tissue type and P supply. Close and Beadle (2004) reported that the proportion of nucleic acid P content of *Eucalyptus globulus* is higher than that of three other *Eucalyptus* species. RNAs accounts for at least 85% of the total nucleic acid P pool (Veneklaas et al., 2012) and RNAs is involved in the biosynthesis of proteins, therefore enhancing the efficiency of RNA usage and the P they contain would regulate the protein biosynthesis during plant development.

Phospholipids are major components of membranes and are another organic P pool. The content of these compounds varies depending on the type of membrane since in some membranes (such as the thylakoid membrane) phospholipids are replaced by others such as sulfolipids and galactolipids (Veneklaas et al., 2012).

Another source of P esters are low molecular weight and water-soluble compounds. These can be important intermediate compounds for metabolic pathways in plants. For example, P sugar esters such as glucose-6-P and fructose-1,6-P are intermediates of cell respiration (Muller et al., 2015). Besides these, nucleotides (ATP, GTP, UTP, CTP) are P-containing molecules for the biosynthesis of nucleic acids (Hollenstein, 2012; Copper, 2002). ATP is the main molecule for energy transfer and is essential for

cellular metabolism (Kim et al., 2006). Phytate is another P pool in plants. Phytate is a complex of calcium or magnesium with myo-inositol and is regarded as the main storage form of P in grains (Wu et al., 2009).

In general, P has a variety of functions in the cell including a structural role, energy and information storage, and energy and information transfer (Blank, 2012). P is a constituent of nucleic acids, phospholipids (a major component of cell membranes) and ATP (Schachtman et al., 1998). P is involved in the regulation of key enzyme reactions and has a key role in metabolic pathways such as respiration and photosynthesis (Theodorou and Plaxton, 1993; Pedas et al., 2011; Kondracka and Rychter, 1997). It was shown that the phosphorylation of protein is involved in most signalling pathways in plants (Luan, 2003) and this protein phosphorylation/dephosphorylation is used to regulate a variety of enzymes (Cheng et al., 2011; Dick et al., 2011). Studies have also shown that a protein phosphorylation/dephosphorylation network controls a plant potassium channel (Lee et al., 2007) and guard-cell protein phosphorylation/dephosphorylation occurs in response to light/dark (Kinoshita et al., 1993).

1.2.2 Nature and dynamics of P in soils

The P content of soils varies between different soil types and in agricultural environments that depend on P fertilization practices (Li et al., 2015; Tóth et al., 2014). A study was able to show that in soils with slow water transport, P leaching is low and results in high P residues (Djodjic et al., 2004). The loss of P is also influenced by soil characteristics. For example, P leaching is low from soils with high P adsorption capacity (as in calcareous soils) and low P saturation (Andersson et al., 2013; Olson et

al., 2010). P application also affects the P content in soils. In developing countries, P content in soils is low due to less P application but increases in developed countries due to excessive P fertilization (Djodjic et al., 2004).

Soil P is found in various forms including inorganic P (P_i) and organic P. P_i is used to indicate P in inorganic compounds and soil P_i often exists in complexes with metal ions classified into several types including aluminium P (Al-P), iron P (Fe-P), Calcium P (Ca-P) and occluded P (Indiati and Sharpley, 1998). Organic P is a term for organic compounds containing P such as phosphate diesters (including DNA, RNA and phospholipids), phosphate monoesters (sugar phosphates, mainly inositol phosphates), organic polyphosphate (ATP) and phosphonate (McKelvie, 2005; Giles et al., 2011; Turner et al., 2005). Organic P accounts for a major part of the total P content of the soil and derives from plant residues, microbial cells and metabolic products. Forms of organic P include inositol phosphates (up to 60% of the organic P), nucleic acids (5-10% of the organic P) and phospholipids (1% of the organic P) (Turner et al., 2002; Sanchez, 2007).

Phospholipids and nucleic acids that enter the soil are rapidly broken down by the activity of soil microorganisms (Sanchez, 2007), while the inositol phosphates are more stable and therefore more abundant. This group exists in various forms depending on the level of inositol phosphorylation (from one to six phosphate residues) and isomeric forms (Turner et al., 2002). The soil phosphates can be hydrolyzed by microbial phytases and result in a huge source of orthophosphate (Giles et al., 2011).

Plant and animal materials are major sources of organic P in the soil environment, however in the organic forms, P is generally unavailable to living organisms. It needs

to be mineralized into the inorganic orthophosphate form that can be then taken up by living organisms. This mineralization is carried out by soil microorganisms capable of producing phosphatase enzymes to release inorganic P from organic materials. Phosphatases include phosphomonoesterase, phosphodiesterases, nuclease, nucleotidase and phytase (Mackey and Paytan, 2009). Released P_i from the mineralization of plant and animal detritus might be utilized by the soil microorganisms, utilized by plants, transferred to the soil inorganic pool, or lost through leaching (Sanchez, 2007).

Opposite to the mineralization, P in soils can also undergo immobilization whereby P is converted into fixed forms. Immobilization processes are classified into two categories; transitory immobilization (or cellular assimilation) and mineral formation. The first category involves all processes where P is used by soil microbes for their growth. Soil microorganisms assimilate P into biological macromolecules such as phospholipids and nucleic acids in the cells. The second category, mineral formation refers to processes where P_i is transformed into insoluble forms by reacting with cations in the environment (Mackey and Paytan, 2009).

The conversion of P_i into less available forms by reaction with cations is related to sorption and precipitation reactions in the soil. P sorption is closely associated with the presence of Fe^{3+}, Al^{3+} and Ca^{2+} (Singh and Gilkes, 1991; Hossain et al., 2012). Agbenin (2003) indicated that the increased concentration of Fe and Al cations in the soil enhances the capacity of P sorption. Calcium is also a factor that results in both sorption and precipitation of P. In calcareous soils, the interactions between P and calcium take place by both surface reactions and precipitation (Djodjic et al., 2004; Tunesi et al., 1999). In soils containing clay, soil P was adsorbed by Fe and Al

(Souliyavongsa et al., 2015). There are many complex factors which determine the availability of P in soil, and understanding soil characteristics and P adsorption in soils can enable appropriate P fertilizer management (Pinto et al., 2013).

P sorption and precipitation are also related to soil pH where studies have shown that in the range of acidic to neutral pH, P sorption declines as pH increases, while the P sorption increases as pH increases from neutral to alkaline conditions (Sato and Comerford, 2005; Pardo et al., 1992).

1.2.3 P dynamics in the rhizosphere

The rhizosphere is a critical zone around plant roots where there are interactions between plants, soil and microorganisms. This region is very important for plant growth as it impacts on nutrient acquisition (Richardson et al., 2009). Features of rhizosphere are driven by different factors mainly including soil characteristics, plant roots and soil microbial populations. Soil is a shared environment for growth of plant roots and microorganisms, therefore, their characteristics such as the physical architecture of the soil matrix, have an effect on their growth (Hinsinger et al., 2009). Plant roots can greatly alter the rhizosphere environment through their physiological activities, especially the release of exudates such as organic acids and phosphatases, and can enhance P uptake under P starvation (Yan et al., 2010). Rhizosphere microorganisms can also modify the rhizosphere zone (Richardson and Simpson, 2011; Marschner, 2008). For example, the presence of phosphate-solubilizing bacteria has been shown to increase P availability in soils (Panhwar et al., 2011; Xie et al., 2013).

In the soil, soluble P_i can be rapidly converted into unavailable forms by reacting with

soil components (Mackey and Paytan, 2009). Therefore, available P in soils is often limited although the total P content usually exceeds the plant's demands. The P_i is principally transported to plant roots via diffusion rather than mass flow due to its strong reactions with soil components (Hinsinger, 2001). P can be quickly taken up in the rhizosphere by plant roots, resulting in a P_i gradient between the root surface and further regions out from the root. In order to meet plant requirements, P_i from areas away from the roots, needs to be transported to the rhizosphere. P dynamics in the rhizosphere are primarily monitored by root growth and function (i.e. organic acid exudation) (Shen et al., 2012). Soil microbial activities, interactions between soil microbes and plants roots can also impact on P dynamics in the rhizosphere (Hinsinger, 2001). For example, the activities of soil microflora can lead to the dissolving of insoluble inorganic P compounds by secreting organic acids or the release of enzymes (i.e. phytases and phosphatases) to convert P in organic compounds into P_i available for plants (Sharma et al., 2013). Nazeri et al. (2014) have indicated that P availability in the rhizosphere was higher when plants were colonized by arbuscular mycorrhizal (AM) fungi with a symbiotic relationship with plants (Nazeri et al., 2014).

P dynamics in the rhizosphere can also be impacted by soil properties such as texture and the presence of metal cations (Achat et al., 2016). Plant root activities, soil microbial activities and soil properties all impact on P availability in the rhizosphere (Figure 1.1) (Richardson, 2001).

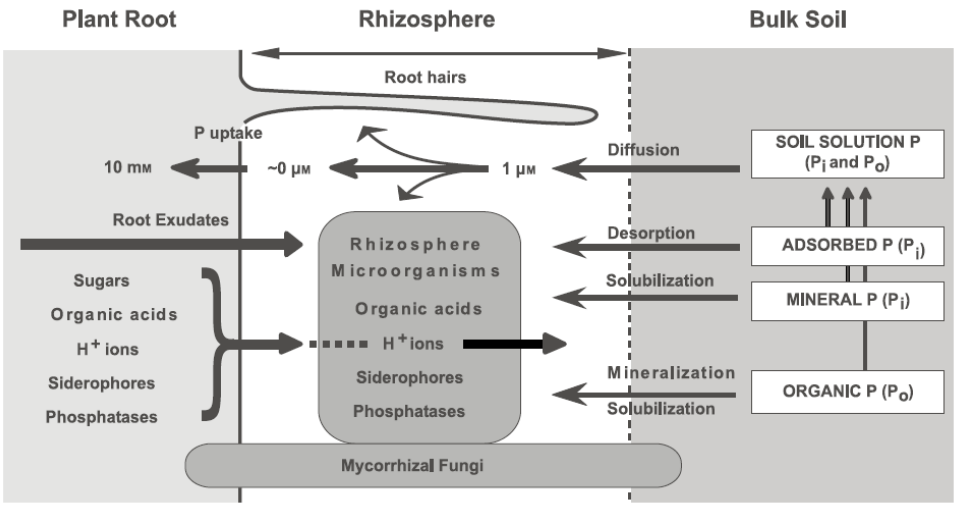


Figure 1.1. Physiological and chemical processes affect the availability of P in the rhizosphere (Richardson, 2001)

1.2.4 P uptake and utilization by plants

Phosphorus is taken up by plant roots in the form of P_i which exists in different forms depending on the pH of the soil solution. H_3PO_4 dissociates into $H_2PO_4^-$ at pH 2.1 and then into HPO_4^{2-} at pH 7.2 (Schachtman et al., 1998). Various authors have suggested that P_i is most likely absorbed as $H_2PO_4^-$ since most studies found that the rate of P_i uptake was highest between pH 5 and 6 (Furihata et al., 1992; Tu and Ma, 2003). In the range of pH 5 to 6, $H_2PO_4^-$ is the prevalent form.

The concentration of P_i in the soil is generally much lower than the P_i concentration in root cells, thus plants must have active transport systems to acquire P from the soil solution. This process is carried out by high-affinity P_i/H^+ symporters that are controlled by two major gene families (*Pht1* and *Pht2*) (Cui et al., 2011). The high-affinity P_i/H^+ symporters are involved in P uptake and translocation throughout the plant. These transporters are predicted to be membrane-spanning proteins with 12 domains (Rausch and Bucher, 2002). The role of P transporter genes has been studied

in various plants. *Arabidopsis Pht1;4* was expressed primarily under P_i starvation and found mainly in the epidermis, the cortex and the root cap. It is suggested that this gene is associated with P uptake in roots (Misson et al., 2004). Recently, a study in *Arabidopsis thaliana* showed that *At Pht1;1* to *At Pht1;4* are involved in P_i acquisition from the rhizosphere while *AtPht1;8* and *AtPht1;9* translocate P_i from the roots to the shoots (Lapis-Gaza et al., 2014). The expression of *OsPht1;6,* a rice P transporter gene, was enhanced under P deficiency in roots and shoots (Ai et al., 2009). Studies in wheat indicate that *TaPht1;4* is involved in P acquisition (Liu et al., 2013) and *TaPht2;1* is involved in the long-distance P translocation of P within plants (Guo et al., 2013).

P_i absorbed by roots is unloaded into the xylem and then transferred to the leaves (Poirier and Bucher, 2002). Genes involved in P unloading into the xylem have been investigated. In *Arabidopsis,* the *PHO1* gene is responsible for transport of P into the xylem; demonstrated by a reduction of P transport from root epidermal and cortical cells to the xylem in a *PHO1* mutant (Hamburger et al., 2002). Stefanovic et al. (2007) reported that the transport of P into the xylem was controlled by two distinct pathways. Their research showed that although *PHO1* makes a major contribution to the P_i unloading into the xylem, *PHO1;H1* also controls some transportation of P_i into the xylem.

P_i translocated to different parts of plants is used for various purposes such as structural and energy storage and transfer (Veneklaas et al., 2012; Blank, 2012). In the cell, P_i can be stored in the vacuole from where it can be utilized to adapt to P deficiency (Rebeille et al., 1983).

Tissue P can be remobilized from one part to other parts of plants. P_i remobilization

from the older leaves to the younger leaves in response to P starvation has been observed in barley (Mimura et al., 1996). P_i was also shown to be re-translocated from the older to younger leaves and from the above parts to the root parts in *Brassica* cultivars under P deprivation (Akhtar et al., 2008). The remobilization of P_i from the older leaves to shoots then roots was also observed in castor bean *(Ricinus communis* L.) (Jeschke et al., 1997). Similarly, P_i from the shoots was recycled through the phloem to the roots in wheat cultivars suffering P deficiency (Peng and Li, 2005). The remobilization of P was also reported in wheat during leaf senescence (Maillard et al., 2015; Stigter and Plaxton, 2015).

Genes associated with P remobilization have been studied and are generally low-affinity P_i transporters belonging to the *Pht1* family. Rae et al. (2003) indicated that *HORvu;Pht1;6* is a low-affinity transporter in barley and probably functions in the re-translocation of stored P from leaves. Rice *OsPT2* is also a low-affinity transporter and is involved in the remobilization of the stored P in plants (Ai et al., 2009). A study in barley showed that the expression of *HvPHT1;6* and *HvPHT1;3*, low-affinity transporters, was correlated with P utilization efficiency (PUtE) (Huang et al., 2011). PUtE is related to the P remobilization (Akhtar et al., 2008); therefore, these genes appear to be involved in the redistribution of P.

1.3 Phosphorus use efficiency (PUE) and measurement

There are various definitions for PUE (Gourley et al., 1993), therefore it is important to consider how PUE is measured when it is evaluated. Adapted from a definition of nutrient use efficiency in plants by Ciarelli et al. (1998), PUE is defined as the efficiency of P acquisition and P utilization for plant improvements in dry matter/or

grain yield under P sufficient or insufficient conditions. Ortiz-Monasterio et al. (2011) defined PUE in the plant as grain yield per unit of nutrient supplied to the crop, a definition adapted from the definition of nitrogen use efficiency by Moll et al. (1982). Wang et al. (2010) defined PUE as the ability of the plant to produce biomass or grain yield under certain supplied P conditions. PUE can therefore be divided into two components: P acquisition efficiency (PAE) and P utilization efficiency (PUtE). PAE is defined as the quantity of the nutrient extracted by the plant from the nutrient source available to the plant in the soil. PUtE is referred to as internal utilization efficiency and defined as the biomass or the yield of grain produced per unit of nutrient absorbed by the plant from the soil. PUtE designates the ability of the plant to convert the acquired P into plant biomass or yield.

The following equations for PUE are adapted from equations for nutrient efficiency by Ortiz-Monasterio et al. (2011).

$$PUE = PAE \times PUtE_{GY}$$

$$GY/P_s = P_{sm}/P_s \times GY/P_{sm}$$

Where: GY: grain yield (g plant^{-1}), P_s: P supplied (g pot^{-1}) and P_{sm}: shoot P uptake (mg shoot^{-1}).

PUE is calculated for vegetative stages and shoot matter at maturity as follow:

$$PUE = PAE \times PUtE_{SM}$$

$$SM/P_s = P_{sm}/P_s \times SM/P_{sm}$$

Where: SM: shoot dry matter (g plant^{-1}), P_s: P supplied (g pot^{-1}) and P_{sm}: shoot P uptake

(mg shoot^{-1}).

The definition for PUE adapted from Ortiz-Monasterio et al. (2011) is able to be used for both low and high P supply conditions.

There are also other PUE classification systems. For instance, a classification system was proposed based on the plant responsiveness under both normal and stress conditions. This system divides genotypes into four groups: (1) efficient, responsive; (2) inefficient, responsive; (3) efficient, non-responsive, and; (4) inefficient, non-responsive. An efficient genotype produces higher yield than the average yield at low P supply, while a responder genotype produces higher yield than the average yield at high addition. This means that group 1 produces high yield at both low and high nutrient supply; group 2 has low yield at low nutrient supply but high yield at high nutrient supply; group 3 produces high yield at low nutrient supply but low yield at high nutrient supply; group 4 produces low yield at both low and high nutrient supply (Korkmaz et al., 2010; Fageria and Baligar, 1999).

Internal PUE and external PUE have also been used to measure PUE. They are defined as the critical values of tissue P concentration and P supply respectively, at which plants obtain a specific percentage (i.e. 90%) of maximum yield (biomass) (Abbadi and Gerendas, 2015; Osborne and Rengel, 2002a). Internal PUE is associated with PUtE and external PUE is related to PAE (van de Wiel et al., 2016). PUE is also measured as the ratio of yield (biomass) at low P supply to the yield at sufficient P supply (Gunes et al., 2006).

1.4 Mechanisms of PUE in plants

It is crucial to understand mechanisms that plants use to adapt to P stress in order to produce cultivars with high PUE. As described above, PUE has two components, PAE and PUtE, therefore, the mechanisms for both PAE and PUtE should be considered.

1.4.1 Mechanisms of PAE

Available P is very limited in the soil, even in soils with regular phosphate addition, since P is rapidly made unavailable by reactions with soil components. Therefore, the ability to acquire P is vital for the healthy growth of plants. In order to cope with the problem of P availability, plants develop different mechanisms to extract more P under limited P supply. There are some major mechanisms for PAE including the modification of root architecture, the secretion of exudates, associations with microorganisms, changes in the membrane transporters related to the uptake of P. Microbial activity in the soil is also involved in improving P acquisition.

1.4.1.1 Root traits and P acquisition

Plants can adapt to P depleted conditions by altering root traits including root architecture, morphology and anatomy (van de Wiel et al., 2016; Niu et al., 2013). P starvation enhances root development, resulting in increases in root/shoot ratio, root elongation and root branching (Peret et al., 2011). Under low P conditions, enhanced root length and root to shoot ratio were found in wheat (Horst et al., 1996). Low P resulted in greater root length in barley under field conditions (Steingrobe et al., 2001) and Gaume et al. (2001) showed that root to shoot ratio increased in maize. Lateral roots are important for P acquisition by increasing soil exploration (Zhu et al., 2005a).

Plants have been shown to respond to P deprivation by increased root branching, such as in *Cucumis melo* L. (Fita et al., 2011) and in cucumber (Zhang et al., 2012). Some plants are able to develop cluster roots, characterized by the formation of compact clusters of root branch or root hairs in a small soil volume, therefore leading to an increase in the surface area of the root system and allowing greater access to soil P (Lambers et al., 2006). In calcareous soil, a maize line with higher root length had greater P uptake and shoot biomass than a maize line with shorter root length (Zhang et al., 2013). Thus, enhanced root growth appears to be important for PAE.

Although most studies have indicated an enhanced plant root growth under low P, research on barley has shown dramatic reductions in total root length and root surface area under limited P (Wang et al., 2015). Furthermore, da Silva et al. (2016) observed that wheat genotypes with shorter root length and smaller root volume had higher P uptake than did wheat genotypes with longer root length and larger root volume. This means that root size is not the determining factor for PAE in these wheat genotypes; possibly due to genetic variation between plants or differences in methodologies (i.e. growth conditions or harvest stages). Besides, Jones et al. (1989) indicated that root efficiency (mg shoot P uptake per g root dry matter) is a useful criterion for screening P efficiency in wheat. Therefore, more research is needed to resolve these discrepancies.

Root hairs are important for P acquisition (Wang et al., 2004) and enhanced root hair length (RHL) and root hair density (RHD) in response to P starvation have been observed (Bates and Lynch, 1996; Bates and Lynch, 2000a). A study in wheat and barley showed that RHL was positively correlated with P depletion from the rhizosphere at three P levels, and was particularly evident under low P conditions

(Gahoonia et al., 1997). Therefore, longer root hairs seem to contribute to improved P uptake. Gahoonia and Nielsen (2004a) were also able to show that barley genotypes with longer root hairs were better adapted to low P supply and produced higher yield in comparison with genotypes with shorter root hairs. Keyes et al. (2013) observed that root hairs and roots (root surface area) contribute equally in P uptake but root hairs are more important for local P acquisition. Although root hair features may be important markers for selection of P efficient plants, root hair characteristics are difficult to measure as they are lost or aggregated after harvest. Growing plants in rhizotrons can be used to solve these dilemmas (Judd et al., 2015). Rhizosheath size measurement is also used to indicate root hair length (Delhaize et al., 2015). Recently, James et al. (2016) have shown on acid soils that a large rhizosheath size improved shoot biomass under both low and adequate P supply. Five major quantitative trait loci (QTLs) were linked to rhizosheath size in this study. Although most studies show that longer root hairs promote P uptake in plants, recently a study in wheat found increased root hair length and density as P supply increased (Yuan et al., 2016). This suggests more research need to be done to find out whether root hair features affect PUE.

Root traits associated with P uptake efficiency are genetically inherited. de Sousa et al. (2012) demonstrated that a P efficient maize line possesses greater root/shoot ratio and root volume than does the inefficient line. Similarly, a study in soybean showed that P efficiency is highly related to root morphology and root architecture and there was genetic variation in root architectural traits (Ao et al., 2010). These traits may be controlled by QTLs that can be used for the early screening of P use efficient genotypes. Another study on white clover (*Trifolium repens* L.) was also able to show that the genotypes with longer roots and more branching exhibited greater P

acquisition (Crush et al., 2008). Recently, a study showed a wide variation in root biomass and root efficiency in rice (Mori et al., 2016).

Root cortical aerenchyma (RCA) formation has been observed to be induced under nutrient stress conditions, including P deprivation. RCA is defined as large gas spaces in the root cortex that are produced through either cell death or cell separation in order to respond to hypoxia (Postma and Lynch, 2011; Fan et al., 2007). Spaces formed via cell separation are produced by differential growth, in which adjacent cells separate from one another (Evans 2003). It is reported that the formation of RCA helps reduce root respiration and P demands and supports plants to adapt to low P conditions (Fan et al., 2003). RCA formation is associated with a decrease in root hydraulic conductivity, suggesting that the transport of water in the root cylinder was limited by the formation of RCA under P stress (Fan et al., 2007). Another study indicated that the development of RCA in wheat, soybean and sunflower significantly increased under P starvation (Fernandez and Rubio, 2015).

1.4.1.2 Root activities and P acquisition

P bioavailability is significantly impacted by changes in pH and root exudates in the rhizosphere (Richardson et al., 2009). Plant roots have been reported to secrete organic acids in order to respond to P deprivation, as the release of organic acids could result in the mobilization of P from fixed forms. In fact, the secretion of organic acids from Lupin roots enhanced the solubility of P_i in different types of soil (Li et al., 1997). Radish (*Rahanus sativus* L.) and rapeseed (*Brassica napus* L.) exuded various organic acids such as tartaric, malic, succinic and citric acids under P starvation. Under P deprivation, tartaric acid was the major exudate of radish, a crop commonly grown in

acid soils, whereas malic and citric acids were the main exudates of oilseed, which is commonly grown on calcareous soils. Tartaric acid may react with Fe^{3+} and Al^{3+} to generate chelates and thereby release P from Fe-P and Al-P complexes. The release of malic and citric acids by oilseed acidifies the rhizosphere or react with Ca^{2+}; hence increasing the solubility of P in calcareous soils (Zhang et al., 1997). Neumann and Römheld (1999) showed that P deficiency induced the release of protons and the exudation of carboxylic acids in chickpea (*Cicer arietinum)* and white lupin (*Lupinus albus* L. cv. Amiga) roots. Similarly, the exudation of organic acids by plant roots under P depletion has been observed in other studies (Shen et al., 2004; Shahbaz et al., 2006). Carvalhais et al. (2011) reported that P starvation stimulated the exudation of γ-aminobutyric acid and the release of oxalic acid increased as P supply decreased in wheat (Dotaniya et al., 2013).

The root secretion of acid phosphatase is considered another adaptive mechanism of plants in response to P deficiency. Acid phosphatases are a group of enzymes which can hydrolyse different forms of organic P at low pH (Olczak et al., 2003; Li and Tadano, 1996), therefore the presence of these enzymes improves the bioavailability of P in the soil, especially under P deficiency. Tadano and Sakai (1991) reported that nine crops, including wheat presented higher acid phosphatase activity in exudates when they were grown under P deficiency. Lupin roots enhanced the release of acid phosphatase in the rhizosphere under P shortage even though seeds and soils were sterilized. It has been demonstrated that acid phosphatase in the rhizosphere primarily originated from the secretion by lupin roots (Li et al., 1997). A study in white lupin (*Lupinus albus* L.) indicated that there was an increase in the activity of extractable acid phosphatase in the lupin roots under low P supply, particularly in the proteoid

roots. Low P also resulted in greater acid phosphatase activity in root exudates (Gilbert et al., 1999). Phosphate starvation resulted in enhanced extracellular and intracellular acid phosphatase activity in comparison to phosphate-sufficient wheat plants (Ciereszko et al., 2011). Transgenic expression of acid phosphatase genes led to the improved P acquisition in white clover (*Trifolium repens* L.) (Ma et al., 2009). The exudation of acid phosphatase can be different among plant genotypes. Low P tolerance maize genotypes secreted greater acid phosphatase activity in comparison to low P susceptible genotypes (Gaume et al., 2001).

1.4.1.3 Interactions between plant roots, microorganisms and P acquisition

Studies have illustrated that arbuscular mycorrhizas (AM) play an important role in plant P acquisition. AM are a group of fungi belonging to the class Glomeromycetes of the phylum Glomeromycota which can form a mutualistic symbiosis with plant roots (Willis et al., 2013). AM develop mycelia both inside and outside plant roots (Smith and Smith, 2012). The external hyphal network extends far away from the plant roots, and can improve P uptake for plants. In fact, plants can take up P from soil in two ways; direct uptake from the rhizosphere via roots or the AM uptake pathway via AM symbiosis. The direct uptake results in the P depletion around the roots, whereas the hyphal network of AM fungi reaches further zones where larger soil area is available for uptake via the AM pathway. P is taken up via fungal extra-radical hyphae and transported to the plant roots (Figure 1.2) (Sawers et al., 2008). A study in wheat showed that over 50% of P uptake was acquired via AM, even under P addition (Li et al., 2006a), demonstrating the importance of AM fungi in assisting plants to exploit P from a greater area.

A number of studies have evaluated the contribution of AM inoculation to the improved P uptake in various crops. AM fungi significantly contributed to P uptake by wheat (Smith et al., 2015). Also, there were significant increases in the P acquisition of pigeonpea and peanut from low P soil modified with non-labile phosphate sources including Fe-P, Al-P and Ca-P (Shibata and Yano, 2003). Neumann and George (2004) reported that under dry conditions, *Sorghum bicolor* L. inoculated with *Glomus mosseae* (AM fungal species) acquired almost twice as much P as uninoculated plants. The growth of grapevine genotypes was significantly influenced by AM inoculation, with two AM fungi *Glomus mosseae* and *Glomus intraradices*, both enhancing shoot and root growth and resulting in increased leaf P content (Ozdemir et al., 2010). Hill et al. (2010) showed that mycorrhizal colonisation affected root hair morphology. The AM inoculation generally increased the density and length of root hairs under P starvation. This demonstrates the utility of inoculating soil with AM fungi promote P uptake in plants.

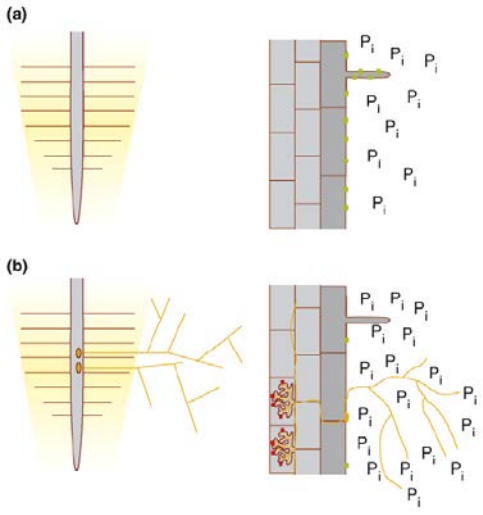


Figure 1.2. Symbosis between plant roots and AM (Sawers et al., 2008). (a) no presence of AM, (b) presence of AM.

1.4.1.4 Soil microbial activities and P acquisition

As discussed earlier, activity of soil microorganisms can contribute to the improvement of P acquisition by plants. Microbes use two mechanisms to enhance P bioavailability: secretion of organic acids and release of acid phosphatase. The secretion of organic acids is assumed to be a major factor (Khan et al., 2007), solubilizing various forms of precipitated P. Xiao et al. (2009) reported that fungi isolated from phosphate mines released organic acids including citric, oxalic and gluconic acids, and these increased soil available P and promoted wheat growth. Another study indicated the improved P solubility from insoluble P forms by bacteria was associated with a drop in the pH of growth media (Yu et al., 2011). The release of acid phosphatase is another mechanism to increase available P, leading to the mineralization of organic P. The fungus *Chaetomium globosum* released higher acid phosphatase in inoculated soil and improved the mineralization of organic P and growth of wheat and pearl millet crops compared to uninoculated soil (Tarafdar and Gharu, 2006).

Microbes that enhance P solubilisation are called phosphate-solubilizing microorganisms. These microbes have recently been the focus of several studies with the purpose of increasing plant growth under P limitation through inoculation. For example, phosphate solubilizing rhizobacteria isolated from the rhizosphere of field-grown crops are potential inoculants to improve the growth and yield of canola (*Brassica napus* L.) (deFreitas et al., 1997). Inoculation with *Penicillium radicum* sp. *nov.*, a phosphate solubilizing strain, led to significantly increased yields and P acquisition in wheat (Whitelaw et al., 1997). Rhizobium inoculation has been observed to increase P uptake in soybean, especially in soils with Ca-P as primary P source.

These rhizobia acidified the rhizosphere, and increased P availability in soils with Ca-P source (Qin et al., 2011). Research has also shown that the combination of inoculants resulted in greater effect in comparison with a single inoculant. In fact, dual inoculations between *Pseudomonas fluorescens* BAM-4 and *Burkholderia cepacia* BAM-6 or *Pseudomonas fluorescens* BAM-4 and *Aeromonas vaga* BAM-77 performed more effectively than did single treatments in both the presence and absence of tricalcium phosphate (Jha et al., 2012).

1.4.2 Mechanisms of PUtE

P utilization efficiency (PUtE) is another factor contributing to the improvement of crop yield. Plants with higher PUtE are mainly associated with efficient remobilization, re-use of stored P and the capacity to maintain normal metabolism with low P content in the tissue.

1.4.2.1 P mobilization and remobilization

The remobilization of P in plants has been reported (Peng and Li, 2005). Absorbed P in the plant can be translocated from inactive sites to active sites (Akhtar et al., 2007), thereby assisting the plant to use internal P more efficiently. For example, P in old leaves is remobilized to young leaves where more nutrients are needed for their higher metabolic activities (Akhtar et al., 2008; Stigter and Plaxton, 2015; Maillard et al., 2015). In turn, the healthy development of young leaves helps plant photosynthesis takes place more productively, thus resulting in better yield. P can also so be recycled from the above-ground parts of plant to the roots in order to cope with P deprivation (Peng and Li, 2005). Acid phosphatases that release P_i from organic P are associated with the remobilization of P in the plant. In fact, the higher activity of acid phosphatase

in the leaf of *Brassica napus* resulted in an increase in P re-translocation (Zhang et al., 2010).

The release of stored P_i is another important factor for the plant to adapt to stress conditions. P_i stored in the vacuole under sufficient conditions is released into the cytoplasm under P deprivation in order to a fulfil plant P demand and maintain P homeostasis. However, the mechanisms for this translocation are still not fully understood (Panigrahy et al., 2009).

1.4.2.2 The activation of alternative pathways

Plants can cope with P deficiency through the activation of alternative carbohydrate metabolism pathways. P_i plays an important role in all major metabolic pathways in the plant, including respiration and photosynthesis. Thus, the normal metabolism of carbohydrates is affected under P starvation since the low concentration of P_i and/or organic P such as ATP influences these pathways. Indeed, P deficiency results in a significant decline in the intracellular level of P_i, ATP and ADP (Theodorou and Plaxton, 1993). This could inhibit the activities of enzymes for classical glycolysis that are dependent on adenylates (i.e., hexokinase, 3-phosphoglycerate (3-PGA) kinase) or P_i (i.e., phosphorylating NAD^+-dependent glyceraldehyde-3-phosphate dehydrogenase (GAPDH)). This inhibition then hinders the carbohydrate metabolism in the plant (Plaxton and Podesta, 2006). Although intracellular P_i and adenylate pools are depleted, P_i-stress plants must continue to produce energy and C-skeletons for central metabolic pathways. Studies have shown that in spite of a reduction in the intracellular level of P_i and adenylates, the level of pyrophosphate (PP_i) remains stable (Duff et al., 1989; Fernie et al., 2002). It is proposed that plants can use PP_i as P source

for their activities during P deprivation. In fact, P deficiency induces the activation of several adenylate or P_i-independent enzymes (i.e., Sucrose synthase, UDP-Glc pyrophosphorylase, PP_i-phosphofructokinase, nonphosphorylating NAD-GAPDH, PEP carboxylase, PEP phosphatase, and NAD-ME) that enable carbohydrate metabolism in alternative pathways (Plaxton and Podesta, 2006). These enzymes are called bypass enzymes and can employ PP_i to perform cellular work, while conserving ATP and recycling P_i (Plaxton and Tran, 2011).

1.4.2.3 Remobilisation and modifications of metabolites

Mobilization of sugars from leaves or shoots to roots is also related to a plant mechanism for P deficiency tolerance (Plaxton and Tran, 2011). Sugars in the plant, synthesized via photosynthesis are major sources of energy for plant metabolism. Therefore, the translocation of these sugars to roots is essential for the plant to adapt to P starvation since the presence of sugars assists the roots to grow and extract more P. P deprivation induces the accumulation of sugars and starch in leaves and this accumulation could be remobilized to roots (Hermans et al., 2006). In fact, more sucrose is transported from leaves to roots via the phloem under P stress (Hammond and White, 2008). Liu et al. (2005) reported that the presence of exogenous sugars (sucrose, glucose and fructose) in roots induces the expression of the phosphate transporter gene, *LaPT1* and acid phosphatase gene, *LaSAP1* in white lupin.

Another way that plants adapt to P starvation is by altering metabolites. Although metabolite profiles under P starvation have not been available in wheat, several reports in other plants have been published. A study on barley indicated that mild P starvation at 17 days of growth stimulated plants to accumulate di- and trisaccharides (sucrose,

maltose, raffinose and 6-ketose) in both shoots and roots. Severe P deprivation enhanced the levels of di- and trisaccharides and metabolites associated with ammonium metabolism, but reduced levels of phosphorylated intermediates such as glucose-6-P, fructose-6-P, inositol-1-P, glycerol-3-P and organic acids (α-ketoglutarate, succinate, fumarate and malate) (Huang et al., 2008). Organic acids were also reduced under low P in the *Brachiaria* hybrid and rice (Nanamori et al., 2004). Research in white lupin showed that after 14 days of P starvation, concentrations of phosphorylated intermediates were reduced in both shoots and roots and further decreased after 22 days of P deficiency (Muller et al., 2015). However, in contrast to barley, sugar levels including sucrose in white lupin shoots reduced and organic acid levels increased under P deficiency (Muller et al., 2015). The reduction of sucrose were also found in *Brachiaria* hybrid and rice under P deficiency (Nanamori et al., 2004). Ganie et al. (2015) observed changes in the metabolite profile of maize leaves under low P supply. Similar to the study in barley, di- and trisaccharides increased while phosphorylated intermediates and some organic acids (ketoglutarate, succinate and malate) decreased under P deficiency. This study also indicated that there was a greater increase (low P/sufficient P) in di- and trisaccharides in low P-tolerance maize in comparison with low P-sensitive maize. The accumulation of sugars and the reduction of phosphorylated intermediate levels support plants to reduce P_i consumption to adapt to P deficiency. As previously mentioned, the secretion of organic acids into the rhizosphere is a mechanism for plants to cope with P deficiency. This could result in the reduction of organic acids in plant tissues under P starvation.

Plants can also use non-P lipids as alternatives in the membrane to sustain P_i levels. Phospholipids are replaced with sulfolipids and galactolipids in the membrane in order

to adapt to P starvation (Fang et al., 2009b). Essigmann et al. (1998) reported that the content of sulfolipids increased, while there was a decrease in phospholipid content in *Arabidopsis thailiana* during P depletion. Also, a recent study indicated that perennial ryegrass (*Lolium perenne*) replaced phospholipids with sulfolipids in order to cope with P stress (Byrne et al., 2011). During P shortage, a phospholipase C that hydrolyzes phosphatidylcholine was significantly induced in roots of *A. thaliana*. The induction of this enzyme could result in the degradation of phospholipids and release of P_i for plant activities (Nakamura et al., 2005). During P depletion, the level of phospholipids decreased, whereas the level of galactolipids increased in *Arabidopsis rosettes* (Li et al., 2006b). P deficiency also led to a significant increase in galactolipid content in *Azolla caroliniana* (Ismail and Mohamed, 2010).

1.5 Genetic variation in responses to P of plants

Variation in PUE has been investigated in various crop species. Montenegro and Zapata (2002) reported that there was variation in P acquisition from the source of phosphate rocks between rapeseed genotypes (*Brassica napus* L.). A study in 21 genotypes of pigeonpea (*Cajanus cajan* (L.) Millsp.) also identified variation in PUE. The short-duration genotypes produced greater biomass and showed higher PAE and PUtE than did medium-and long-duration genotypes (Vesterager et al., 2006). Other research showed wide variation in PUE (defined as the ratio of shoot dried weight at low P to shoot dried weight at high P) occurred across 96 soybean genotypes (*Glycine max* (L.) Merrill) on P-deficient soil and PUE was correlated with high biomass production, higher root to shoot ratio, longer root length and greater root surface area (Pan et al., 2008). Genotypic variation in P uptake from a Fe-P source was found in rice where P uptake was associated with root surface area and root volume (Li et al.,

2007). Variation in PUtE was investigated in rice (Wissuwa et al., 2015; Rose et al., 2016). For example, PUtE varied from 1.38 to 2.82 g biomass mg^{-1} P within 292 accessions (Wissuwa et al., 2015). A study in a maize population indicated that genotypes varied in plant growth, root architecture and P uptake, in which P efficient accessions produced greater biomass and a greater root to shoot ratio (Bayuelo-Jiménez et al., 2011).

Natural PUE variation in wheat has been documented. PUE (relative shoot growth) of wheat genotypes, determined as the ratio of shoot dry matter under P deficiency to that under adequate P, varied widely and ranged from 51% to 71% for 39 bread wheat and 47% to 79% for 34 durum wheat (Ozturk et al., 2005). Ninety-nine wheat genotypes grown in a poorly available P source (Fe-PO_4) differed widely in PAE and PUtE, varying from 40.7 to 104.8 (mg P in plant g^{-1} P supplied) for PAE and from 425 to 1076 (mg shoot DM mg^{-1} P in plant) for PUtE under P deficient conditions (Osborne and Rengel, 2002a). Yaseen and Malhi (2009) also found significant differences in PUtE among 20 wheat genotypes in both low P (211 to 365 kg grain kg^{-1} P shoot P uptake) and adequate P (206 to 325 kg grain kg^{-1} P shoot P uptake). Gunes et al. (2006) reported wide variations in the tolerance to P deficiency and P response among 25 wheat genotypes, but they showed no correlation in PUE between greenhouse and field-grown genotypes, therefore growth conditions need to be considered for screening P efficient wheat. Four groups including efficient – responsive, inefficient – responsive, efficient – nonresponsive, and inefficient – nonresponsive were classified from 10 genotypes grown in calcareous soils with different P treatments (Korkmaz et al., 2009). Recently, field research indicated that genotypic variation in grain yield is strongly correlated with variation in PUtE than to variation in P acquisition among 53

wheat genotypes. Therefore, an increase in PUtE is beneficial for PUE improvements (McDonald et al., 2015).

Different traits may be associated with P efficiency in plants. Under low P, common bean genotypes with long root hairs and shallow roots had higher shoot biomass and shoot P accumulation than genotypes with short root hair and deep roots (Miguel et al., 2015). Segregation in root morpho-architecture and root growth traits was observed among 88 soybean recombinant inbred lines and shoot P uptake was positively correlated with root length, root surface area and root volume (Ao et al., 2010). In contrast, a study in wheat showed short-root length and small-root volume cultivars had greater P uptake efficiency (da Silva, Bruno et al. 2016). Bayuelo-Jiménez et al. (2011) reported that P efficient maize genotypes had a greater root to shoot ratio. In another study, P efficient maize genotypes showed significantly higher in root to shoot ratio, specific root surface area, specific root length, specific projected area and specific root volume than the inefficient genotype (de Sousa et al., 2012). In the same study, expression levels of genes, *Rtcs*, *Bk2* and *Rth3*, which are associated with root morphology, were evaluated and showed higher expression levels in efficient genotypes than inefficient genotypes (de Sousa et al., 2012).

Quantitative trait loci (QTLs) associated with PUE have been identified. In phenotyping, different criteria were used for QTL analysis including biomass, P uptake, PUtE and root traits. Wissuwa, Yano, et al. (1998) found three QTLs for dry weight and four QTLs for P uptake, altogether accounting for 45.4% and 54.5% of the variation for respective traits in rice. Three QTLs for internal P use efficiency were found and two of these QTLs coincided with QTLs for P uptake (Wissuwa et al., 1998). This study showed that differences in P uptake was a major factor contributing to P

deficiency tolerance. In a study in soybean, seven QTLs were identified for three traits of shoot biomass, leaf and root P content (Li et al., 2005). Six QTLs linked to rhizosheath size that was correlated with shoot biomass under low P were identified in wheat (James et al., 2016).

Although most studies have focused on PAE, some research on PUtE has been conducted. Screening with low grain P concentration is a strategy for improved PUtE (Ortiz-Monasterio et al., 2011). Indeed, PUtE is measured by grain yield or biomass per unit of P uptake and grain P accounts for most of P content in wheat, therefore, if grain P concentration is low, PUtE is high. Grain P concentration varied widely in rice but was not correlated with either reductions in grain yield or seed size or significantly associated with harvest index (Rose et al., 2010). Similarly, grain P concentration showed significant differences in wheat grain between genotypes, but their grain yields were not different (Schulthess et al., 1997). Therefore, grain P concentration seems to be a criterion for screening and generating crops with low P concentration to reduce P taken up from soil.

It is difficult to evaluate PUtE since it is confounded by differences in P uptake. It appears that genotypes taking up more P from the soil suffer a lower degree of P deprivation, hence their PUtE is lower (Rose et al., 2011). It means that the more P plants absorb, the lower PUtE is present. Rose et al. (2011) suggested that only solution culture growth methods assures all plants accumulate the same amount of P and there make it suitable for PUtE evaluation.

1.6 PAE or PUtE, what is important?

PUE can be enhanced though the improvement of PAE and PUtE. PAE is a plant's

ability to extract and take up P in the soil, while PUtE is the ability to convert P absorbed into yield. Therefore, PAE is determined by root traits and root activities, while PUtE is determined by P remobilization and re-use. It is ideal if both PAE and PUtE are improved. However, it is more difficult to target both at once. Wang et al. (2010) suggested that the importance of PAE or PUtE depends on P status in soils.

The relative importance of PAE and PUtE seems to vary with P status in the soil. In low P soils, PAE could play a more important role than PUtE. Manske et al. (2001) reported that PAE was a major factor contributing to PUE in wheat under low P supply in both acidic and calcareous soils. PAE, but not PUtE, contributed to greater biomass production under low P supply in pigeonpea (Fujita et al., 2004). Higher P uptake produced greater grain yield in a P efficient rice line under P deficiency (Wissuwa and Ae, 2001). Under low P, common bean with PAE had greater shoot biomass (Miguel et al., 2015). A study in maize indicated that PAE was positively correlated with PUE, while PUtE was negatively associated with PUE under low P (DoVale and Fritsche-Neto, 2013). The screening of genotypes with high PAE has been suggested as a means to improved PUE in rapeseed (*Brassica napus* L.) in low P soils (Zhang et al., 2009).

When P supply is adequate, PUtE could be more important for PUE than PAE. Indeed, with adequate P, genotypes that use less P and produce higher yield should be considered for planting since this could reduce the cost of fertilizers or can save P in soils. PUtE was the main factor accounting for variations in wheat grain yield under P adequate supply (Manske et al., 2001). Balemi and Schenk (2009) stated that PUE was associated with PUtE in potato. PUtE was mainly responsible for PUE in wheat (McDonald et al., 2015) and Faba bean (*Vicia faba* L.) (Daoui et al., 2012).

In maize, PUE was positively correlated with PAE, but negatively related to PUtE in both low and high P conditions (DoVale and Fritsche-Neto, 2013). PAE was important for PUE in some wheat genotypes while PUtE was more important for PUE in other wheat genotypes in the germplasm tested (Ozturk et al., 2005). Vesterager et al. (2006) reported that PUE was negatively correlated with PAE in pigeonpea. Therefore, it seems to be important to simultaneously consider both PAE and PUtE. High PAE crops are vital for growing in soil with high soil P content but low available P, while high PUtE plants are necessary in reducing P application. Genotypes harbouring both PAE and PUtE traits are ideal for plant breeding. Manske et al. (2001) suggested that these genotypes require selection under both low and adequate P conditions. Although PAE and PUtE were reported to be negatively associated in some studies (Su et al., 2006; Rose et al., 2011), Su et al. (2009) found positive linkages between QTLs for PAE and PUtE at two loci in winter wheat. This would allow the potential to improve PAE and PUtE simultaneously.

1.7 Effect of light intensity on plant growth and responses to P of plants

Light intensity is one of the most important factors for plant growth and development but it varies between regions over the world and affects the growth of plants. For example, the problem of low light intensity in Bangladesh impacts the growth of wheat (Saifuzzaman et al., 2004). Furthermore, light intensity has an influence in responses to P in plants (Yin et al., 2012). Therefore, light intensity would need to be considered when evaluating PUE in plants.

Effect of light intensity on plant growth has been documented in various studies. For

example, in a study by Koksal et al. (2015) supplemental LED light was used to promote plant growth in pansy (*Viola cornuta*) or high light intensity stimulated both root and shoot growth in wheat (Page and Feller, 2016). Another study in wheat showed that low light intensity increased the time of ear emergence (Evtushenko and Chekurov, 2004). Light quality also affects yield and nutrient profiles in plants. The combination of red and white light were shown to increase harvest index and reduce lignin in wheat (Dong et al., 2014a). Dong et al. (2014c) also reported that wheat grew and developed more efficiently under red-white light and white light.

Light intensity and P supply affect plant growth, P uptake and photosynthetic parameters. Photosynthetic rate (P_n) increased under high light intensity in rice but higher P_n did not affect plant growth under P starvation (Wissuwa et al., 2005). This study also found that although shoot P uptake under high light intensity was greater than that under low light at high P supply, light intensity did not lead to differences in shoot P uptake under low P treatments. However, a study in white lupin indicated increased P uptake with increasing light intensity under both P deficient and P sufficient conditions (Cheng et al., 2014). In a study in rice plants, P deficiency resulted in a reduction in P_n (Xu et al., 2007). Interestingly, Li et al. (2006c) have shown that a low P tolerant rice had greater P_n than a low P sensitive genotype under P deficiency. However, intercellular CO_2 and stomatal conductance were not different between these two rice genotypes under both low and high P treatments. P supply and light intensity affected growth of two weed species *Veronica perisca* and *Chorispora tenella* quite differently. Under low P supply, growth of *V. perisca* was more limited when compared to *C. tenella* under high light intensity, but their responses were reversed under low light intensity (Yin et al., 2012), suggesting that light intensity may

affect responses to P of plants.

1.8 Root trait measurement

The root system plays a very important role for plants to acquire nutrients from the soil. Therefore, an investigation of root traits related to P uptake could contribute to improved PUE in plant. A number of studies have tried to understand how roots develop under nutrient deficiency (Lambers et al., 2006; Peret et al., 2011). Root architecture and morphology traits have been used to elucidate how roots adapt to stress conditions. Root architectural and morphological traits have normally been reported together and are often not clarified. However, according to Lynch (1995) and Bucksch et al. (2014), root morphology is described as the surface features of a single root axis as an organ including characteristics of root hairs, while root architecture traits refer to geometric or topological measures of the root shape (i.e. root angle, total root length, root tip number and convex hull area) generally at the scale of the whole root system or a large subset of the root system. With continuous development of root analysis software tools, more root architectural and morphological traits have been investigated such as convex area, projected root area, perimeter and root tip number (Galkovskyi et al., 2012). Recently, Bucksch et al. (2014) developed software that can elucidate root density, root angle, root width accumulation at a specific depth and other traits. Root hair length and root hair density can also be measured using a microscope (Gahoonia and Nielsen, 2004a; Yuan et al., 2016).

In order to elucidate root architectural and morphological traits, three main steps need to be carried out: elaborate experimental work to obtain root systems, appropriate root-imaging techniques and image analysis using software (Leitner and Schnepf, 2012).

Roots can be obtained by various methods. Hydroponic growth is a simple method for root trait analysis (Petrarulo et al., 2015), however root traits of plants cultured in solution may be different from those of plants cultured in soil since solution cultivation is very different from soil cultivation. Besides, root architecture is altered after harvest if plants are grown under both soil and solution culture, especially fine roots as in wheat and rice, thus spatial distribution could not be analyzed. Plants can be grown on transparent media (gel) to make it easy to image the root systems (French et al., 2009; Naeem et al., 2011; Iyer-Pascuzzi et al., 2010). Although plant roots grown in the transparent media may not behave similarly to those grown in soil. This method has some advantages such as real time measurement without destroying plant root systems. Courtois et al. (2013) developed the hydroponic system with glass beads to solve some limitations of hydroponic and media growth system. In this type of cultivation, plants were grown in rhizoboxes that contain nails, which kept roots in place after removing glass beads. This method can measure root spatial distribution and is more similar to soil growth in comparison with hydroponic and medium growth.

When roots are obtained, the root system is captured using flat-bed scanners (Bengough et al., 2004; Lobet et al., 2011) or cameras (Clark et al., 2013). The root system images are then analyzed by suitable software tools.

The number of software tools for root analysis is quite substantial. In general, root analysis software tools are classified into three main types including manual, semi-automated and fully automated methods, according to the manual levels of user interaction (Ristova et al., 2013; Lobet et al., 2015). The first approach is the traditional measurement of root traits, which relies on manual procedures such as DART (Data Analysis of Root Tracing) (Le Bot et al., 2010). Although SmartRoot is a semi-

automated software tool, it has manual analysis functions (Lobet et al., 2011). These

Table 1.1. Software tools for root analysis

Software tool	Automation	Image type	Reference
Aria	Automated	2D	(Pace et al., 2014)
EZ-Rhizo	Automated	2D	(Armengaud et al., 2009)
DART	Manual	2D	(Le Bot et al., 2010)
DIRT	Automated	2D	(Bucksch et al., 2014)
GiA Roots	Automated	2D	(Galkovskyi et al., 2012)
RootGraph	Automated	2D	(Cai et al., 2015)
Rootnav	Semi-automated	2D	(Pound et al., 2013)
RootReader2D	Automated	2D	(Clark et al., 2013)
RootReader3D	Automated	3D	(Clark et al., 2011)
RootSystemAnalyser	Automated	2D	(Leitner et al., 2014)
RooTrak	Automated	3D	(Mairhofer et al., 2012)
RooTrace	Automated	2D	(French et al., 2009)
RootSmart	Semi-automated	2D	(Lobet et al., 2011)

classical methods have an advantage of accurately measuring the root systems but they are very time consuming and are not suitable for analyzing complicated root systems.

Several semi-automated software tools that reduce manual interactions during analysis have been released. RootSmart (Lobet et al., 2011) and RootNav (Pound et al., 2013) are two semi-automated image analysis tools. In another approach, automatic root analysis tools have been developed for further reducing analysis time and these software tools can meet the requirements of measuring a large number of roots such as in a larger population. These image analysis tools include RooTrace, EZ-Rhizo, GiA Roots, RootGraph, and DIRT (Table 1.1).

Although most root analysis tools have been developed for 2D analysis,

RootReader3D and RooTrak, can be used for 3D analysis (Table 1.1). Plants need to grow with nondestructive root growth systems to obtain 3D images or advanced imaging techniques need to be applied. Growing plants in transparent gel cylinders is a simple method to obtain 3D root images (Clark et al., 2011). X-ray computed tomography was used to observe root system in their natural state within soil (Mooney et al., 2012). Magnetic resonance imaging (MRI) is another method that can be applied for root trait analysis (Nagel et al., 2009; van Dusschoten et al., 2016). A three-dimensional laser scanner can also be utilized to capture root system in transparent-gel based cylinders (Fang et al., 2009a). Although three-dimensional approaches are more advanced, they are expensive methods (Iyer-Pascuzzi et al., 2010). Thus, two-dimensional methods are still more prevalent since they are inexpensive and accessible.

Aims of this research

This research firstly aims to examine variation in PUE of six selected wheat genotypes which have been reported in field studies, to differ in PUE. Evaluations take place under controlled environments to identify suitable conditions that give similar results to field results and to further select two wheat genotypes with contrasting PUE for mechanistic studies of P efficiency. Secondly, a simple soil-based cultivation is developed for elucidating root architectural traits related to PUE of the selected wheat genotypes. Differences in root hair features of two wheat genotypes are also estimated. Lastly, variations in metabolite profiles between the two wheat genotypes are investigated to identify metabolic responses which are associated with PUE.

The following work is divided into four research chapters describing distinct yet

related aspects of research into PUE: (i) variation in PUE of six selected wheat genotypes under controlled environments (Chapter 2); (ii) effect of light intensity on phosphorus responses and photosynthetic parameters of two wheat genotypes differing in phosphorus use efficiency (Chapter 3); (iii) variation in root architecture and morphology of two wheat genotypes with contrasting PUE (Chapter 4); (iv) phosphorus use efficiency in RAC875 is associated with the accumulation of raffinose and maintenance of phosphorylated sugars (Chapter 5).

CHAPTER 2

Variation in phosphorus use efficiency among six wheat genotypes under two different controlled environment conditions

Abstract

Wheat (*Triticum aestivum* L.) is one of the world's major crops and is susceptible to various abiotic stresses, including phosphorus (P) deficiency. Developing wheat genotypes that can tolerate P deficiency will assist in improve yields under P deficient conditions. Measuring P use efficiency (PUE) is not an easy task as PUE is affected by the environment. A more controlled environment such as a greenhouse or a growth room is ideal for screening but may not reflect field responses and any controlled environment need also to be evaluated against published responses for the same genotypes. This then acts as a validation for the screening method. Six selected wheat genotypes were used for the examination of PUE and P responsiveness under two growth conditions; greenhouse and growth room. Plants were grown at six different rates of P supply (2.5, 5, 10, 20, 30 and 40 mg P kg^{-1} soil) under greenhouse conditions and the results showed a P supply of 30 mg P kg^{-1} soil was optimal for plant growth. The external P requirement (defined as amount of P supplied to achieve 90% of maximum grain yield) varied from 13.6 to 19.9 mg P kg^{-1} soil among wheat genotypes. External P requirement and $PUE_{GY10/30}$ (calculated as relative grain yield at P supply of 10 to 30 mg P kg^{-1} soil) showed relatively similar results for the assessment of PUE between the wheat genotypes. In a growth room, plants were also grown at two P

treatments (10 and 30 mg kg^{-1} soil). PUE was evaluated based on the external P requirement and relative shoot biomass (or grain yield) at a P supply of 10 to 30 mg P kg^{-1} soil ($PUE_{10/30}$), while P responsiveness was assessed based on shoot biomass or grain yield at high P supply. Wheat genotypes did not all show consistency in PUE and responses to P between the two growth conditions. Results from the growth room conditions were more consistent with published field results than those under greenhouse conditions. Genotypic variation in root dry matter and root volume was observed, in which RAC875 and Scout had a smaller root system, while Wyalkatchem possessed a large root system under both growth conditions. In contrast, root efficiency (calculated as shoot P uptake per unit of root dry matter) was higher in RAC875 and Scout, while it was the lowest in Wyalkatchem. Root efficiency was positively correlated with PUE at both levels of P supply ($r=0.565^{**}$ and $r=0.432^{*}$ at 10 and 30 mg P kg^{-1} soil, respectively) under growth room conditions. The wheat genotypes with contrasting root size and root efficiency may be beneficial for examining how a small root system contributes to higher grain yield and PUE. Response variations between controlled growth conditions also create an awareness of the need to scrutinize results and validate against published field responses.

2.1 Introduction

Phosphorus (P) is an important macronutrient for plant growth and development (Roberts and Johnston, 2015), however available P is limited in many soils (Shepherd et al., 2016). It was estimated that over 40% of the world agricultural lands are low in P (Balemi and Negisho, 2012) and such shortages are often overcome with the application of P fertilizers (Cordell and Neset, 2014). The main fertilizer source of rock P is a non-renewable resource and may be depleted by 2050 (Vance et al., 2003), therefore, the development of P efficient crops is an approach to cope with the limitation of P resources; where efficiency is defined as the efficiency in P acquisition and P utilization for plant improvements in biomass or grain yield, particularly under low P availability (this definition is adapted from the definition of nutrient efficiency in plant by Ciarelli et al. (1998)).

A number of studies on genotypic variation in phosphorus use efficiency (PUE) have been reported (Subbarao et al., 1997; Osborne and Rengel, 2002b; Sepehr et al., 2009; Bayuelo-Jiménez et al., 2011; Fageria, 2014). Plants often modify their root traits in response to P deficiency and this can be linked to efficiency mechanisms, with greater basal root whorl number enhancing P acquisition under low P soil supply(Miguel et al., 2013), while longer root hairs improved P uptake (Gahoonia et al., 1997) and contributed to higher yields (Gahoonia and Nielsen, 2004a; Miguel et al., 2015). Candidate genes controlling efficiency mechanisms of root traits, have also been reported (de Sousa et al., 2012) and on validation, these heritable traits could be used in marker assisted plant breeding.

Wheat (*Triticum aestivum* L.) is a staple food crop and is one of the most important

cereal crops in the world (Shewry, 2009), therefore the generation of P efficient wheat is important for enhancement of crop yield to meet global food demand. Understanding the mechanisms of PUE is essential to produce P efficient plants. Investigation of genotypic variation in PUE is often targeted first and then genotypes differing in PUE can be used to elucidate the mechanisms of PUE can then be elucidated.

There are reports that wheat genotypes respond differently to P supply and vary in PUE (Akhtar et al., 2011; Ozturk et al., 2005; Liao et al., 2008; da Silva et al., 2016). Recent field experiments of 53 wheat genotypes was able to show that some genotypes produced relatively high yield at low P supply and some were highly responsive to P (McDonald et al., 2015). Therefore, genotypes with contrasting PUE from this research can be used to investigate mechanisms of P efficiency. Although controlled conditions (i.e. greenhouse or growth room) can make ease for mechanistic studies, responses to P in plants may differ between controlled and field conditions. Gunes et al. (2006) observed no correlation in PUE of 25 wheat cultivars between greenhouse and field conditions. Thus, it is crucial to select controlled conditions that show similar PUE to field conditions for screening P efficient wheat or for further mechanistic study of PUE.

da Silva et al. (2016) showed that root volume was negatively correlated with PUE in wheat. It appears that the efficiency of a root system in to acquire P is more important than the actual root size if one is to PUE. Root efficiency is calculated as mg shoot P uptake per unit of g root dry matter or root surface area (Mori et al., 2016). According to Jones et al. (1989) root efficiency is an indication as to the fineness and structure of a root system and its soil explorative capacity and it may be used in breeding programs for improved P efficiency in wheat. Studies in rice have shown that genotypes with

high root efficiency (in this case, calculated as shoot P uptake per unit of root surface area) had greater P uptake efficiency (Mori et al., 2016; Wissuwa, 2005). Thus, root efficiency could be a useful criterion for screening P efficient wheat.

The aim of this study is to test the hypothesis that six wheat genotypes (RAC875, Scout, Gladius, Mace, Correll and Wyalkatchem) reported to vary in P efficiency within the field (McDonald et al., 2015), also exhibit P efficiency in more controlled environment conditions. If there is alignment between field and controlled environments, one can then use the more controlled environment for conducting more controlled environment experiments to identify the mechanisms of PUE in wheat. Root efficiency and the correlation of root efficiency and PUE also were investigated.

2.2 Materials and methods

2.2.1 Materials

Six wheat genotypes (sourced from Dr Glenn McDonald at the Waite Campus, University of Adelaide) with varied responses to P deficiency under field conditions, were used in this experimentation. The genotypes included RAC875, Scout, Gladius, Mace, Correll and Wyalkatchem.

2.2.2 Experiment 1. Greenhouse conditions

Doubled washed sandy soil with characteristics presented in Table 2.1 (bought from Marion Sand & Metal Pty Ltd) was air-dried and 4.2 kg of soil and weighed into plastic bags with a basal nutrient addition (expressed in mg kg^{-1} soil) consisting of $Ca(NO_3)_2.4H_2O$ (918), K_2SO_4 (113.6), $MgSO_4.7H_2O$ (140), $FeSO_4.7H_2O$ (1.4), NaCl (3.2), $CuSO_4.5H_2O$ (2.25), $MnSO_4.4H_2O$ (3.68), $ZnSO_4.7H_2O$ (6.6), H_3BO_3 (0.28).

Nutrient was mixed into the soil by massaging the soil through the outside of the plastic bag. Phosphorus (P) (in the form of KH_2PO_4) was then added to the bags at six different levels, 2.5, 5, 10, 20, 30 and 40 mg P kg^{-1} soil. The added P was then massaged into the soil as mentioned in the method above. The bag of prepared soil was placed in a round-shaped pot with the following dimensions: 18.5-cm high x 17.5-cm diameter at the top x 16.0-cm diameter at the bottom.

Five wheat seeds were sown into each pot in August 2013 in a greenhouse located at Flinders University and the experiment was carried out with four replicates. The temperature in the greenhouse varied during the time of the experiment since the temperature in the greenhouse changed when outdoor temperature changed. Two plants per pot were harvested at 27 days after sowing (DAS) and at 48 DAS, one further plant was harvested per pot and the remaining two plants were allowed to grow to maturity. Plants were watered to 8-10% of the soil weight three times a week. However, at ripening stage plants were watered two times a week since plants required less water.

At maturity, plants were harvested and stem parts close to soils were washed and then rinsed with high purity (> 18 MΩ resistivity) Milli-Q water. The stems were then dried at 85 ^{o}C for 24 h before taking dry weight measurements. Spikelets were dried at 37 ^{o}C and grains were obtained using a Haldrup thresher and the relative grain yield was then measured.

Plant roots were washed to remove excess soil and the volume of roots was measured by measuring the volume of displaced water that roots occupied in a volumetric flask. The dry matter of roots was also measured after 48 h of drying at 85 ^{o}C.

Table 2.1. Some properties of sandy soil analyzed by CSBP Soil & Plant Analysis Laboratory, Western Australia

Parameter	Sandy soil
Texture	1
Gravel (%)	3.3
$pH_{1:5}$ (CaCl2)	6.7
$pH_{1:5}$ (H_2O)	7.3
Electrical conductivity (dSm^{-1})	0.09
Organic carbon (%)	< 0.05
Ammonium nitrogen (mg kg^{-1})	< 1
Nitrate nitrogen (mg kg^{-1})	1.3
Phosphorus Colwell (mg kg^{-1})	< 2
Potassium Colwell (mg kg^{-1})	16.5
Exchangeable potassium (meq $100g^{-1}$)	0.04
Boron (mg kg^{-1})	0.23
DTPA copper (mg kg^{-1})	0.18
DTPA iron (mg kg^{-1})	4.08
DTPA manganese (mg kg^{-1})	0.17
DTPA zinc (mg kg^{-1})	0.08
Exchangeable aluminium (meq $100g^{-1}$)	0.2
Exchangeable magnesium (meq $100g^{-1}$)	0.21
Exchangeable sodium (meq $100g^{-1}$)	0.38
Exchangeable calcium (meq $100g^{-1}$)	0.27

2.2.3 Experiment 2. Growth room conditions

Plants were grown in pots as described in Experiment 1 and filled with 4.2 kg of sandy soil with two P levels of 10 and 30 mg P kg^{-1} soil. Basal nutrients (expressed in mg kg^{-1} of soil) consisting of $Ca(NO_3)_2.4H_2O$ (918), K_2SO_4 (113.6), $MgSO_4.7H_2O$ (140), $FeSO_4.7H_2O$ (1.4), $Na_2MoO_4.2H_2O$ (0.61), $CuSO_4.5H_2O$ (2.25), $MnSO_4.4H_2O$ (3.68), $ZnSO_4.7H_2O$ (6.6), H_3BO_3 (0.28) were also added and mixed into the soil.

Wheat grains were sterilized in 2% hypochlorite for 10 min and were then rinsed with Milli-Q water. Grains were then germinated in petri disks lined with moisturized filter papers that were kept in a dark place at room temperature for three days. One germinated grain was sown per pot and the experiment was conducted with four replicates in a growth room (temperature: 10 ^{o}C night time, 20 ^{o}C day time; 13 h light period with a light intensity of 700 µmol m^{-2} s^{-1} at the leaf surface. The type of light is fluorescent and incandescent. Plants were watered to 8-10% of the soil weight three times a week. However, at ripening stage, plants were watered two times a week since plants required less water.

Plants were harvested at maturity. Spikelets were detached from stems and stems were detached from roots. Spikelets were then dried at 37 ^{o}C for five days and grains were obtained using a Haldrup thresher and grain yield was then measured. Stem parts close to the soil were rinsed with Milli-Q water. Plant roots were obtained and washed to remove excess soil and the volume of root was measured by volume of displaced water that roots occupied in a volumetric flask. Stems and roots were then dried at 85 ^{o}C for 48 h to gain the dry matter.

2.2.4 Measurements

The P concentration in shoot, grain and straw was determined by ICP-MS (Agilent Technologies, Model 7500cx) using the digestion method (proposed closed-tube method) of Wheal et al. (2011). Various criteria were used for PUE evaluation, including: 1. external P requirement; 2. internal P requirement; 3. relative growth (calculated as ratio between low P and adequate P); 4. P acquisition efficiency (PAE); 5. P utilization efficiency (PUtE) and 6. shoot biomass (or grain yield) under low P.

External P requirement (defined as amount of P supplied to achieve 90% of maximum grain yield or shoot dry matter) and internal P requirement (defined as shoot P concentration to achieve 90% of grain yield or shoot dry matter) were calculated based on the Mitscherlich response curve (Dobermann et al., 2011; Bolland and Brennan, 2008). Relative growth was calculated as the shoot biomass ratio (or grain yield) between a P supply of 10 and 30 mg P kg^{-1} soil ($PUE_{10/30}$). PAE was calculated as the percentage of total P uptake to P supplied per pot. PUtE was calculated based on shoot dry matter at the vegetative and maturity stages ($PUtE_{SM}$; mg shoot dry matter per mg total P taken up by the shoots) as well as on grain yield ($PUtE_{GY}$; mg grain yield per mg total P taken up) (Ortiz-Monasterio et al., 2011). PUE was then derived from grain yield per unit of P supply ($PAE*PUtE_{GY}/100$). Harvest index (HI, % of the grain yield to the shoot dry matter) and P harvest index (PHI, % of P content in the grain to P content in the shoot) were also calculated.

2.2.5 Statistical analysis

Statistical analyses were conducted by using IBM SPSS v23. The normality of data was tested using Kolmogorov-Smirnov and Shapiro-Wilk test ($P<0.05$). Root efficiency was not normal distributed and was transformed using $\log_{10}$. Plant indices were analysed by two-way independent ANOVA (Genotype x P supply). Significant differences between the means were analysed by Tukey's test ($P<0.05$). The particular sets of variables was subjected to Pearson's correlation analysis (Field, 2013).

2.3 Results

2.3.1 Experiment 1. Greenhouse responses

2.3.1.1 Shoot dry matter (DM), grain yield, tiller number, root volume, root DM, shoot to root ratio, harvest index (HI) and phosphorus harvest index (PHI) under greenhouse conditions

There was a significant (P<0.001) effect of P supply on grain yield, shoot DM and tiller number (Table 2.2, Table 2.3). Grain yield and shoot DM increased dramatically from the P supply of 2.5 mg P kg^{-1} soil to the P supply of 10 mg P kg^{-1} soil then slightly rose and reached the plateau at a P supply of 30 mg P kg^{-1} soil (Figure 2.1, Figure 2.2). Similar to grain yield and shoot DM, tiller number also increased with an increase in P supply but reached the plateau at 20 mg P kg^{-1} soil. Across all genotypes, plants did not produce tillers at the extreme low P supply of 2.5 mg P kg^{-1} soil (Figure S 2.1).

Significant (P<0.001) genotypic variation in grain yield, shoot DM and tiller number were observed between wheat genotypes (Table 2.2, Table 2.3). Scout and Mace produced high grain yield and shoot DM while RAC875 and Wyalkatchem produced low grain yield and shoot DM. Scout also had a high tiller number. Strong genotype x P supply (G x P) interactions (P<0.001) were observed for grain yield, shoot DM and tiller number (Table 2.2, Table 2.3) and this indicated that wheat genotypes behaved differently under different P treatments. Grain yield and shoot DM in Scout was relatively low at 2.5 and 5 mg P kg^{-1} soil but high at higher P supply. RAC875 showed significantly (P<0.05) lower grain yield and shoot DM in comparison to Mace, Correll and Wyalkatchem at the P supply of 5 mg kg^{-1} soil, while there was no difference at the P supply of 30 mg kg^{-1} soil (Figure 2.1, Figure 2.2).

Table 2.2. Grain yield, shoot dry matter (DM), shoot P uptake, P concentration in grain, straw and shoot, HI, PHI, PAE and PUtE at maturity of six wheat genotypes grown in a greenhouse at different P treatments

Genotype	Grain yield (g plant^{-1})	Shoot matter (g plant^{-1})	P uptake (mg shoot^{-1})	P concentration (mg kg^{-1})			HI	PHI	PAE (P uptake/ P supply, %)	PutE	
				Grain	Straw	Shoot				Shoot (mg DM mg^{-1} P uptake)	Grain (mg grain mg^{-1} P uptake)
RAC875	3.2^{a}	5.8^{a}	11.2	2754^{a}	270	1602^{a}	51.4^{acd}	91.2^{a}	14.2	843^{a}	388^{ab}
Scout	4.0^{b}	7.9^{b}	11.2	2370^{b}	259	1267^{cd}	46.8^{b}	88.7^{b}	14.5	934^{b}	410^{ab}
Gladius	3.7^{bc}	6.9^{c}	11.5	2465^{bc}	263	1428^{bd}	51.3^{acd}	90.7^{a}	15.2	832^{a}	406^{ab}
Mace	3.9^{b}	7.0^{c}	11.3	2344^{b}	277	1379^{d}	51.7^{acd}	89.7^{b}	14.6	857^{ab}	417^{a}
Correll	3.6^{c}	6.4^{d}	11.4	2604^{abc}	280	1551^{ab}	53.1^{c}	91.4^{a}	15.2	777^{a}	390^{ab}
Wyalkatchem	3.5^{c}	6.7^{cd}	11.9	2718^{c}	291	1525^{ab}	49.8^{d}	90.0^{ab}	15.8	802^{a}	379^{b}
F Ratio											
Genotype (G)	16.225***	41.606***	1.096 ns	6.919***	1.538 ns	12.355***	11.922***	9.564***	2.2021 ns	5.347***	3.028*
G × P	3.640***	6.176***	1.984**	2.651***	1.437 ns	2.837***	1.053 ns	1.958*	3.209***	6.828***	5.981***
P treatment (P)											
2.5	0.4^{a}	0.95^{a}	0.6^{a}	1516^{a}	153^{a}	665^{a}	37.4^{a}	84.9^{a}	6.1^{a}	1545^{a}	570^{a}
5.0	1.6^{b}	3.4^{b}	3.1^{b}	1790^{b}	189^{b}	949^{b}	47.4^{b}	89.4^{b}	15^{b}	1069^{b}	504^{b}
10	4.1^{c}	7.7^{c}	9.1^{c}	2075^{c}	218^{c}	1194^{c}	52.6^{c}	91.2^{c}	21.6^{c}	861^{c}	451^{c}
20	5.2^{d}	9.4^{d}	15.6^{d}	2809^{d}	297^{d}	1680^{d}	55.3^{d}	92.0^{c}	18.6^{d}	606^{d}	335^{d}
30	5.4^{d}	9.6^{d}	19.2^{e}	3508^{e}	386^{e}	2120^{e}	55.7^{d}	91.9^{c}	15.9^{e}	480^{e}	267^{e}
40	5.3^{d}	9.6^{d}	20.8^{e}	3606^{e}	397^{e}	2178^{e}	55.6^{d}	91.8^{c}	12.4^{f}	465^{e}	258^{e}
F Ratio											
P	917.400***	1199.811***	668.273***	189.069***	116.663 ***	354.691***	133.207***	63.067***	184.595***	400.062***	252.718***

Data represent the means of four replicates. Analysis of variance performed on original data. *F* ratios are from two-way ANOVA analysis. *, ** and *** significant at P<0.05, P<0.01 and P<0.001, respectively; ns: not significant. Different letters show significant differences between genotypes or P supply.

Table 2.3. Tiller number, shoot height, root dry matter, root volume, root length, shoot to root ratio and root efficiency at maturity of six wheat genotypes grown in a greenhouse at different P treatments

Genotype	Tiller number (tiller plant^{-1})	Shoot height (cm)	Root dry matter (g plant^{-1})	Root volume (ml plant^{-1})	Root length (cm)	Shoot to root ratio (g g^{-1})	Root efficiency (mg g^{-1})
RAC875	2.3^{a}	59.1^{a}	0.27^{a}	1.4^{a}	35.9^{ab}	23.3^{a}	38.8^{a}
Scout	2.8^{b}	59.1^{a}	0.42^{b}	2.5^{b}	35.3^{a}	18.4^{d}	24.6^{b}
Gladius	2.4^{a}	64.4^{c}	0.46^{bd}	2.2^{b}	39.0^{b}	15.4^{b}	22.3^{bcd}
Mace	2.5^{ac}	61.1^{a}	0.53^{d}	2.7^{b}	41.8^{c}	13.3^{bc}	18.9^{c}
Correll	2.5^{ac}	65.1^{c}	0.42^{b}	2.2^{b}	43.7^{c}	16.4^{bd}	24.4^{b}
Wyalkatchem	2.7^{bc}	52.8^{b}	0.71^{c}	3.7^{c}	40.0^{c}	10.8^{c}	15.4^{d}
F Ratio							
Genotype (G)	7.978***	36.823***	33.461***	25.571***	12.569***	20.486***	28.679***
G × P	2.392**	2.709***	4.085***	2.576***	3.258***	1.329 ns	2.202*
P treatment (P)							
2.5	1^{a}	49.6^{d}	0.07^{a}	0.46^{a}	33.1^{a}	13.9^{a}	9.1^{a}
5.0	1.3^{b}	62.5^{ac}	0.26^{b}	1.54^{b}	43.6^{b}	14.5^{ad}	14.0^{b}
10	2.9^{c}	64.9^{b}	0.45^{c}	2.71^{c}	39.3^{c}	18.4^{b}	22.5^{c}
20	3.4^{d}	62^{ac}	0.54^{d}	2.69^{d}	38.5^{c}	18.5^{b}	30.9^{d}
30	3.3^{d}	61.7^{ac}	0.63^{e}	3.16^{e}	40^{bc}	17.6^{bd}	37.5^{e}
40	3.3^{d}	60.8^{ac}	0.84^{f}	4.14^{f}	41.3^{bc}	14.6^{a}	31.1^{d}
F Ratio							
P	258.475***	53.559***	118.933***	73.867***	14.390***	4.979***	106.796***

Data represent the means of four replicates. Analysis of variance performed on original data except for root efficiency transformed using lg10. *F* ratios are from two-way ANOVA analysis. *, ** and *** significant at P<0.05, P<0.01 and P<0.001, respectively; ns: not significant. Different letters show significant differences between genotypes or P supply.

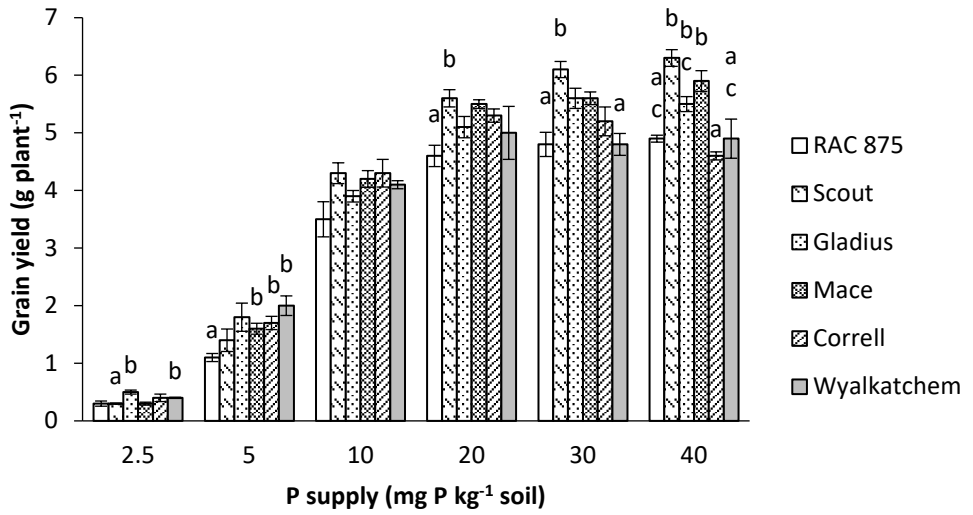


Figure 2.1. The effect of P supply on the grain yield of six wheat genotypes grown in the greenhouse at maturity. Data represent means of four replicates with standard errors. Different letters show significant differences within each P supply ($P<0.05$). Within each P supply, columns without letters show no significant differences among each other and with labelled columns.

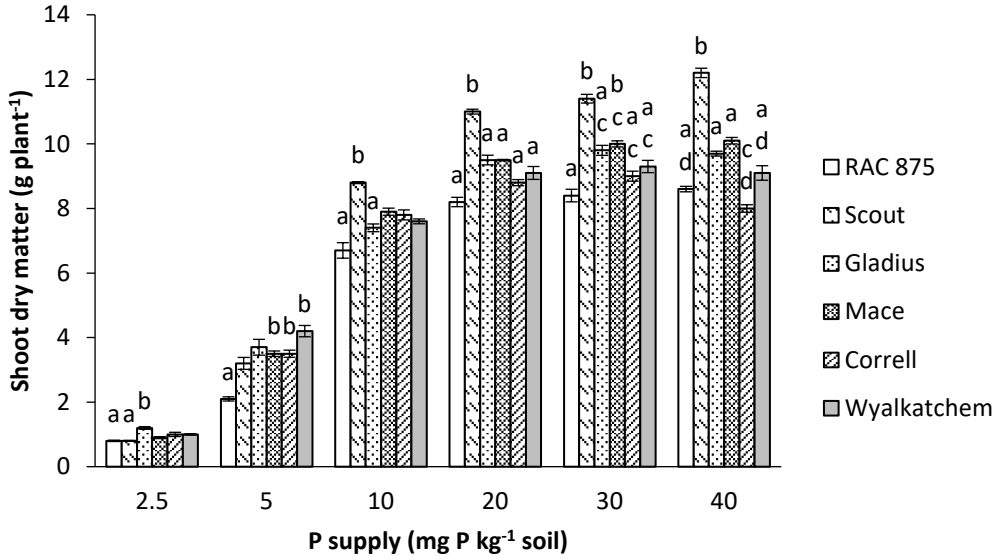


Figure 2.2. The effect of P supply on the shoot matter of six wheat genotypes grown in the greenhouse at maturity. Data represent means of four replicates with standard errors. Different letters show significant differences within each P supply ($P<0.05$). Within each P supply, columns without letters show no significant differences among each other and with labelled columns.

Responses to P supply were also observed in wheat genotypes at growth stages of 27 and 48 DAS (Table S 2.1). Significant genotypic variation in shoot DM was observed, however these differences were not correlated with shoot DM at the maturity stage. Scout produced low biomass at earlier growth stages, but this genotype had high biomass at maturity (Table 2.2, Table S 2.1).

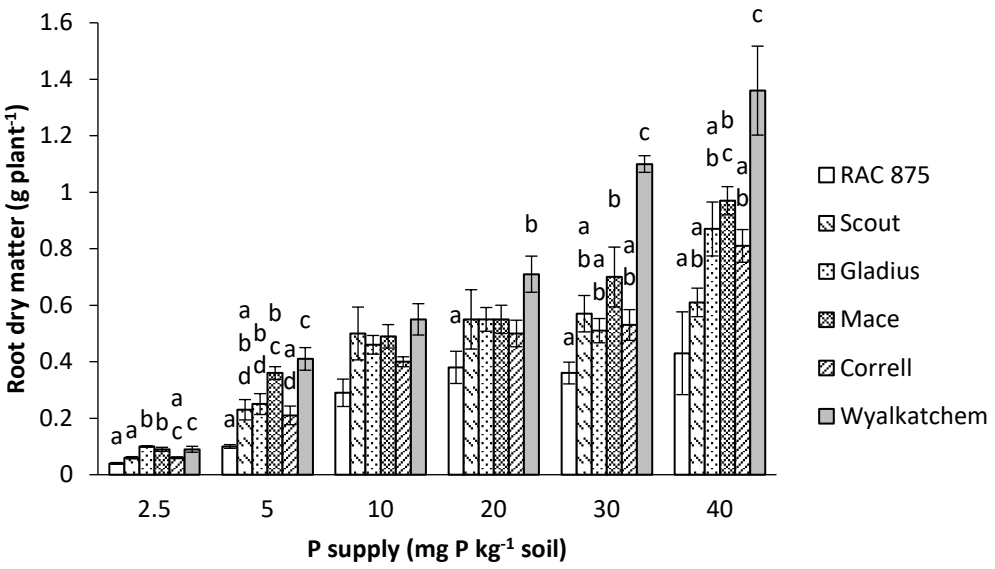


Figure 2.3. The effect of P supply on the root dry matter of six wheat genotypes grown in the greenhouse at maturity. Data represent means of four replicates with standard errors. Different letters show significant differences within each P supply ($P<0.05$). Within each P supply, columns without letters show no significant differences among each other and with labelled columns.

Root DM and root volume were strongly correlated (Table 2.6) and increased steadily with an increase in P supply. Root DM and root volume increased from 0.07 to 0.84 g plant^{-1} and 0.46 to 4.41 mL plant^{-1} respectively, from a P supply of 2.5 to 40 mg P kg^{-1} soil (Figure 2.3, Figure S 2.7). These two parameters significantly ($P<0.001$) varied among wheat genotypes (Table 2.3). Indeed, RAC875 had the smallest root system (0.27 g DM plant^{-1}) and significantly smaller than the other genotypes, while Wyalkatchem had the largest root system (0.71 g DM plant^{-1}) and was significantly

larger than the other genotypes. Mace also had a relatively large root system (0.53 g DM plant^{-1}). G x P interactions were significant (P<0.001) for root DM and root volume. For instance, the root DM of RAC875 and Scout increased from the P level of 2.5 to 20 mg P kg^{-1} soil and then remained stable, while that of other genotypes still increased up to the P level of 40 mg P kg^{-1} soil (Figure 2.3).

Shoot height and root length were significantly (P<0.001) affected by both wheat genotype and P supply (Table 2.3). Wyalkatchem was a short wheat genotype, while Gladius and Correll were taller. Mace, Correll and Wyakatchem had longer root systems than the other wheat genotypes. The extreme P supply of 2.5 mg P kg^{-1} soil resulted in the shortest shoot height. Root lengths were also lowest at the P supply of 2.5 mg P kg^{-1} soil and reached the longest at the P supply of 5 mg P kg^{-1} soil. Significant (P<0.001) G x P interaction was observed for shoot height and root length (Table 2.3). The reduction in shoot height in Wyalkatchem from P supply of 10 mg P kg^{-1} soil was more dramatic than that in other wheat genotypes (Figure S 2.9). Wheat genotypes also behaved differently for root length at different P levels (Figure S 2.8).

There was no significant G x P interaction for shoot to root ratio, however, the shoot to root ratio of the six wheat genotypes showed significant (P<0.001) variation between P treatments (Table 2.3). This ratio at the middle range of P supply (10, 20 and 30 mg P kg^{-1} soil) was significantly higher than that at the lowest and the highest P supply. Shoot to root ratio at 5 mg P kg^{-1} soil was also significantly lower than at a P supply of 10 and 20 mg P kg^{-1} soil. Among wheat genotypes, there was also significant (P<0.001) variation in shoot to root ratio. RAC875 (23.3 g g^{-1}) showed a significantly higher shoot to root ratio than the other genotypes, while the shoot to root ratio in Wyalkatchem (10.8 g g^{-1}) was the smallest and significantly smaller than

Scout, Gladius and Correll (18.4, 15.4 and 16.4 g g^{-1}, respectively) (Table 2.3).

The results indicated a significant ($P<0.001$) variation in HI and PHI among wheat genotypes (Table 2.2). HI in Scout and Wyalkatchem was lower than HI in the other wheat genotypes. Scout also showed the lowest PHI. HI increased from 37.4 % to 55.7 % from the P supply of 2.5 to 30 mg P kg^{-1} soil, and then stayed constant. PHI had an increase from 84.9 to 92.0 % from 2.5 to 20 mg P kg^{-1} soil P supply and then remained stable. G x P interaction was found in PHI ($P<0.05$) but not in HI (Table 2.2).

2.3.1.2 Phosphorus concentration, P uptake and root efficiency under greenhouse conditions

P supply significantly ($P<0.001$) affected grain P concentration, straw P concentration and shoot P concentration at maturity. P concentrations increased by about 2.3, 2.5 and 3.2 times for grain, straw and shoot P concentrations respectively, from the P supply of 2.5 to 30 mg P kg^{-1} soil then slightly rose to the P supply of 40 mg P kg^{-1} soil (Table 2.2). Grain P concentration was almost 10 times higher than straw P concentration. Significant ($P<0.001$) variations in the grain and shoot P concentration and strong G x P interactions ($P<0.001$) in these two indices were observed among wheat genotypes but not in straw P concentration (Table 2.2, Figure S 2.2, Figure S 2.3). In fact, RAC875 and Wyalkatchem had high grain and shoot P concentrations, while Scout and Mace showed low grain and shoot P concentrations. RAC875 had the lowest grain and shoot P concentrations at the P supply of 2.5 mg P kg^{-1} soil, whereas it has the highest grain and shoot P concentration at the P supply of 5, 10 and 30 mg P kg^{-1} soil. Similar to the response at maturity, at 27 DAS, shoot P concentration responded to P supply. Also at this stage, variation in shoot P concentration ($P<0.001$) and a strong G

x P interaction (P<0.01) was observed (Table S 2.1).

At maturity, although there was an increase in P uptake with increasing P addition, no significant variation in P uptake was observed among the wheat genotypes (Figure 2.4, Table 2.2). Wheat genotypes also responded to P supply at 27 DAS in the same pattern as at maturity. However, significant differences in P uptake were observed between the wheat genotypes at 27 DAS. P uptake in RAC875 and Correll, each was 28.6% higher than that in Gladius (Table S 2.1).

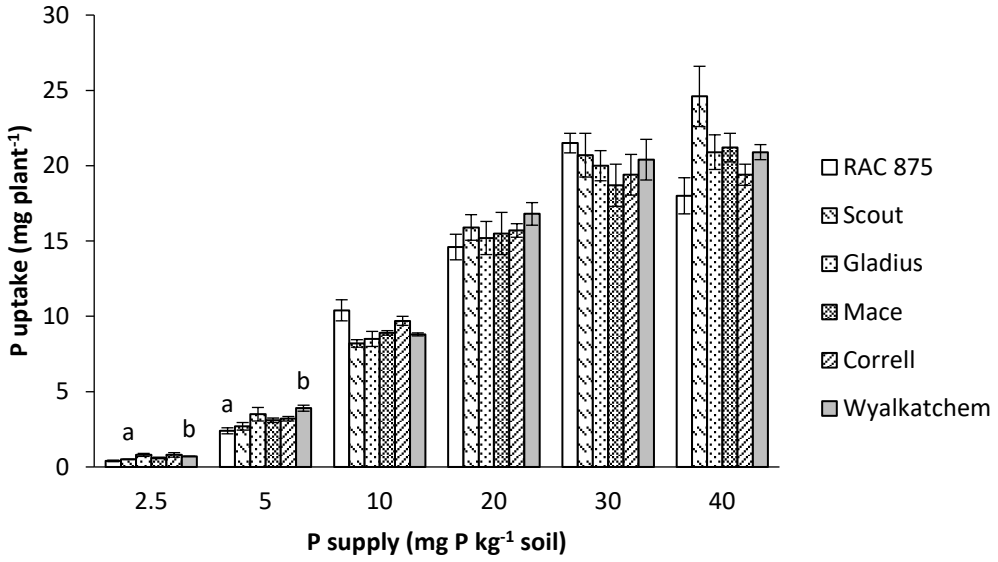


Figure 2.4. The effect of P supply on the P uptake of six wheat genotypes grown in the greenhouse at maturity. Data represent means of four replicates with standard errors. Different letters show significant differences within each P supply (P<0.05). Within each P supply, columns without letters show no significant differences among each other and with labelled columns.

Root efficiency significantly (P<0.001) varied among the wheat genotypes (Table 2.3, Figure 2.5). Root efficiency in RAC875 was 2.5-fold higher than that in both Wyalkatchem and both Scout and Correll also showed relatively high root efficiencies.

Root efficiency was significantly (P<0.001) affected by P deficiency and increased by more than 4-fold from the P supply of 2.5 to 30 mg P kg^{-1} soil. There was a strong G x P interaction (P<0.05) for root efficiency (Table 2.3). This means that wheat genotypes behaved differently at each P level. Indeed, root efficiency of Scout increased steadily from the P supply of 2.5 to 40 mg P kg^{-1} soil, while in the other genotypes, it increased from the P supply of 2.5 to 30 mg P kg^{-1} soil then declined at the P supply of 40 mg P kg^{-1} soil (Figure 2.5).

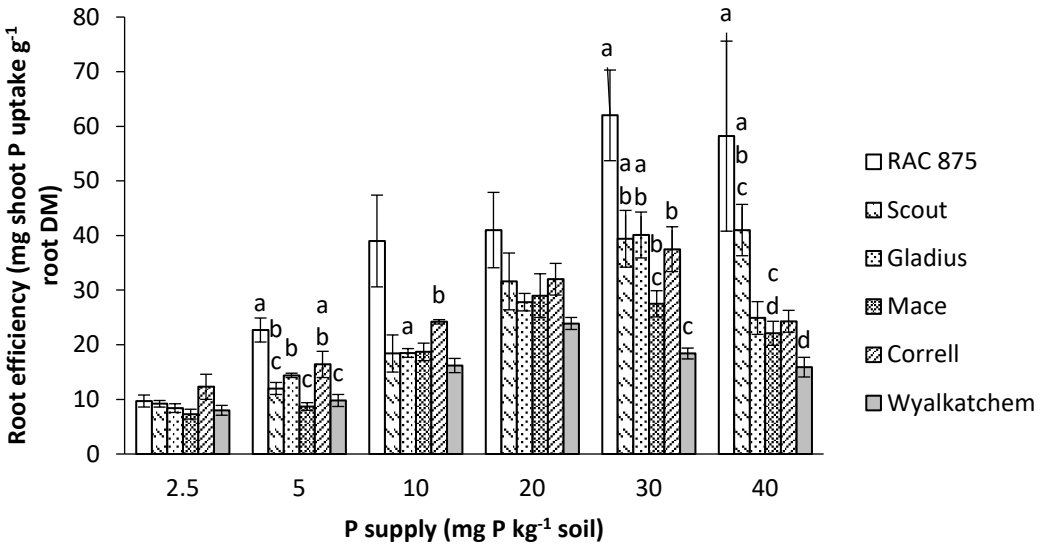


Figure 2.5. The effect of P supply on the root efficiency of six wheat genotypes grown in the greenhouse at maturity. Data represent means of four replicates with standard errors. Different letters show significant differences within each P supply (P<0.05). Within each P supply, columns without letters show no significant differences among each other and with labelled columns.

2.3.1.3 PUE under greenhouse conditions

Mitscherlich response curves were plotted between P supply and grain yield to determine the external P requirement for grain yield based on the critical value (external P supply to achieve 90% of maximum grain yield). The results showed that

the critical values for Correll and Wyalkatchem (13.8 and 13.6 mg P kg^{-1} soil, respectively) were lower than that of other genotypes (Figure 2.6 A). RAC875 and Scout had high critical values (18.6 and 19.9 mg P kg^{-1} soil, respectively). External P requirement (based on yield in RAC875) was greater than in Wyalkatchem although their maximum grain yields were similar. Therefore, Wyalkatchem was more P efficient than RAC875. External P requirements for shoot DM (external P supply to achieve 90% of maximum shoot DM) were also calculated based on the Mitchscherlich response curves plotted between P supply and shoot dry matter. External P requirement for shoot DM showed a similar pattern in comparison with external P requirement for grain yield (Figure 2.6 A & B).

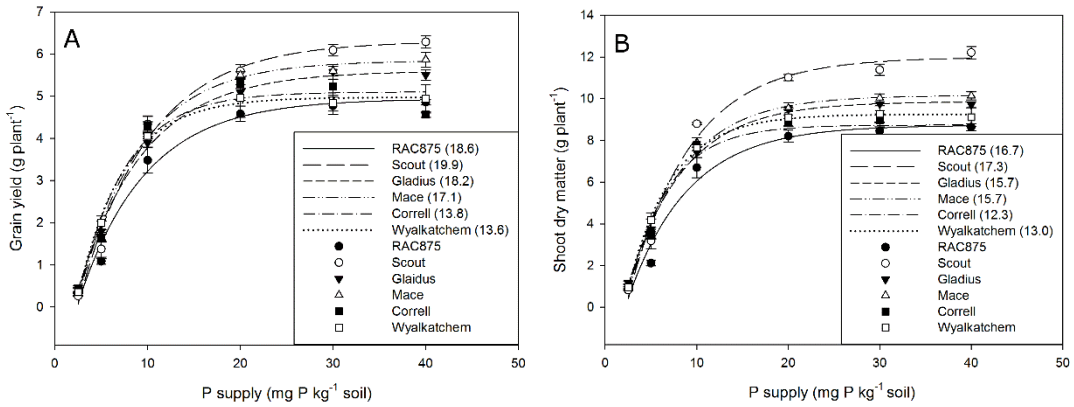


Figure 2.6. The relationship between grain yield (A), shoot dry matter (B) and P supply. Values are means of four replicates with standard errors. Numbers in brackets are the external P requirement ($critical_{90}$). The curves represent Mitscherlich responses.

The internal P requirement was also calculated by using the Mitscherlich response curves in which grain yield and shoot DM were plotted versus shoot P concentrations. Scout and Wyalkatchem showed low internal P requirement for grain yield (1540 and

1478 mg P kg^{-1}, respectively), while RAC875 had the highest internal P requirement for grain yield (2080 mg P kg^{-1}) (Figure 2.7 A). Also, RAC875 had the highest internal P requirement for shoot DM (2024 mg P kg^{-1}) while that in Scout, Mace and Wyalkatchem was low (1507, 1493 and 1424, respectively) (Figure 2.7 B).

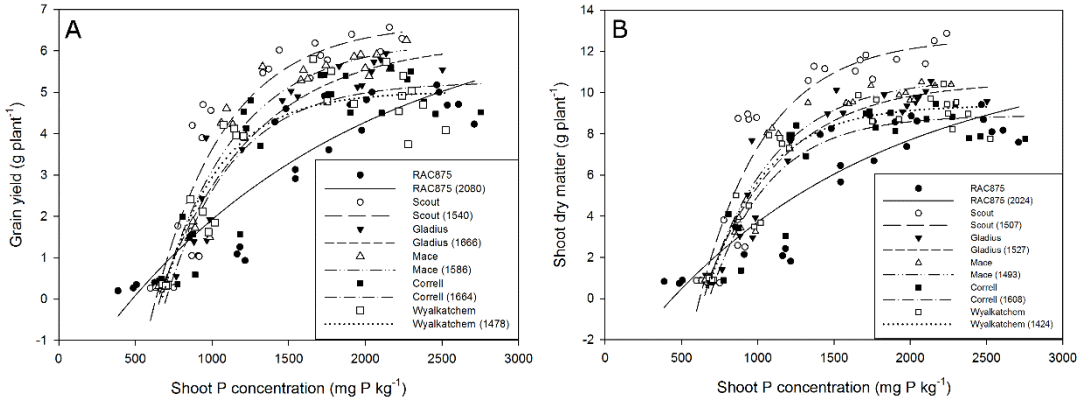


Figure 2.7. The relationship between grain yield (A), shoot dry matter (B) and shoot P concentration. Numbers in the brackets are the internal P requirement ($critical_{90}$). The curves represented Mitscherlich responses.

At maturity, PAE varied with increasing P supply (Table 2.2, Figure S 2.4). It increased dramatically by 3.5-fold over the range of 2.5 to 10 mg P kg^{-1} soil then steadily decreased by around 30% at the P supply of 40 mg P kg^{-1} soil. No significant different in PAE was witnessed between the wheat genotypes. PAE at 27 DAS was also significantly ($P<0.001$) affected by P supply and it had a similar pattern at maturity. However, there was significant ($P<0.001$) variation in PAE between genotypes at 27 DAS (Table S 2.1).

$PUtE_{GY}$ and $PUtE_{SM}$ were significantly ($P<0.001$) affected by P treatments and these parameters were considerably reduced with the increase of P supply (Table 2.2). $PUtE_{GY}$ and $PUtE_{SM}$ also significantly ($P<0.05$ and $P<0.001$, respectively) varied

among the wheat genotypes. Scout and Mace exhibited high $PUtE_{GY}$ and $PUtE_{SM}$, while these indices were low in Wyalkatchem. A significant ($P<0.001$) G x P interaction was found in both $PUtE_{GY}$ and $PUtE_{SM}$, therefore, the results for these parameters were presented at each P level to evaluate their variations at each P treatment (Figure S 2.5, Figure S 2.6). $PUtE_{GY}$ was positively correlated with PUE (grain yield per unit of P supplied) at both P levels of 10 and 30 mg P kg^{-1} soil ($r=0.715^{**}$ and $r=0.563^{**}$, respectively), while PAE had a strong correlation ($r=0.541^{**}$) with PUE at the P supply of 10 mg P kg^{-1} soil only (Table 2.8).

2.3.2 Experiment 2. Growth room responses

2.3.2.1 Grain yield, shoot DM, tiller number, root DM, root volume and shoot to root ratio under growth room conditions

The grain yield and shoot DM of the six wheat genotypes varied depending on P supply. The wheat genotypes produced about 55% and 37.6% higher grain yield and shoot DM respectively, at adequate P supply (30 mg P kg^{-1} soil) in comparison with yield at low P supply (Table 2.4, Figure 2.8, Figure 2.9). Genotypic variation in grain yield was observed between the wheat genotypes ($P<0.01$), while no significant variation was observed in shoot DM. Scout had the highest grain yield (18.5 g $plant^{-1}$), while Wyalkatchem produced the lowest (15.6 g $plant^{-1}$). No significant G x P interactions were found for both grain yield and shoot DM. At low P, RAC875, Scout and Gladius produced higher grain yield than Wyalkatchem ($P<0.05$) (Figure 2.8). At adequate P supply, significant ($P<0.05$) variation in grain yield was only observed between Scout and Wyalkatchem (Figure 2.8).

Table 2.4. Grain yield, shoot dry matter (DM), shoot P uptake, P concentration in grain, straw and shoot, HI, PHI, PAE and PUtE at maturity of six wheat genotypes grown in a growth room at different P treatments

Genotype	Grain yield (g plant^{-1})	Shoot DM (g plant^{-1})	P uptake (mg shoot^{-1})	P concentration (mg kg^{-1})			HI	PHI	PAE (P uptake/P supply, %)	PUtE	
				Grain	Straw	Shoot				Shoot (mg DM mg^{-1} P uptake)	Grain (mg grain mg^{-1} P uptake)
RAC875	17.0^{ab}	36.8	37.9	1890	188^{a}	978	46.0^{ab}	88.9	45.1	1228	559
Scout	18.5^{a}	37.5	40.6	1835	211^{ab}	1022	48.8^{a}	88.8	48.4	1136	539
Gladius	17.2^{ab}	36.3	40.1	1919	235^{ab}	1041	47.0^{ab}	87.8	46.7	1169	538
Mace	17.0^{ab}	35.9	38.1	1794	250^{b}	995	46.9^{ab}	86.7	43.9	1219	552
Correll	16.7^{b}	37.1	37.7	1802	213^{ab}	950	45.5^{b}	87.6	44.7	1229	546
Wyalkatchem	15.6^{b}	36.4	36.4	1858	241^{ab}	1033	45.0^{b}	88.1	43.8	1171	510
F Ratio											
Genotype (G)	6.775***	2.067 ns	1.884 ns	2.046 ns	2.521*	1.768 ns	2.942*	1.333 ns	2.194 ns	0.717 ns	0.733 ns
G × P	2.152 ns	0.943 ns	1.422 ns	0.151 ns	5.111**	1.567 ns	1.347 ns	3.365*	1.715 ns	0.503 ns	0.780 ns
P treatment (P)											
10	13.4	30.6	18.5	1203	140	605	43.8	87.0	43.9	1668	729
30	20.8	42.1	59.1	2534	306	1402	49.3	88.9	46.9	717	353
F Ratio											
P	538.532***	327.710***	3071.280***	1089.658 ***	192.651***	1399.683 ***	72.005***	11.276**	8.861**	653.268***	544.994***

Data represent the means of four replicates. Analysis of variance performed on original data. *F* ratios are from two-way ANOVA analysis. *, ** and *** significant at P<0.05, P<0.01 and P<0.001, respectively; ns: not significant. Different letters show significant differences between genotypes.

Table 2.5. Tiller number, shoot height, root dry matter, root volume, shoot to root ratio and root efficiency at maturity of six wheat genotypes grown in a growth room at different P treatments

Genotype	Tiller number (tiller plant^{-1})	Shoot height (cm)	Root dry matter (g plant^{-1})	Root volume (ml plant^{-1})	Shoot to root ratio (g g^{-1})	Root efficiency (mg g^{-1})
RAC875	8.0^{a}	66.6^{abcd}	3.7^{ab}	27.6^{abc}	10.6^{a}	9.8^{a}
Scout	10.3^{bcd}	63.6^{be}	3.2^{a}	22.1^{b}	11.9^{a}	12.3^{a}
Gladius	9.3^{b}	70.4^{ca}	4.5^{abc}	26.2^{cb}	8.3^{b}	8.6^{b}
Mace	9.5^{b}	62.3^{db}	5.1^{c}	32.3^{cda}	7.2^{b}	7.4^{b}
Correll	9.0^{abc}	68.5^{ea}	4.7^{b}	31.7^{cda}	8.0^{b}	7.6^{b}
Wyalkatchem	11.0^{d}	54.8^{f}	5.3^{c}	38.8^{d}	6.9^{b}	7.0^{b}
F Ratio						
Genotype (G)	11.057***	15.348***	6.760***	11.083***	19.088***	11.397***
G × P	0.771 ns	1.085 ns	1.840 ns	1.645 ns	3.062*	1.987 ns
P treatment (P)						
10	8.5	67.4	3.7	25.5	9.0	5.5
30	10.5	61.3	5.3	34.0	8.5	12.1
F Ratio						
P	61.714***	27.396***	43.440***	36.424***	1.861 ns	207.764***

Data represent the means of four replicates. Analysis of variance performed on original data except for root efficiency transformed using lg10. *F* ratios are from two-way ANOVA analysis. *, ** and *** significant at P<0.05, P<0.01 and P<0.001, respectively; ns: not significant. Different letters show significant differences between genotypes.

Tiller number also varied significantly ($P<0.001$) under different P treatments (Table 2.5). Significant ($P<0.001$) variation in tiller number was also found among wheat genotypes and no significant G x P interaction for tiller number was observed. Wyalkatchem had the highest tiller number (11 tillers plant^{-1}), whereas RAC875 produced the least number of tillers (8 tillers plant^{-1}) (Table 2.5).

P supply significantly ($P<0.001$) affected shoot height, root DM and root volume (Table 2.5). Root DM and root volume were higher at adequate P, whereas plants produced higher shoot height at low P. Significant ($P<0.001$) variations in these parameters were found among the wheat genotypes, however no strong G x P interactions for these parameters were observed. Wyalkatchem was the shortest plant but it had a large root system, whereas RAC875 and Scout produced small root systems (Figure 2.10). Indeed, root DM in Wyalkatchem was 43% and 66% larger than that in RAC875 and Scout, respectively. Mace also produced a large root system (Table 2.5).

P supply significantly affected HI and PHI ($P<0.001$ and $P<0.01$, respectively) and plants produced up to 5.5% and 1.9% higher HI and PHI respectively, at adequate P supply. HI significantly ($P<0.05$) varied among the wheat genotypes and ranged from 45.0% for Wyalkatchem to 48.8% for Scout, however no significant variation was observed for PHI among the wheat genotypes (Table 2.4).

The shoot to root ratio did not significantly respond to P treatments, however there was considerable ($P<0.001$) difference in shoot to root ratio among the wheat genotypes. Shoot to root ratio in RAC875 (10.6 g g^{-1}) and Scout (11.9 g g^{-1}) was

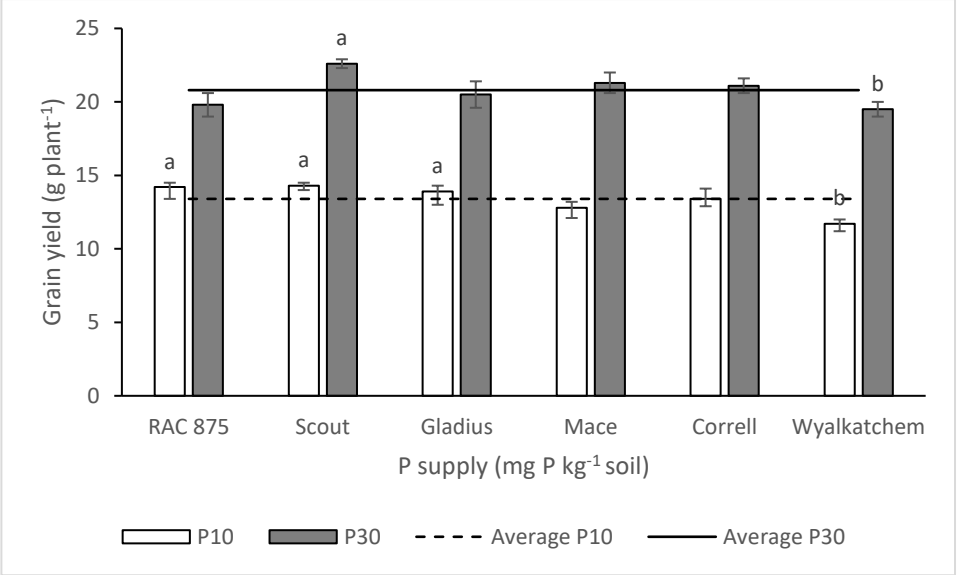


Figure 2.8. The effect of P supply on the grain yield of six wheat genotypes grown in the growth room at maturity. Data represent means of four replicates with standard errors. Different letters show significant differences within each P supply ($P<0.05$).

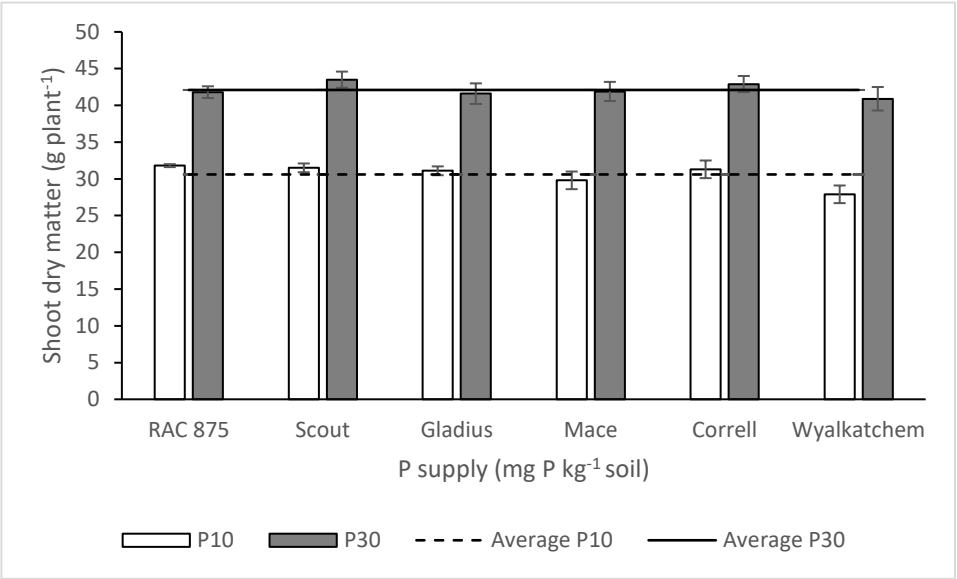


Figure 2.9. The effect of P supply on the shoot dry matter of six wheat genotypes grown in the growth room at maturity. Data represent means of four replicates with standard errors.

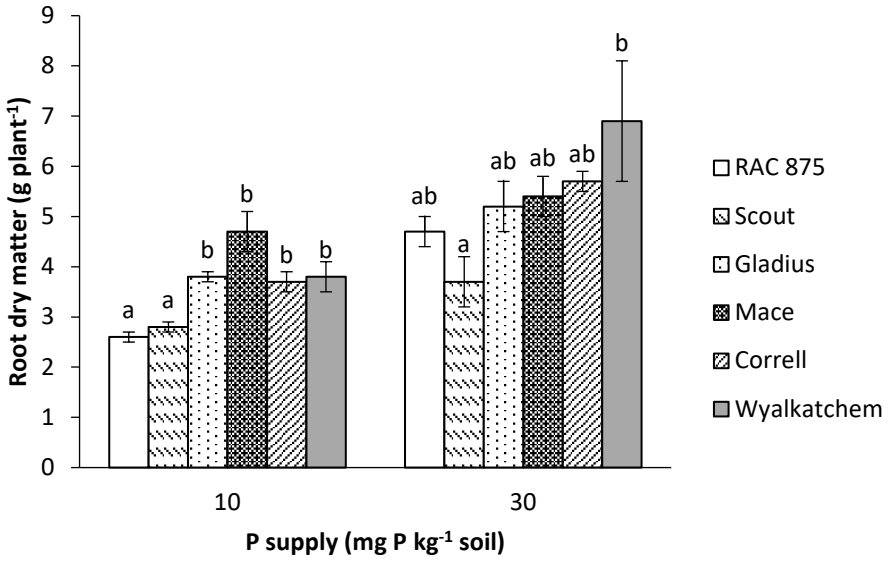


Figure 2.10. The effect of P supply on the root dry matter of six wheat genotypes grown in the growth room at maturity. Data represent means of four replicates with standard errors. Different letters show significant differences within each P supply ($P<0.05$).

significantly higher than that in the other genotypes. Wyalkatchem had the lowest shoot to root ratio (6.9 g g^{-1}) (Table 2.5). A significant ($P<0.05$) G x P interaction was also found for shoot to root ratio. Under low P treatment, RAC875 and Scout had significantly ($P<0.05$) higher shoot to root ratio than the other genotypes, but only Scout showed significantly ($P<0.05$) greater shoot to root ratio than other genotypes under adequate P (Figure S 2.11).

2.3.2.2 P concentration, P uptake, PUE and root efficiency under growth room conditions

The grain P concentration, straw P concentration and shoot P concentration had increases of more than two times under adequate P compared to under low P. There was no significant variation in grain P concentration and shoot P concentration among the wheat genotypes, while significant ($P<0.05$) differences for straw P concentration

were found (Table 2.4). Shoot P uptake was also affected by P supply but not by genotypes (Table 2.4, Figure 2.11).

PAE, $PUtE_{GY}$ and $PUtE_{SM}$ significantly ($P<0.001$) varied under different P treatments, however no significant differences were observed for these parameters among the wheat genotypes (Table 2.4). $PUtE_{GY}$ was positively correlated ($r=0.750^{**}$) with PUE (grain yield per unit of P supplied) under adequate P (30 P mg kg^{-1} soil), while PAE was strongly associated ($r=0.541^{**}$) with PUE under low P (10 mg P kg^{-1} soil) (Table 2.8).

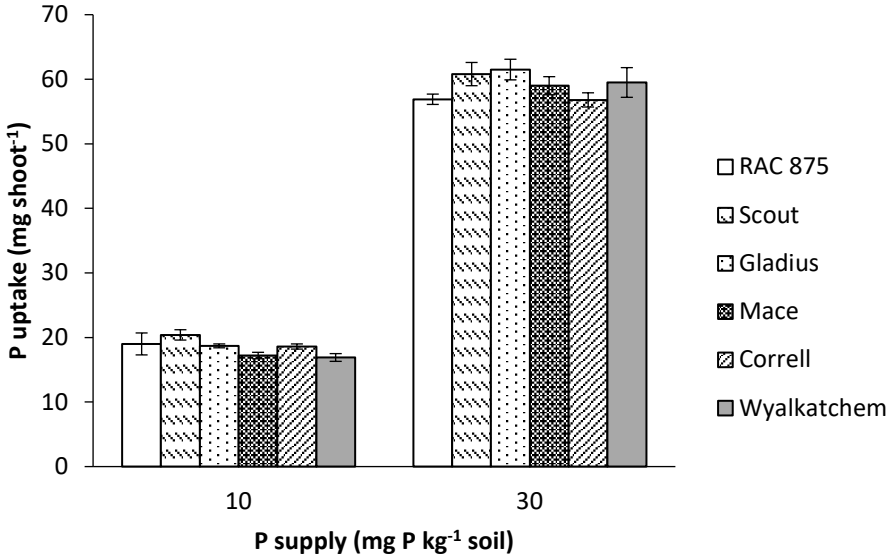


Figure 2.11. The effect of P supply on the P uptake of six wheat genotypes grown in the growth room at maturity. Data represent means of four replicates with standard errors.

Root efficiency was more than double at adequate P in comparison with that at low P (Table 2.5). Root efficiency significantly ($P<0.001$) varied among the wheat genotypes, and RAC875 and Scout showed significantly ($P<0.05$) higher root efficiency when compared to the other genotypes (Table 2.5, Figure 2.12). No

significant G x P interaction for root efficiency was observed. Root efficiency in RAC875 and Scout was about 40% and 76% respectively, greater than that in Wyalkatchem. Root efficiency was positively correlated with PUE under both low and adequate P ($r=0.565^{**}$ and $r=0.432^{*}$, respectively) (Table 2.8).

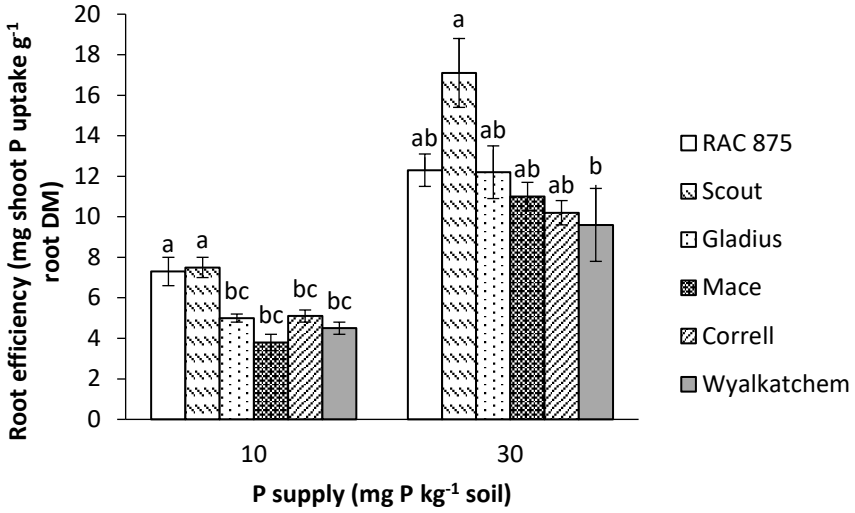


Figure 2.12. The effect of P supply on the root efficiency of six wheat genotypes grown in the growth room at maturity. Data represent means of four replicates with standard errors. Different letters show significant differences within each P supply ($P<0.05$).

PUE were also evaluated as $PUE_{GY10/30}$ and $PUE_{SM10/30}$. RAC875 and Gladius were more P efficient genotypes in comparison with the other genotypes. Wyalkatchem was the least P efficient genotype with $PUE_{GY10/30}$ of 60%, which was 11.7% and 7.8% lower than $PUE_{GY10/30}$ of RAC875 and Gladius, respectively. PUE_{SM} had a similar pattern to PUE_{GY} and varied from 68.2% for Wyalkatchem to 76.1% for RAC875 (Table 2.7).

Table 2.6. Pearson correlation coefficients between root dry matter (DM), root volume, grain yield

	P supply (mg P kg^{-1} soil)	Greenhouse			Growth room		
		Root volume	Grain yield	P uptake	Root volume	Grain yield	P uptake
Root DM	10	0.877**	0.238	-0.385	0.757**	-0.445**	-0.453**
	30	0.963**	-0.138	0.099	0.890**	-0.342	0.108
Root volume	10		0.142	-0.340		-0.581**	-0.471*
	30		-0.141	0.084		-0.268	-0.004

*, ** significant at P<0.05 and P<0.01, respectively

2.4 Discussion

2.4.1 Variation in responses to P under controlled conditions

In general, the results in the greenhouse experiment showed that shoot DM and grain yield responded to P in a similar pattern at maturity. Although significant (P<0.001) G x P interactions for shoot DM and grain yield were observed, in general these two indices responded to P supply and reached a maximum at 30 mg P kg^{-1} soil (Figure 2.1, Figure 2.2). Thirty kg P ha^{-1} (ca. 30 mg P kg^{-1} soil) was also used as adequate P in field experiments (McDonald et al., 2010). However, Korkmaz (2009) reported that a P application rate of 100 mg P kg^{-1} soil was optimum for wheat growth. Soil type could result in these differences. The soil used in Korkmaz's experiment was a high Ca clay, while the soil used in this experiment was sandy soil that was very low in clay and calcium. P availability can be reduced by its adsorption to iron and calcium (Osborne and Rengel, 2002a).

Wheat genotypes responded differently under greenhouse conditions. For example, Scout and Mace gained high grain yields at high P rates (30 and 40 mg P kg^{-1} soil), while RAC875 and Wyalkatchem showed low grain yield at these levels of P (Figure

2.1). However, at extremely low rates of supply (i.e. 5 mg P kg^{-1} soil), Wyalkatchem produced greater grain yield, whereas RAC875 and Scout had lower grain yield. At a P supply of 10 mg P kg^{-1} soil, Scout gained higher grain yield, while grain yield of RAC875 remained low. Similar to greenhouse conditions, in the growth room, Scout produced high grain yield under both low P (10 mg P kg^{-1} soil) and high P (30 mg P kg^{-1} soil). However, in contrast to greenhouse conditions, RAC875 had high grain yield under low P but showed little responsiveness to high P in the growth room (Figure 2.8). This result is similar to reports from field experiments showing that RAC875 produced high grain yield under low P and was not responsive to high P (McDonald et al., 2015). In agreement with the results from field experiments (McDonald et al., 2015), Mace showed a higher responsiveness to high P under both greenhouse and growth room conditions. However, Wyalkatchem was responsive to added P in the field but did not showed responsiveness under both greenhouse and growth room conditions.

Plants produced both higher grain yield and shoot DM under growth room conditions, when compared to the greenhouse. The major yield component that differed between the two growth conditions was tiller number and this would explain the different responses of RAC875 and Wyalkatchem under the two different environments. What was evident was a lower light intensity within the greenhouse area which could be due to the polycarbonate walls that could reduce light intensity. Differences in temperature between greenhouse and growth room may also affect responses to P.

PUE was measured using a variety of methods. For example, PUE was measured based on external and internal P requirements, or $PUE_{GY10/30}$ (calculated as the ratio of grain yield between low P (10 mg P kg^{-1} soil) and high P (30 mg P kg^{-1} soil)), or PAE and

PUtE. In this discussion, all these measures will be examined. The results show that under greenhouse conditions, external P requirement for grain yield varied widely among genotypes from 13.6 mg P kg^{-1} soil for Wyalkatchem to 19.9 mg P kg^{-1} soil for Scout (Figure 2.6 A). The wide variation in external P requirement has previously been observed (Bell et al., 2013; Poulton et al., 2013). Also, Bell et al. (2013) reported that the external P requirement diverged depending on soil types; Grey Vertosols resulted in low critical values (16-22 mg P kg^{-1} soil), while Supracalcic Calcarosols showed high external P requirement (24-92 mg P kg^{-1} soil). Adsorption of P by calcium could lead to higher external P requirement in Supracalcic calcarosols than in Grey Vertosols. Based on the external P requirement and maximal grain yield, the lower external P requirement means the higher PUE and the higher maximal grain yield indicates the greater P responsiveness. According to this classification, Correll and Wyalkatchem were efficient and non-responsive genotypes, while Scout and Mace were inefficient and responsive genotypes and RAC875 was inefficient and non-responsive in the greenhouse. When $PUE_{GY10/30}$ was used for evaluation of PUE, genotypes with high $PUE_{GY10/30}$ are more efficient. The results demonstrated that Correll and Wyalkachem were more P efficient than RAC875, Scout and Mace (Table 2.7). This indicates that external P requirement and $PUE_{GY10/30}$ had relatively similar results for PUE evaluation.

In the growth room, plants were grown at two P rates, low P (10 mg P kg^{-1} soil) and adequate P (30 mg P kg^{-1} P) due to space limitations. P efficiency and P responsiveness are classified based on the ratio of grain yield between low P and adequate P ($PUE_{GY10/30}$) and grain yield at high P. Genotypes with high $PUE_{GY10/30}$ are efficient genotypes and genotypes with high grain yield under adequate P are responsive.

Table 2.7. $PUE_{GY10/30}$ (%) and $PUE_{SM10/30}$ (%) (ratio of grain yield to grain yield and ratio of shoot DM to shoot DM at supply of 10 to 30 mg P kg^{-1} soil)

Genotype	Greenhouse		Growth room	
	$PUE_{GY10/30}$	$PUE_{SM10/30}$	$PUE_{GY10/30}$	$PUE_{SM10/30}$
RAC875	72.9	79.8	71.7	76.1
Scout	70.5	77.2	63.3	72.4
Gladius	69.6	75.5	67.8	74.8
Mace	75.0	79.0	60.1	71.1
Correll	82.7	86.7	63.5	73.0
Wyalkatchem	85.4	81.7	60.0	68.2

Although Scout and Mace showed consistency in PUE and were highly responsive to adequate P under both growth room conditions, PUE in RAC875 and Wyalkatchem was not consistent between the two growth conditions. RAC875 was an efficient and non-responsive genotype, while Wyalkatchem was an inefficient and non-responsive genotype under growth room conditions. However, the results were the opposite under greenhouse conditions. The results in the growth room agree with data from fields in which RAC875 was more P efficient than Wyalkatchem. Indeed, in the fields, PUE (calculated as relative growth at low P/high P) of RAC875 was 90.8%, whereas that in Wyalkatchem was 76.6% (results calculated from supplemental data provided by McDonald et al. (2015)). Furthermore, RAC875 and Wyalkatchem showed consistent $PUE_{10/30}$ at different growth stages under growth room conditions (Chapter 4) but not under greenhouse conditions. Thus, growth room appears to provide a better condition than the greenhouse for elucidating mechanisms of PUE.

$PUtE_{GY}$ at high P (30 mg P kg^{-1} soil) was positively correlated with PUE in both greenhouse ($r=0.563^{**}$) and growth room ($r=0.750^{**}$) (Table 2.8). In contrast, PAE showed a positive correlation ($r=0.541^{**}$) with PUE under low P (10 mg P kg^{-1} soil) in the growth room but no correlation was observed in the greenhouse (Table 2.8).

These correlations under growth room conditions were consistent with that from field results (Manske et al., 2001). Other studies have also shown that under low P supply, PAE made a major contribution to PUE (Fujita et al., 2004; DoVale and Fritsche-Neto, 2013), while PUtE was more important for PUE under adequate P (Balemi and Schenk, 2009).

2.4.2 Root efficiency is important for PUE rather than root size

Root dry matter and root volume increased steadily with the addition of P under both growth conditions. Wyalkatchem had a large root system, while RAC875 and Scout had small root systems under both growth conditions (Table 2.3, Table 2.5, Figure 2.3, Figure 2.10). Although Wyalkatchem possessed a large root system, its grain yield was relatively low (Figure 2.1, Figure 2.8). Conversely, Scout had the highest grain yield, whereas its root was small. RAC875 with the small root system also produced higher grain yield under low P in the growth room. Thus, smaller root systems may contribute to higher grain yield production under low P supply since plants do not need increased energy for root development. Previous studies have shown contrasting results for correlation between root size and P uptake. Positive correlations between P uptake and root volume have been observed in soybean (Ao et al., 2010), while small root volume contributing to a higher P uptake was reported in wheat (da Silva et al., 2016). In this study, P uptake was negatively correlated with root DM (r=-0.385 in the greenhouse, $r=-0.453^{*}$ in the growth room) and root volume (r=-0.340 in the greenhouse, $r=-0.471^{*}$ in the growth room) at the P supply of 10 mg P kg^{-1} (Table 2.6). Furthermore, in the growth room, RAC875 and Scout produced higher grain yield at low P, even with a small root system, while Wyalkatchem had lower grain yield although harbouring a large root system. Therefore, a larger root system may not contribute to greater PUE.

Table 2.8. Pearson correlation coefficients between $PUtE_{GY}$, PAE and PUE

	P supply (mg P kg^{-1} soil)	PUE	
		Greenhouse	Growth room
$PUtE_{GY}$	10	0.715**	0.391
	30	0.563**	0.750**
PAE	10	-0.002	0.541**
	30	0.136	0.276
Root efficiency	10	-0.306	0.565**
	30	-0.004	0.432*

PUE=$PUtE_{GY}$*PAE/100; *, ** significant at P<0.05 and P<0.01, respectively

The shoot to root ratio illustrated great variation among wheat genotypes. RAC 875 and Scout demonstrated higher shoot to root ratio, while this ratio was the lowest in Wyalkatchem under both growth conditions, suggesting that RAC875 and Scout had greater ability to produce shoot biomass per unit of root dry matter (Table 2.3, Table 2.5).

In this study, a large variation in root size was observed, although P uptake was not significantly different among wheat genotypes. Therefore, it is useful to evaluate root efficiency (referred to as shoot P uptake per unit of root size (Mori et al., 2016)) to examine the ability of roots for increased P uptake. In this study, root efficiency was calculated as shoot P uptake per unit of root DM. The results showed that root efficiency significantly varied among genotypes under both growth conditions with efficiency higher in RAC875 and Scout, while this index was low in Mace and reached the lowest in Wyalkatchem. Jones et al. (1989) have shown that root efficiency varied among wheat genotypes and root efficiency can be used for screening of P efficient wheat. Mori et al. (2016) measured root efficiency as shoot P uptake per unit of root

surface area in rice and found a wide variation between 196 rice accessions. In addition, the rice genotypes with the low-P-tolerance *Pup1* locus had greater root efficiency than the rice genotypes without this locus. Thus, root efficiency can be of benefit for the selection of P efficient crops. In this study, root efficiency was positively associated with PUE at both P supply ($r=0.565^{**}$ and $r=0.432^{*}$ at 10 and 30 mg P kg^{-1} soil, respectively) under growth room conditions, however no significant correlation between root efficiency and PUE at these P levels were witnessed under greenhouse conditions (Table 2.8).

Conclusions

Six wheat genotypes were evaluated for P efficiency and P responsiveness under two growth conditions, in a greenhouse and in a growth room. P efficiency was evaluated by external P requirement (amount of P supplied at which grain yield achieved 90% of maximal grain yield) and relative grain yield ($PUE_{GY10/30}$, yield at 10 mg P kg^{-1} soil/ yield 30 mg P kg^{-1} soil). These two criteria showed relatively similar results. Not all wheat genotypes showed consistent PUE and responses to P between two growth conditions. In fact, PUE in RAC875 was low in the greenhouse but high in the growth room, while that in Wyalkatchem showed contrasting results. However, both RAC875 and Wyalkatchem were not highly responsive under both growth conditions. Scout and Mace were also P inefficient and highly responsive to P supply under both growth conditions. The growth room appears to be more optimal than the greenhouse for the screening of P efficient wheat since it maintained consistency with field experiments and consistency in PUE at different growth stages (Chapter 4). Variation in root dry matter was observed, in which RAC875 and Scout had small root systems, while Wyalkatchem possessed large root systems under both growth conditions. In contrast,

root efficiency (calculated as shoot P uptake per unit of root dry matter) was high in RAC875 and Scout, while it was the lowest in Wyalkatchem. Root efficiency was positively correlated with PUE at both P treatments ($r=0.565^{**}$ and $r=0.432^{*}$ at 10 and 30 mg P kg^{-1} soil, respectively) under growth room conditions. The wheat genotypes with contrasting root size and root efficiency would be beneficial for examining how small root systems contribute to higher grain yield and PUE.

Supplemental tables and figures

Table S 2.1. Shoot dry matter, shoot P concentration, P uptake, PAE, PUtE at 27 DAS and shoot dry matter at 48 DAS of six wheat genotypes grown in a greenhouse at different P treatments

Genotype	Shoot dry matter (g plant^{-1})	Shoot P concentration (mg kg^{-1})	P uptake (mg shoot^{-1})	PAE (P uptake/P supply, %)	PUtE (mg DM/mg P uptake)	Shoot dry matter at 48 DAS (g plant$^{-1)}$
RAC875	0.116^{a}	5289^{a}	0.63^{a}	1.2^{a}	244^{acd}	0.81^{ab}
Scout	0.096^{b}	5361^{acd}	0.54^{ab}	0.9^{b}	238^{acd}	0.75^{a}
Gladius	0.112^{ac}	4228^{b}	0.49^{b}	1.0^{b}	289^{b}	1.01^{b}
Mace	0.108^{bc}	5723^{acd}	0.61^{a}	1.0^{ab}	254^{c}	0.77^{ab}
Correll	0.117^{a}	5289^{c}	0.63^{a}	1.2^{a}	250^{acd}	0.92^{ab}
Wyalkatchem	0.091^{b}	5823^{d}	0.56^{ab}	1.0^{b}	228^{d}	0.75^{a}
F Ratio						
Genotype (G)	14.722***	17.507***	5.667***	6.874***	13.160***	2.617*
G × P	2.503**	2.220**	1.582 ns	2.646***	1.982**	1.269 ns
P treatment (P)						
2.5	0.078^{a}	1925^{a}	0.15^{a}	1.4^{a}	529^{a}	0.31^{a}
5.0	0.102^{b}	3314^{b}	0.34^{b}	1.6^{b}	305^{b}	0.73^{b}
10	0.111^{bc}	4183^{c}	0.47^{c}	1.1^{c}	243^{c}	0.93^{c}
20	0.116^{dc}	5976^{d}	0.69^{d}	0.8^{d}	171^{d}	1.02^{c}
30	0.124^{ed}	7420^{e}	0.91^{e}	0.7^{e}	138^{e}	1.06^{c}
40	0.103^{fb}	8741^{f}	0.9^{e}	0.5^{f}	171^{e}	0.99^{c}
F Ratio						
P	30.514***	372.528***	175.317***	122.449***	694.113***	39.370***

Results are original data and represent the means of four replicates. Analysis of variance performed on original data. *, ** and *** significant at P<0.05, P<0.01 and P<0.001, respectively; ns: not significant. Different letters show significant differences between genotypes or P supply.

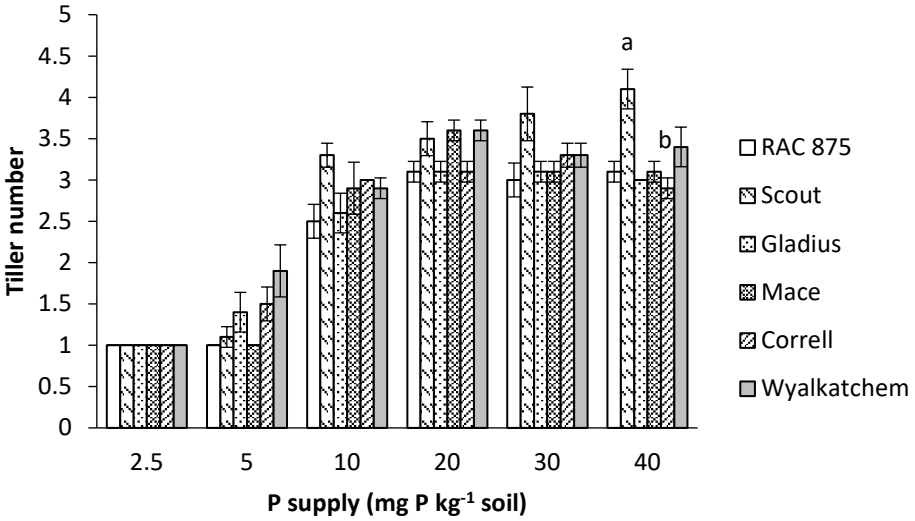


Figure S 2.1. The effect of P supply on the tiller number of six wheat genotypes grown in the greenhouse at maturity. Data represent means of four replicates with standard errors. Different letters show significant differences within each P supply ($P<0.05$). Within each P supply, columns without letters show no significant differences among each other and with labelled columns.

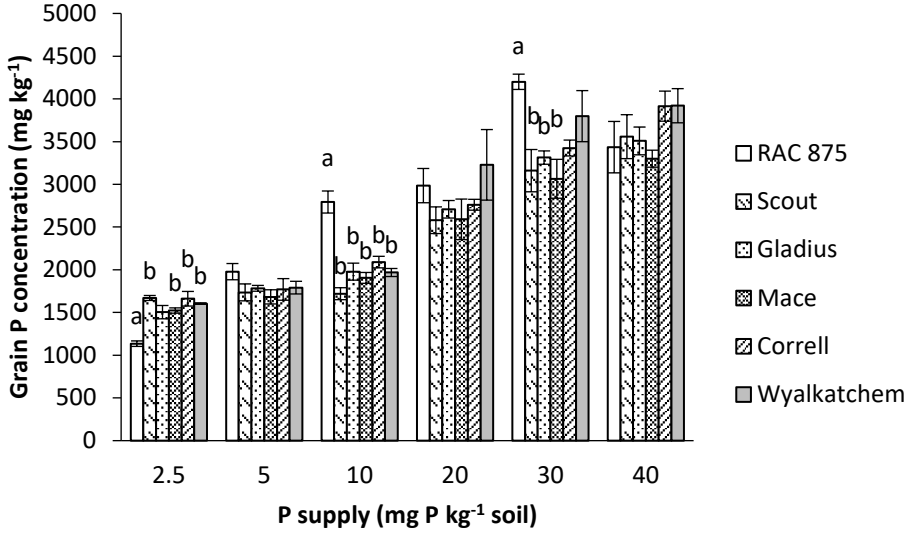


Figure S 2.2. The effect of P supply on the grain P concentration of six wheat genotypes grown in the greenhouse at maturity. Data represent means of four replicates with standard errors. Different letters show significant differences within each P supply ($P<0.05$). Within each P supply, columns without letters show no significant differences among each other and with labelled columns.

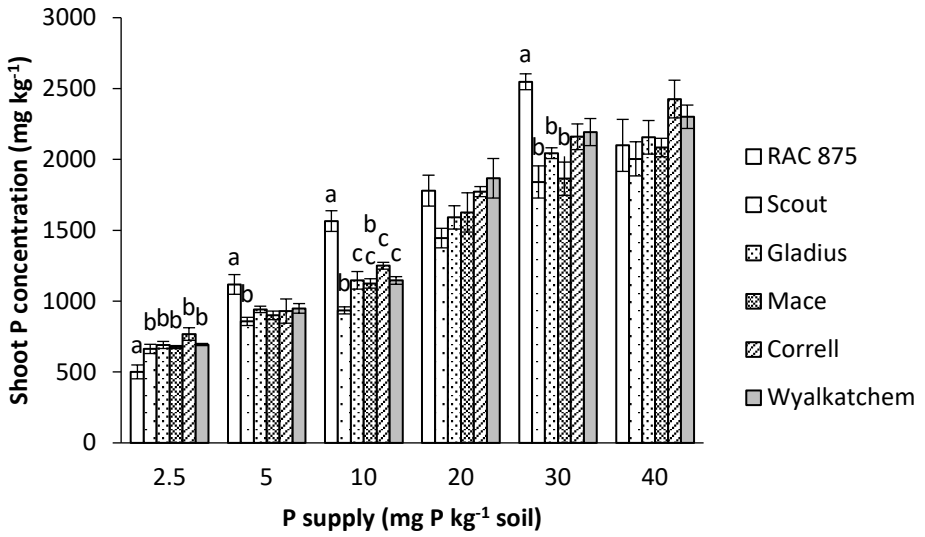


Figure S 2.3. The effect of P supply on the shoot P concentration of six wheat genotypes grown in the greenhouse at maturity. Data represent means of four replicates with standard errors. Different letters show significant differences within each P supply ($P<0.05$). Within each P supply, columns without letters show no significant differences among each other and with labelled columns.

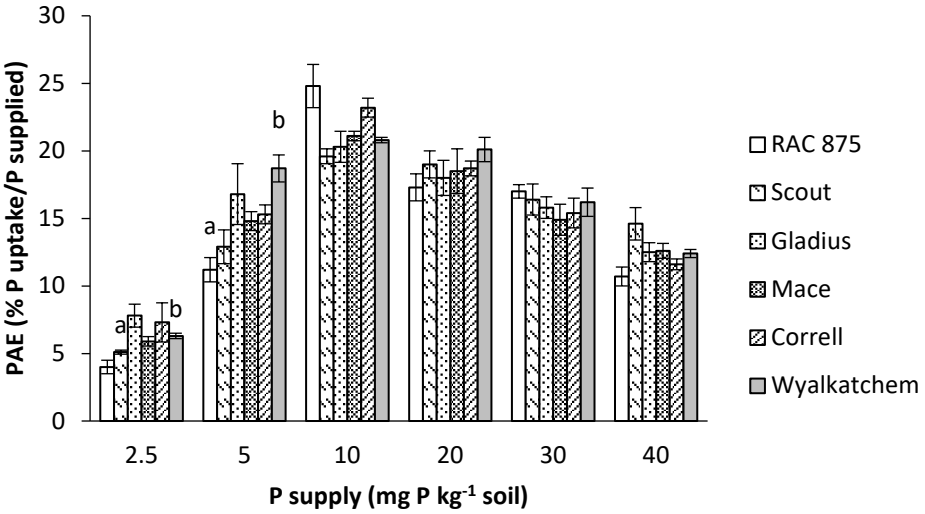


Figure S 2.4. The effect of P supply on the P acquisition efficiency (PAE) of six wheat genotypes grown in the greenhouse at maturity. Data represent means of four replicates with standard errors. Different letters show significant differences within each P supply ($P<0.05$). Within each P supply, columns without letters show no significant differences among each other and with labelled columns.

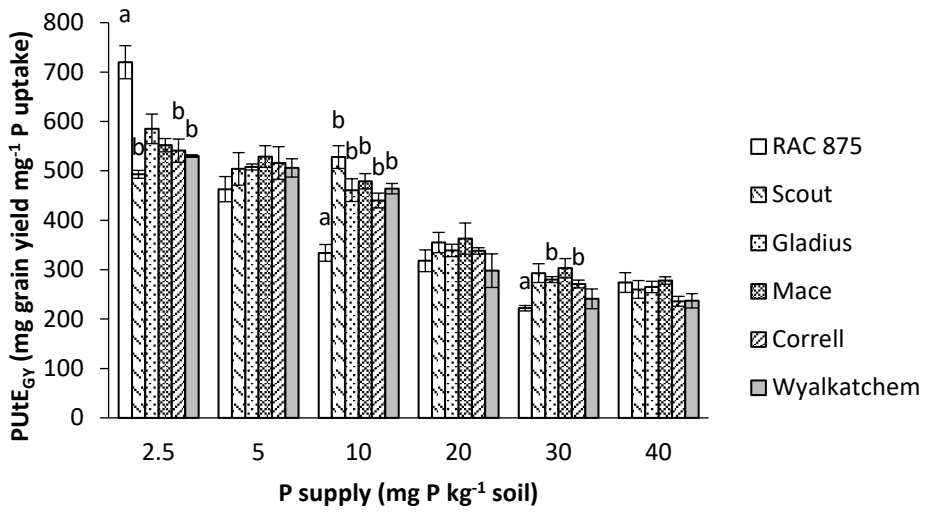


Figure S 2.5. The effect of P supply on the P utilisation efficiency – grain yield ($PUtE_{GY}$) of six wheat genotypes grown in the greenhouse at maturity. Data represent means of four replicates with standard errors. Different letters show significant differences within each P supply ($P<0.05$). Within each P supply, columns without letters show no significant differences among each other and with labelled columns.

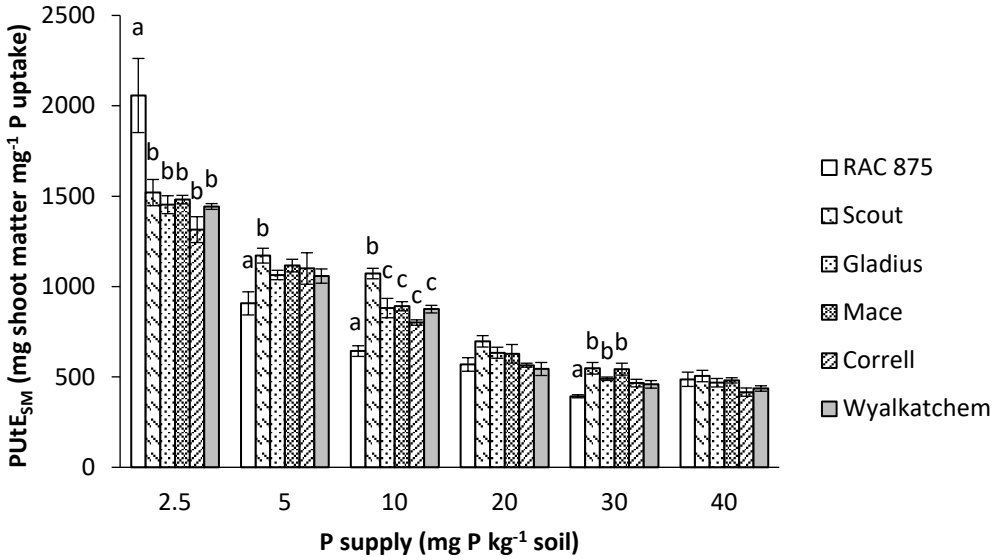


Figure S 2.6. The effect of P supply on the P utilisation efficiency – shoot dry matter ($PUtE_{SM}$) of six wheat genotypes grown in the greenhouse at maturity. Data represent means of four replicates with standard errors. Different letters show significant differences within each P supply ($P<0.05$). Within each P supply, columns without letters show no significant differences among each other and with labelled columns.

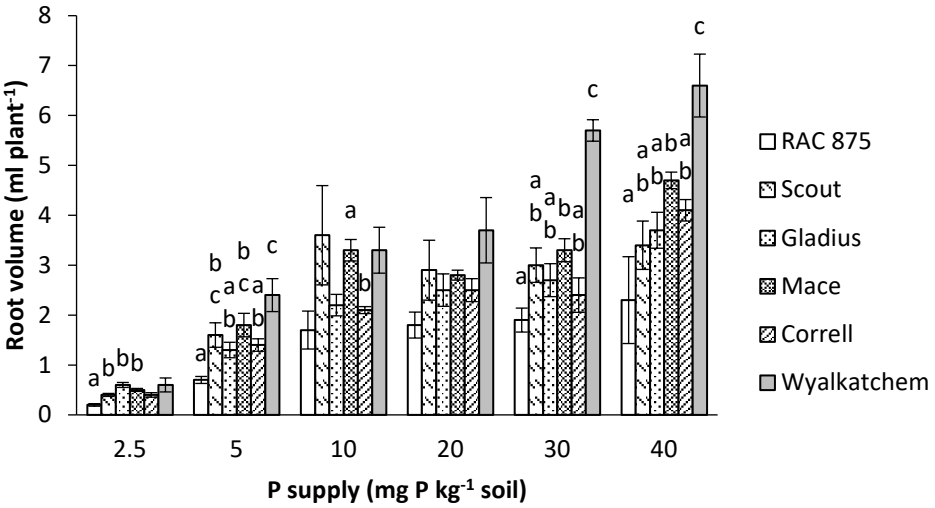


Figure S 2.7. The effect of P supply on the root volume of six wheat genotypes grown in the greenhouse at maturity. Data represent means of four replicates with standard errors. Different letters show significant differences within each P supply (P<0.05). Within each P supply, columns without letters show no significant differences among each other and with labelled columns.

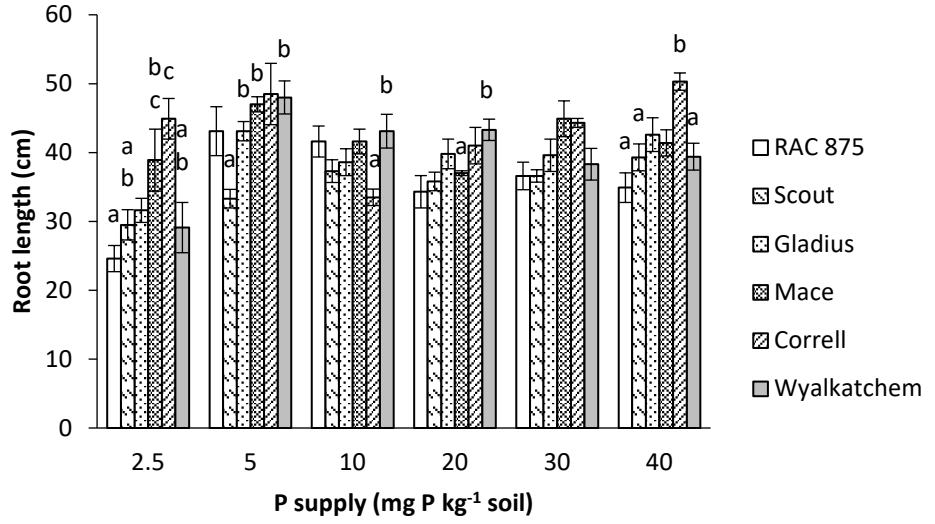


Figure S 2.8. The effect of P supply on the root length of six wheat genotypes grown in the greenhouse at maturity. Data represent means of four replicates with standard errors. Different letters show significant differences within each P supply (P<0.05). Within each P supply, columns without letters show no significant differences among each other and with labelled columns.

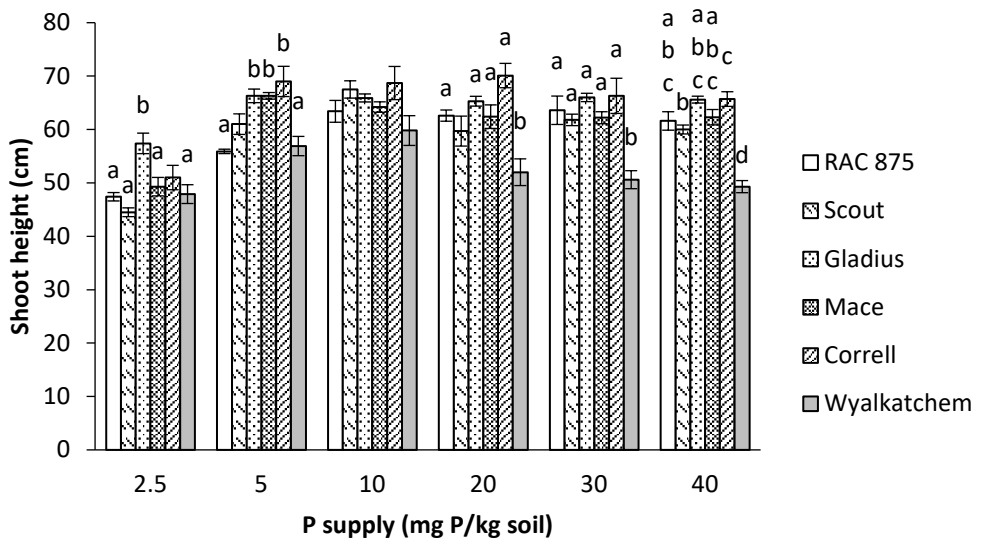


Figure S 2.9. The effect of P supply on the shoot height of six wheat genotypes grown in the greenhouse at maturity. Data represent means of four replicates with standard errors. Different letters show significant differences within each P supply ($P<0.05$). Within each P supply, columns without letters show no significant differences among each other and with labelled columns.

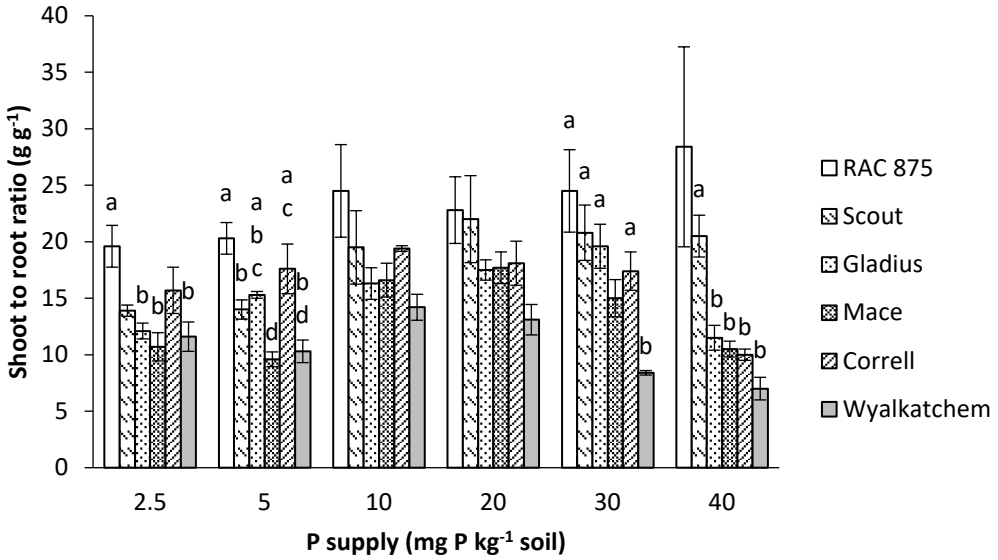


Figure S 2.10. The effect of P supply on the shoot to root ratio of six wheat genotypes grown in the greenhouse at maturity. Data represent means of four replicates with standard errors. Different letters show significant differences within each P supply ($P<0.05$). Within each P supply, columns without letters show no significant differences among each other and with labelled columns.

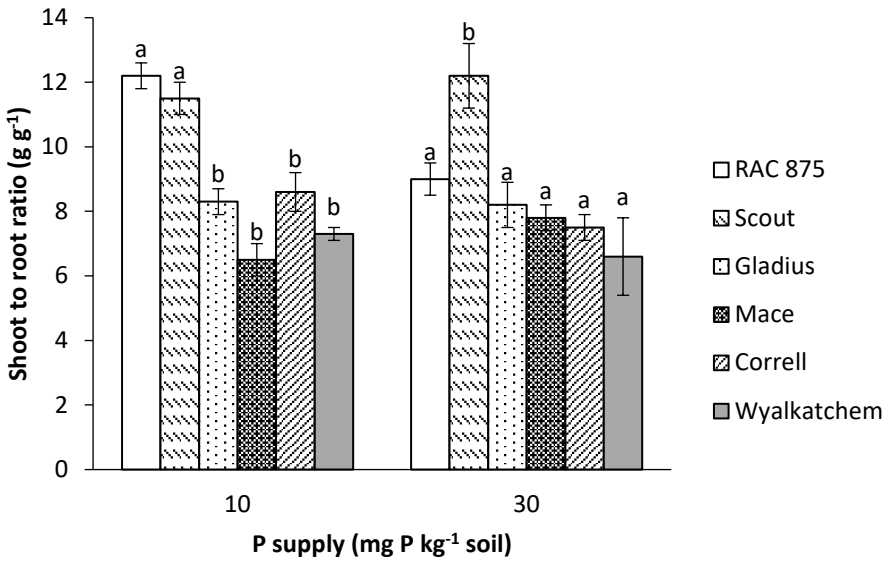


Figure S 2.11. The effect of P supply on the shoot to root ratio of six wheat genotypes grown in the growth room at maturity. Data represent means of four replicates with standard errors. Different letters show significant differences within each P supply ($P<0.05$).

CHAPTER 3

Effect of light intensity on phosphorus responses and photosynthetic parameters of two wheat genotypes differing in phosphorus use efficiency

Abstract

The effects of light intensity can vary depending on the degree of shading and is a common problem in foggy areas such as South Asia. The results from Chapter 2 showed that responses to P of two wheat genotypes RAC875 and Wyalkatchem were opposite between the growth room and green house. When light intensity measurements were taken within the greenhouse, the levels were below optimal for plant growth and this could help to explain the lower yield of the plants growing in the greenhouse. The light effects on genotype response needed to be evaluated to confirm the efficiency of the wheat genotypes. In this study, greenhouse responses (low light intensity) were evaluated against growth room (adequate light intensity) responses in the two wheat genotypes, Wyalkatchem and RAC875. Low light intensity decreased grain yield, shoot dry matter (DM) and tiller number, while increased flag leaf length and maximum flag leaf width. The photosynthetic rate (P_n) increased, but stomatal conductance (Cond) and intercellular CO_2 (C_i) declined under high light intensity. Low P reduced grain yield, shoot DM, tiller number and P_n but increased C_i. Responses to P within the two wheat genotypes varied under different light intensities, where Wyalkatchem produced higher grain yield and shoot dry matter than RAC875 under low light intensity, but not under high light intensity. Wyalkatchem also had greater P_n than RAC875 under low light intensity, while no difference occurred at high light

intensity. Wyalkatchem showed greater C_i and Cond than RAC875 and under both light conditions, RAC875 showed greater PUE than Wyalkatchem since RAC875 had a lower external P requirement and greater relative grain yield ratio (yield at low P/yield at adequate P). This indicates that P use efficiency of the two wheat genotypes was not impacted by light intensity and that Wyalkatchem is more tolerant to low light than RAC875.

3.1 Introduction

Light intensity and its quality are important environmental factors affecting plant growth, morphogenesis and other morphological responses (Dong et al., 2014b). For example, an increased light intensity enhanced branching, leaf area and yield in cowpea but reduced plant height (Tarila et al., 1977). Improved plant biomass with higher light intensity have also been observed in common sage (Zervoudakis et al., 2012) and higher light intensity also resulted in increased tiller number in wheat (Tamaki et al., 2001; Page et al., 2012). In a study by Xu et al. (2016), mid-level and severe shading after anthesis reduced wheat grain yield but slight shading improved photosynthetic productivity and grain yield due to the delaying of leaf senescence. It is also reported that light quality (i.e. blue light or red light) also affects plant morphogenesis (Fukuda et al., 2008) and light spectrum impacts on the growth and development of plants (Cope and Bugbee, 2013).

Phosphorus (P) is an essential macronutrient for plants and it plays a variety of cellular functions including structural, energy storage, information storage, information transfer and regulation of metabolic pathways (Blank, 2012; Kamerlin et al., 2013). ATP and phosphorylated compounds (i.e. sugars) are important for plant photosynthesis (Arnon, 1956), therefore P deficiency would lead to a reduction in photosynthetic parameters relating to plant growth. Thus, the measurement of photosynthetic parameters could evaluate how P deficiency affects plant growth. Photosynthetic rate decreased under P starvation in sugar beet (Terry and Ulrich, 1973). A decrease in photosynthetic rate and biomass also occurred in maize under low P treatment (Usuda and Shimogawara, 1991). Other studies have shown that the addition of P enhanced photosynthetic rate, stomatal conductance (Cond) but

decreased intercellular CO_2 (C_i) in rice (Xu et al., 2007) and in *Brachiaria brizantha* (Martins et al., 2015). Interestingly, Li et al. (2006c) have shown that a P efficient rice had greater P_n than an inefficient genotype under P deficiency.

A light intensity by P supply interaction for plant growth has also been documented. For instance, when two weed species, *Veronica persica* and *Chorispora tenella* were grown together, *C. tenella* showed a greater competitive capacity under high light intensity and low P supply, but its competitive advantage was lost under low light intensity and higher P supply (Yin et al., 2012). The light intensity and P supply interaction has also been observed in pak-choi (Yang et al., 2009). A study in lupin was able to show that shoot and root biomass increased dramatically under adequate P and high light intensity when compared to low P, whereas these parameters were enhanced slightly under adequate P and low light intensity (Cheng et al., 2014). Thus, light intensity can affect P use efficiency (PUE) (relative growth at low P/high P).

Two wheat genotypes, RAC875 and Wyalkatchem exhibit differences in PUE under two controlled environments, in a greenhouse and in a growth room (Chapter 2). RAC875 had lower PUE than Wyalkatchem in the greenhouse but higher efficiency in the growth room. It was noticed that light intensity in the greenhouse was lower than in the growth room and our preliminary results showed that plants tillered better in the growth room than in the greenhouse. The light intensity in the greenhouse measured in September 2015 was 270 μmol m^{-2} s^{-1} while in the growth room it was 700 μmol m^{-2} s^{-1}. Therefore, light intensity could have an impact on differences in PUE of these two wheat genotypes under these two growth conditions.

In this study, two wheat genotypes, RAC875 and Wyalkatchem were grown in the

growth room at different light intensities and P supply to examine effects of light intensity on PUE of these wheat genotypes. Also, as low light intensity is a common problem in various wheat growing regions of the world (i.e. Bangladesh, where they get excessive fog that reduces light penetration to the wheat canopy) (Saifuzzaman et al., 2004), it's worthwhile knowing whether PUE changes under varied light intensity.

3.2 Materials and methods

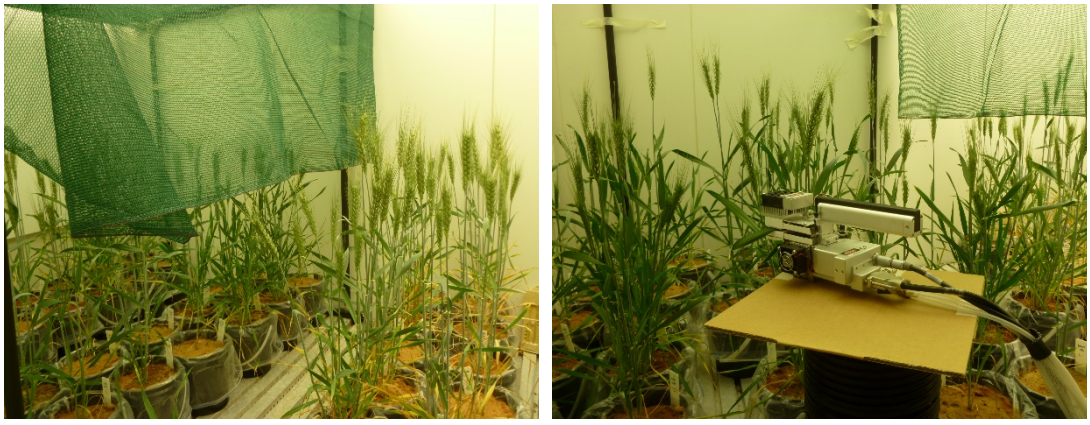
3.2.1 Experiment 1

3.2.1.1 Experiment design

Two wheat genotypes, RAC875 and Wyalkatchem were grown at three different P rates of 5, 10 and 30 mg P kg^{-1} soil and two different light intensities. Plants were sown into pots filled with 4.2 kg of doubled washed yellow sandy soil with the following added basal nutrients (expressed in mg kg^{-1} soil): $Ca(NO_3)_2.4H_2O$ (918), K_2SO_4 (113.6), $MgSO_4.7H_2O$ (140), $FeSO_4.7H_2O$ (1.4), $Na_2MoO_4.2H_2O$ (0.61), $CuSO_4.5H_2O$ (2.25), $MnSO_4.4H_2O$ (3.68), $ZnSO_4.7H_2O$ (6.6), H_3BO_3 (0.28). Phosphorus (P) (in the form of KH_2PO_4) was then added to pots with three different levels of 5, 10 and 30 mg P kg^{-1} soil. The added P was then massaged into the soil. Pot size and shape were described in the Experiment 1 in Chapter 2. The experiment was carried out in a growth room and the day length was 13 h and the day/night temperature was 20/10 ^oC. The experiment was split in two, with equal number of pots at two different light intensities. Low light intensity was imposed by shading plants with commercially bought shade-cloth (Figure 3.1 A). The light intensity measured at the flag leaf level was 285±4 and 624±14 μmol m^{-2} s^{-1} for low and high light intensities, respectively. The sort of light was the combination of fluorescent and incandescent.

Wheat seeds were germinated in petri disks lined with moisturized filter papers and were kept in the dark at room temperature for three days prior to planting. One germinated seed was sown per pot and the experiment was conducted with four replicates. Plants were watered to 8-10% of the soil weight three times a week. At ripening stage, however, pots were water two times a week since plants required less water. Pots were randomly arranged and moved around frequently.

Plants were harvested at maturity. Stems were cut at the crown level and stem parts close to the soil were washed and then rinsed in Milli-Q water. Stems were dried at 85 ^{o}C for two days for dry matter measurement. Spikelets were dried at 37 ^{o}C for five days and spikelet dry matter was then measured. Wheat grains were obtained using a Haldrup thresher and the grain yield was then measured.



A B

Figure 3.1. Plants were grown under two different light intensities by the use of shade-cloth (A). Gas exchange measurements were taken with the use of a LiCor LI-6400XT portable photosynthesis system (B).

3.2.1.2 Gas exchange measurement

Gas exchange was measured using LI-6400XT portable photosynthesis system (Figure 3.1 B) at anthesis from a time of 09:00 to 10:30 h and measurements were randomly broken into four days (78, 79, 80 and 81 DAS) due to a large number of measurements.

Measurements were made on flag leaves with the light intensities of 285 µmol $m^{-2}s^{-1}$ and 624 µmol m^{-2} s^{-1} for plants grown at low light intensity and high light intensity, respectively. All photosynthetic measurements were taken after two minutes at a constant airflow rate of 400 µmol s^{-1}. CO_2 concentration was 400 µmol mol^{-1} and the temperature was 20 ºC and the sample humidity was 62 ± 2%.

3.2.2 Experiment 2

This experiment was designed to elucidate phosphorus distribution in two wheat genotypes, RAC875 and Wyalkatchem. The plants were sown and grown in the same soil as in Experiment 1 and also with 4.2 kg soil per pot. Pot size and shape were described in the Experiment 1 in Chapter 2. Three rates of P were applied; 5, 10 and 30 mg P kg^{-1} soil with the addition of basal nutrients as described in Experiment 1. One germinated grain was sown per pot and the experiment was conducted in a growth room with the temperature of 20/10 ºC (day/night) and the day-length of 13 h. The light intensity was 700 µmol m^{-2} s^{-1} at the leaf level. The sort of light was the combination of fluorescent and incandescent. The primary tiller of each plant was harvested at anthesis. Then leaves and stem were sectioned. Leaf tissue was further sectioned in to flag leaves (F), leaf immediately below the flag leaf (F-1) and leaf immediately below the F-1 leaf (F-2). The P concentration of leaf and stem material was then analyzed using ICP-OES (SPECTRO, Model was a CIROS CCD Radial ICPOES) at the Waite Analytical Service (WAS).

3.2.3 Statistical analysis

Statistical analyses were conducted by using IBM SPSS v23. The normality of data was tested using Kolmogorov-Smirnov and Shapiro-Wilk test ($P<0.05$). Major growth

traits and gas exchange parameters including P_n, Cond and C_i were analysed by three-way ANOVA (Genotype x Light intensity x P treatments). These indices and parameters were also analysed by two-way ANOVA (Genotype x P supply) at each light intensity. P concentration was analysed by three-way ANOVA (Genotype x Part of plant x P supply). Significant differences between the means between P treatments were analysed by Tukey's test ($P<0.05$). Mean comparisons between genotypes at each P treatment were performed by independent t-test ($P<0.05$). The particular sets of variables was subjected to Pearson's correlation analysis (Field, 2013).

3.3 Results

3.3.1 Responses of wheat genotypes to light intensity and phosphorus

Both light intensity and P supply had significant ($P<0.001$) impact on grain yield, shoot dry matter (DM) and tiller number (Table 3.1). Plants produced higher grain yield (29.6%), shoot DM (34.6%) and tiller number (32.6%) under higher light intensity and increasing P supply also resulted in greater grain yield, shoot DM and tiller number production (Table 3.1, Table 3.2).

There was a significant interaction between light intensity (L) and P supply (P) for grain yield ($P<0.001$), shoot DM ($P<0.001$) and tiller number ($P<0.05$) (Table 3.1). Increased light intensity did not significantly improve grain yield, shoot DM and tiller number at the P supply of 5 mg P kg^{-1} soil, but higher light intensity showed greater grain yield, shoot DM and tiller number production at the P supply of 10 and 30 mg kg^{-1} soil (Table S 3.2).

Table 3.1. The effect of genotype, light intensity and P supply on grain yield, shoot dry matter (DM), tiller number at maturity and shoot height, flag leaf length and maximum (Max) flag leaf width at anthesis of two wheat genotypes. Plants were grown under two different light intensities (low light intensity: 285 μmol m^{-2} s^{-1}, high light intensity: 624 μmol m^{-2} s^{-1}) and three different P supply (5, 10 and 30 mg P kg^{-1} soil).

Effect	Grain yield (g plant^{-1})		Shoot DM (g plant^{-1})		Tiller number (tiller plant^{-1})		Shoot height (cm)		Flag leaf length (cm)		Max flag leaf width (cm)	
	df	*F* Ratio	df	*F* Ratio	df	*F* Ratio	df	*F* Ratio	df	*F* Ratio	df	*F* Ratio
Genotype (G)	1	2.488 ns	1	0.721 ns	1	17.322***	1	62.936***	1	1.450 ns	1	58.086***
Light intensity (L)	1	49.180***	1	44.345***	1	21.483***	1	4.225*	1	109.782***	1	16.451***
P supply (P)	2	689.646***	2	443.110***	2	217.696***	2	2.213 ns	2	6.098**	2	20.419***
G × L	1	5.135*	1	5.913*	1	2.013 ns	1	1.156 ns	1	0.036 ns	1	0.070 ns
G × P	2	4.442*	2	3.103 ns	2	3.717*	2	0.593 ns	2	4.451*	2	0.067 ns
L × P	2	11.547***	2	9.728***	2	4.917*	2	0.450 ns	2	7.712**	2	0.343 ns
G × L × P	2	0.367 ns	2	2.212 ns	2	4.798*	2	1.764 ns	2	3.050 ns	2	2.307 ns
Error	34		34		34		34		34		34	
Total	46		46		46		46		46		46	

Results are from three-way ANOVA analysis. *, ** and *** significant at P<0.05, P<0.01 and P<0.001, respectively; ns: not significant.

Table 3.2. The effect of P supply on grain yield, shoot dry matter (DM) and tiller number at maturity of two wheat genotypes grown under different two different light intensities (low light intensity: 285 μmol m^{-2} s^{-1}, high light intensity: 624 μmol m^{-2} s^{-1})

Genotype	P supply (mg P kg^{-1} soil)	Grain yield (g $plant^{-1}$)		Shoot DM (g $plant^{-1}$)		Tiller number (tiller $plant^{-1}$)	
		Low light	High light	Low light	High light	Low light	High light
RAC875	5	2.0 ± 0.32	2.6 ± 0.35	4.8 ± 0.97	5.7 ± 0.79	1.5 ± 0.29	1.5 ± 0.29
	10	6.1 ± 0.65	9.9 ± 0.84	11.9 ± 1.39	20.3 ± 2.02	3.0 ± 0.41	4.3 ± 0.33
	30	14.1 ± 1.04	19.3 ± 0.93	25.4 ± 1.94	37.8 ± 1.14	6.0 ± 0.71	10.0 ± 0.58
Wyalkatchem	5	2.0 ± 0.25	1.7 ± 0.16	3.8 ± 0.68	3.8 ± 0.48	1.5 ± 0.29	1.8 ± 0.25
	10	6.9 ± 0.68	9.3 ± 0.16	13.1 ± 1.98	19.3 ± 0.81	4.3 ± 0.63	6.3 ± 0.48
	30	17.3 ± 0.26	20.1 ± 0.60	33.0 ± 0.73	36.9 ± 2.06	9.7 ± 1.2	10.3 ± 0.25
Average		**8.1**	**10.5**	**15.3**	**20.6**	**4.3**	**5.7**
F Ratio							
Genotype (G)		6.715*	0.154 ns	4.854*	1.405 ns	11.084**	6.323*
P supply (P)		234.011***	480.676***	146.35***	331.500***	54.962***	249.114***
G × P		3.181 ns	1.207 ns	4.526*	0.079 ns	4.597*	2.857 ns

Data represent the means of four replicates with standard errors. *F* Ratio are from two-way ANOVA analysis. *, ** and *** significant at P<0.05, P<0.01 and P<0.001, respectively; ns: not significant.

In general, Wyalkatchem had significantly (P<0.001) higher tiller number than RAC875, but no significant differences in grain yield and shoot DM were observed between the genotypes (Table 3.1). However, under low light intensity, Wyalkatchem produced significantly (P<0.05) greater grain yield and shoot DM than RAC875 (Table 3.2). In regards to PUE, RAC875 was more P efficient than Wyalkatchem under both light conditions. Indeed, RAC875 exhibited a slightly lower external P requirement (calculated as mg P supply at which plants achieve 90% of maximum grain yield) than Wyalkatchem under both light conditions. Under low light intensity, the external P requirement was 24.2 mg P kg^{-1} soil for RAC875 and 24.9 mg P kg^{-1} soil for Wyalkatchem, while at higher light intensity, the external P requirement was 21.4 and 22.5 mg P kg^{-1} soil for RAC875 and Wyalkatchem, respectively (Figure 3.2). RAC875 also had a greater relative grain yield (yield at low P/yield at high P) than Wyalkatchem under both light conditions (Figure 3.3).

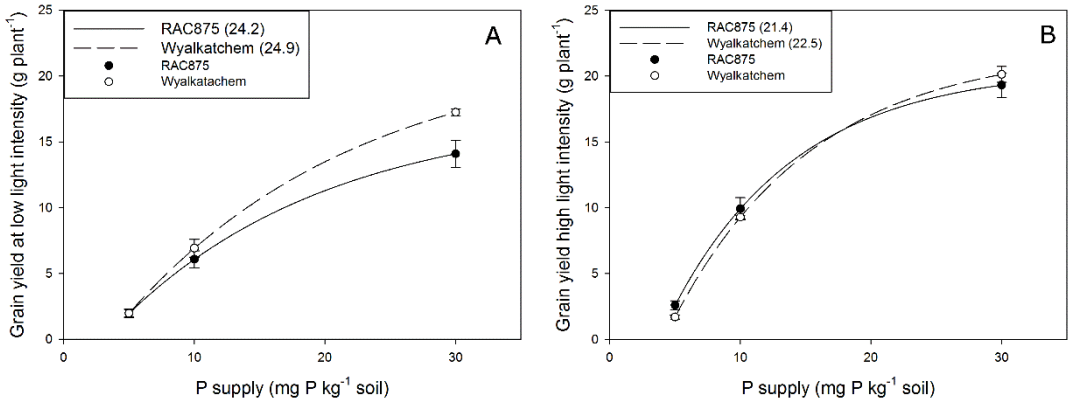


Figure 3.2. Responses to P of two wheat genotypes under two different light intensities (low light intensity: 285 µmol m^{-2} s^{-1}, high light intensity: 624 µmol m^{-2} s^{-1}). Values are the means of four replicates with standard errors, numbers in brackets were external P requirement ($critical_{90}$). The curves represent Mitscherlich responses.

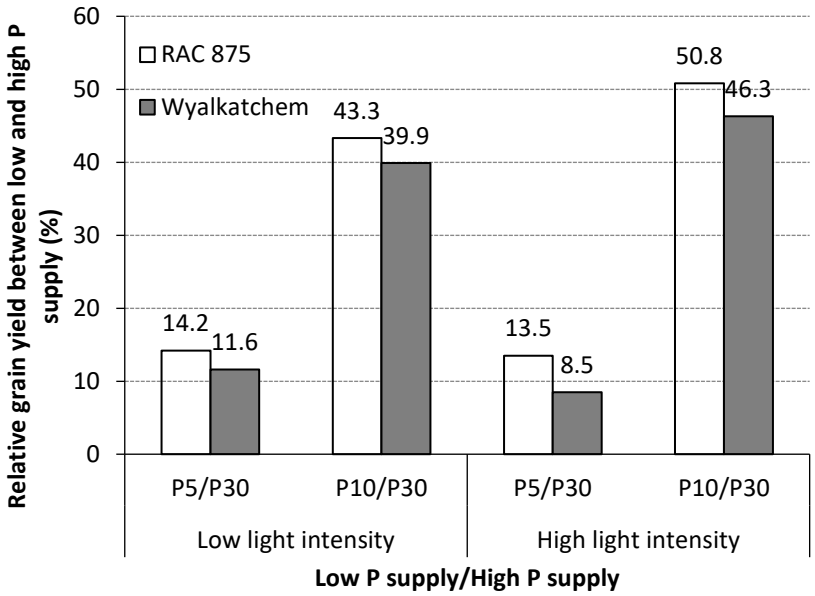


Figure 3.3. Relative grain yield between low P and high P supply of two wheat genotypes under different light intensities (low light intensity: 285 μmol m^{-2} s^{-1} and high light intensity: 624 μmol m^{-2} s^{-1}). P5, P10 and P30 are P supply of 5, 10 and 30 mg P kg^{-1} soil, respectively. Results were the ratio of the means.

Shoot height was not significantly affected by P supply but was significantly ($P<0.05$) impacted by light intensity (Table 3.1). RAC875 also showed significantly ($P<0.001$) greater shoot height than Wyalkatchem under both light intensities (Table 3.3).

Flag leaf length and maximum flag leaf width were significantly impacted by both light intensity and P supply (Table 3.1). Under very low P (5 mg P kg^{-1} soil), flag leaf length and maximum flag leaf width was smaller than that under higher P treatments (Table 3.1, Table 3.3, Table S 3.1). Low light intensity led to 45.1% longer flag leaf ($P<0.001$) and 10.3% greater maximum flag leaf width ($P<0.001$) (Table 3.1, Table 3.3).

Significant ($P<0.05$) L x P interaction for flag leaf length was observed. Indeed, under low light intensity, plants produced longer flag leaf under higher P supply when compared to P supply of 5 mg P kg^{-1} soil ($P<0.05$), but no considerable difference in flag leaf length between P supply of 10 mg P kg^{-1} soil and 30 mg P kg^{-1} soil occurred. However, under high light intensity, there was significant ($P<0.05$) difference in flag leaf length between these two levels of P (10 and 30 mg P kg^{-1} soil) (Table S 3.3). There was no considerable difference in flag leaf length between the two wheat genotypes, but RAC875 had significantly ($P<0.001$) larger maximum flag leaf width under both light intensities (Table 3.3).

Table 3.3. The effect of P supply on shoot height, flag leaf length and maximum (Max) leaf width at anthesis of two wheat genotypes grown under two different light intensities (low light intensity: 285 μmol m^{-2} s^{-1}, high light intensity: 624 μmol m^{-2} s^{-1})

Genotype	P supply (mg P kg^{-1} soil)	Shoot height		Flag leaf length (cm)		Max leaf width (cm)	
		Low light	High light	Low light	High light	Low light	High light
RAC875	5	55.1 ± 5.8	59.4 ± 1.9	13.9 ± 0.8	12.2 ± 0.9	1.42 ± 0.05	1.41 ± 0.06
	10	61.5 ± 1.5	61.5 ± 4.2	20.5 ± 1.6	10.8 ± 0.1	1.69 ± 0.12	1.54 ± 0.01
	30	61.9 ± 2.6	62.5 ± 0.9	19.8 ± 1.2	14.5 ± 1.0	1.79 ± 0.03	1.54 ± 0.12
Wyalkatchem	5	45.6 ± 3.6	45.1 ± 0.7	16.8 ± 1.3	12.1 ± 0.8	1.24 ± 0.02	1.01 ± 0.03
	10	46.6 ± 3.9	52.3 ± 1.0	18.0 ± 0.9	11.5 ± 1.3	1.42 ± 0.03	1.30 ± 0.04
	30	41.0 ± 0.3	51.6 ± 2.3	17.1 ± 0.7	12.4 ± 0.4	1.45 ± 0.03	1.33 ± 0.03
Average		**52.0**	**55.4**	**17.7**	**12.5**	**1.50**	**1.36**
F Ratio							
Genotype (G)		26.086***	52.894***	0.829 ns	0.621 ns	29.118***	29.043***
P supply (P)		0.578 ns	4.081*	9.155**	4.002*	13.265***	7.738**
G × P		1.205 ns	0.895 ns	5.366*	1.512 ns	0.956 ns	1.382 ns

Data represents the means of four replicates with standard errors. *F* Ratio are from two-way ANOVA analysis. *, ** and *** significant at P<0.05, P<0.01 and P<0.001, respectively; ns: not significant.

3.3.2 Photosynthetic parameters

In general, the photosynthetic rate (P_n) and intercellular CO_2 concentration (C_i) were significantly affected by light intensity and P supply. Higher light intensity led to higher P_n ($P<0.001$) (Table 3.4, Figure 3.4 A). P_n was significantly ($P<0.05$) reduced under very low P supply (5 mg P kg^{-1} soil) when compared to adequate P supply (30 mg P kg^{-1} soil) (Figure 3.4 A, Table S 3.1). In contrast to P_n, plants showed greater C_i under low light intensity ($P<0.001$) (Table 3.4, Figure 3.4 C). Increasing P supply resulted in a lower C_i. G x L, G x P, L x P, and G x L x P interactions were not significant for P_n and C_i (Table 3.4). Between two genotypes, Wyalkatchem had significantly ($P<0.01$) higher P_n than RAC875 under low light intensity, while C_i was greater in Wyalkatchem than in RAC875 at both light intensities (Table 3.5, Figure 3.4 A & C). Light intensity and P supply had no impact on stomatal conductance (Cond), but Cond was significantly affected by genotypes ($P<0.001$) in which Wyalkatchem had a significantly higher Cond than RAC875 (Table 3.4, Figure 3.4 B).

Table 3.4. The effect of genotype, light intensity and P supply on photosynthetic rate (P_n), stomatal conductance (Cond) and intercellular CO_2 concentration (Ci) at anthesis of two wheat genotypes. Plants were grown under two different light intensities (low light intensity: 285 µmol m^{-2} s^{-1}, high light intensity: 624 µmol m^{-2} s^{-1}) and three different P supply (5, 10 and 30 mg P kg^{-1} soil).

Effect	P_n (µmol CO_2 m^{-2} s^{-1})		Cond (µmol H_2O m^{-2} s^{-1})		Ci (µmol CO_2 m^{-2} s^{-1})	
	df	*F* Ratio	df	*F* Ratio	df	*F* Ratio
Genotype (G)	1	7.698**	1	18.705***	1	22.194***
Light intensity (L)	1	128.016***	1	1.395 ns	1	30.132***
P supply (P)	2	3.877*	2	0.825 ns	2	5.260*
G × L	1	0.027 ns	1	0.232 ns	1	1.128 ns
G × P	2	1.459 ns	2	0.163 ns	2	0.167 ns
L × P	2	2.885 ns	2	0.206 ns	2	0.239 ns
G × L × P	2	0.888 ns	2	0.460 ns	2	2.892 ns
Error	34		34		34	
Total	46		46		46	

F Ratio are from three-way ANOVA analysis. * and *** significant at P<0.05 and P<0.001, respectively; ns: not significant.

Table 3.5. The effect of genotype and P supply on photosynthetic rate (P_n), stomatal conductance (Cond) and intercellular CO_2 concentration (C_i) at anthesis of two wheat genotypes. Plants were grown under two different light intensities (low light intensity: 285 µmol m^{-2} s^{-1}, high light intensity: 624 µmol m^{-2} s^{-1}) and three different P supply (5, 10 and 30 mg P kg^{-1} soil).

Genotype	P_n (µmol CO_2 $m^{-2}s^{-1}$)		Cond µmol (H_2O m^{-2} s^{-1})		C_i (µmol CO_2 m^{-2} s^{-1})	
	Low light	High light	Low light	High light	Low light	High light
F Ratio						
Genotype (G)	10.740**	2.564 ns	7.531*	11.333**	5.885*	19.181***
P supply (P)	0.521 ns	4.109*	0.812 ns	0.212 ns	2.365 ns	3.227 ns
G × P	1.064 ns	1.214 ns	0.066 ns	0.539 ns	1.099 ns	2.079 ns
df_G	1	1	1	1	1	1
df_P	2	2	2	2	2	2
df_E	17	17	17	17	17	17

F Ratio are from two-way ANOVA analysis. *, ** and *** significant at P<0.05, P<0.01 and P<0.001, respectively; ns: not significant.

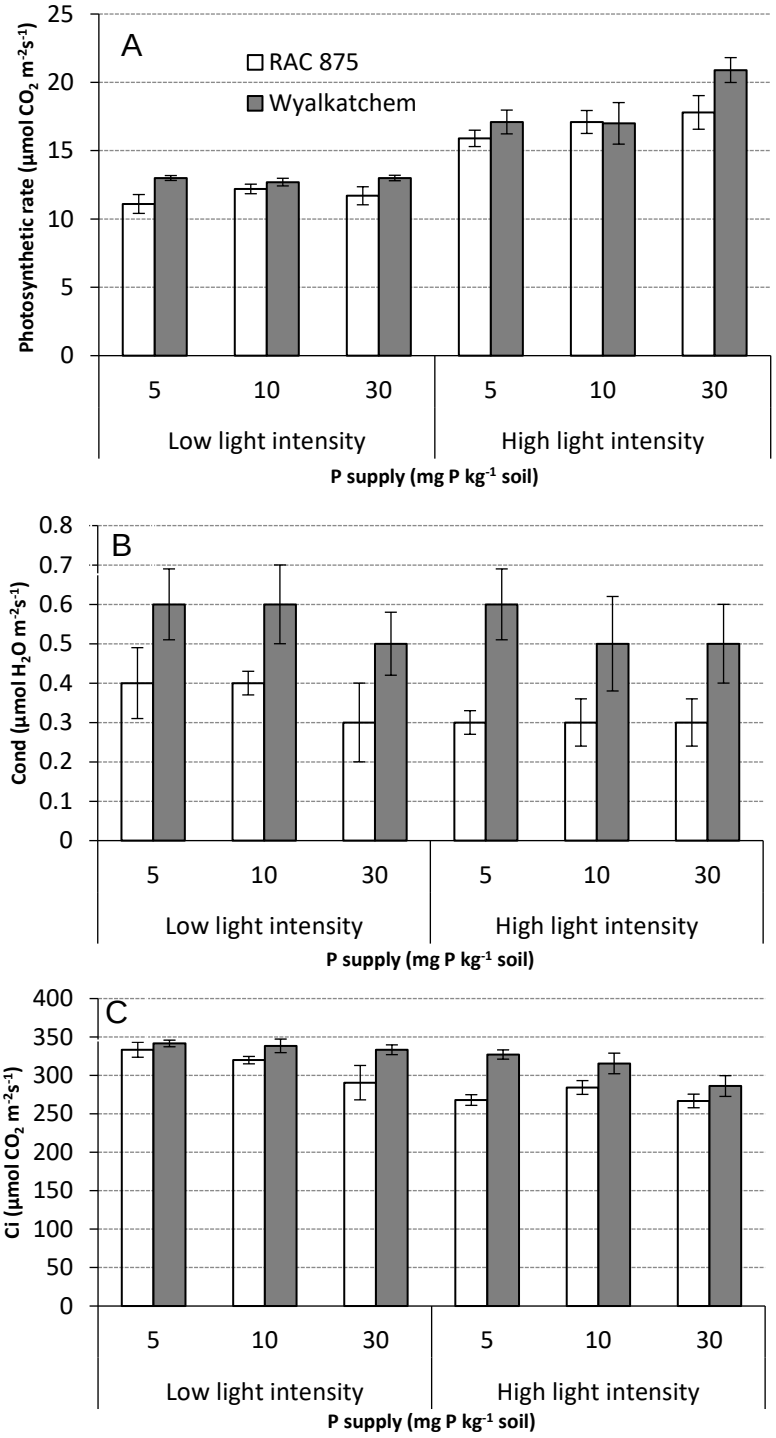


Figure 3.4. The effect of light intensity and P supply on the photosynthetic rate (P_n) (A), stomatal conductance (Cond) (B) and intercellular CO_2 (C_i) (C) of two wheat cultivars. Low light intensity (285 µmol $m^{-2}s^{-1}$), high light intensity (624 µmol $m^{-2}s^{-1}$). Data represent the means of four replicates with standard errors.

P_n and Cond was positively correlated under both light intensities, but P_n had a strongly positive correlation with C_i only under low light intensity (Table 3.6). C_i was positively correlated with Cond at both low and high light intensities.

Table 3.6. Pearson correlation coefficients between photosynthetic rate (P_n), stomatal conductance (Cond) and intercellular CO_2 (C_i) of two wheat genotypes grown under three different P supply (5, 10 and 30 mg P kg^{-1} soil) and different light intensities (low light intensity: 285 μmol m^{-2} s^{-1}, high light intensity: 624 μmol m^{-2} s^{-1}). *, ** significant at P<0.05, P<0.01, respectively.

	Low light intensity		High light intensity	
Parameter	Cond	C_i	Cond	C_i
P_n	0.624**	0.546**	0.500*	0.243
Cond		0.860**		0.917**

3.3.3 Phosphorus distribution

P concentration varied significantly (P<0.001) between the different plant parts (Table 3.7). P concentration significantly (P<0.01) decreased in the order from the flag leaf (F) to the second oldest leaf (F-1) to the third oldest leaf (F-2) (Figure 3.5). This is indicative of the level of P mobility in leaf tissues.

The stem had a lower P concentration than the flag leaf (P<0.001), but higher than the F-2 leaf (P<0.001) (Table S 3.4). P concentration in all parts (F, F-1, F-2 and stem) significantly increased as P supply increased (P<0.001) (Table 3.8, Figure 3.5). Wyalkatchem showed a significantly (P<0.01) greater P concentration in the F-2 leaf and stem (P<0.05) than RAC875, but the P concentration in the flag leaf and F-1 leaf was not significantly different between the two wheat genotypes (Table 3.8).

Table 3.7. The effect of genotype and P supply on P concentration in different parts of plants

Effect	P concentration (mg kg^{-1})	
	df	*F* Ratio
Genotype (G)	1	3.859 ns
Parts of plant (PP)	3	69.850***
P supply (P)	2	446.087***
G × PP	3	2.185 ns
G × P	2	0.704 ns
PP × P	6	10.146***
G × PP × P	6	0.337 ns
Error	96	
Total	120	

F: flag leaf, F-1: the leaf under the flag leaf, F-2: the leaf under the F-1 leaf.
F Ratio are from three-way ANOVA analysis. *** significant at P<0.001; ns: not significant.

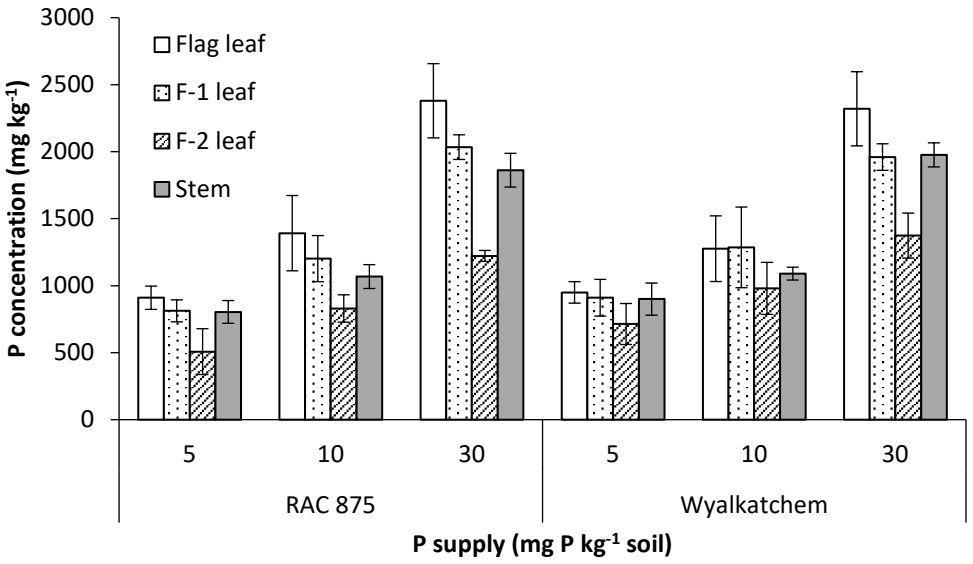


Figure 3.5. The effect of P supply on the P concentration in different leaves and stem of two wheat cultivars grown in the growth room. Plant tissues were sampled at anthesis (Zadoks scale 60-62). F-1: the leaf under the flag leaf; F-2: the leaf under the F-1 leaf. Data represent the mean with standard deviation of five replicates. Different letters show significant differences within each P supply (*P<0.05*); within each P supply, columns without letters show no significant differences among each other and with labelled columns.

Table 3.8. The effect of genotype and P supply on P concentration in flag leaf, the leaf (F-1) under flag leaf, the leaf (F-2) under the F-1 leaf and stem

Effect	P concentration (mg kg^{-1})			
	F	F-1	F-2	Stem
RAC875	1561	1349	853	1245
Wyalkatchem	1515	1385	1023	1322
F Ratio				
Genotype (G)	0.302 ns	0.356 ns	9.884**	4.819*
P supply (P)	104.724***	122.431***	54.604***	339.412***
G × P	0.305 ns	0.836 ns	0.116 ns	0.638 ns
df_G	1	1	1	1
df_P	2	2	2	2
df_E	24	24	24	24

Values are means at each part of plants. *F* Ratio are from two-way ANOVA analysis. *, ** and *** significant at P<0.05, P<0.01 and P<0.001, respectively; ns: not significant.

3.4 Discussion

This study was conducted to identify whether light intensity affects P responses and photosynthetic parameters of two wheat genotypes, which might help to explain previous results witnessed between greenhouse and growth room conditions.

Low light intensity showed a reduced grain yield, shoot DM and tiller number (Table 3.1, Table 3.2). This effect agrees with previous findings in wheat that tiller number declined under low light intensity (Page et al., 2012; Tamaki et al., 2001). These results are also consistent with other reports that low light intensity resulted in a reduction in shoot biomass (Zervoudakis et al., 2012; Cheng et al., 2014) and grain yield (Tarila et al., 1977). In another research, Xu et al. (2016) have shown that a slight shading after anthesis increased wheat grain yield due to the delay of leaf senescence, but mid-level

and severe shading negatively affected grain yield.

In this study, plants under low light intensity had greater flag leaf length and maximum leaf width (Table 3.1, Table 3.3). Greater leaf expansion under low light intensity was also observed in *Aeschynanthus longicaulis* (Li et al., 2014). Plants appear to expand their leaves to obtain more light under low light intensity.

The results presented here show that high light intensity increased P_n (Table 3.4, Figure 3.4 A). Cheng et al. (2014) reported that a high light intensity enhanced P_n and sucrose levels in plant leaves and roots. Wang et al. (2005) reported that a light shading led to a reduced concentration of sucrose and the activity of sucrose synthase in nectarine leaves, which could consequently inhibit the plant growth rate. In contrast to P_n, C_i decreased under high light intensity (Table 3.4, Figure 3.4 C). Higher P_n under high light intensity could result in greater use of C_i and lead to lower C_i. Cond was not affected by light intensity and this result agrees with a finding in maize (Raschke et al., 1978).

Light intensity affected the growth of the two wheat genotypes in different ways. No significant differences in grain yield and shoot DM occurred under high light intensity, but Wyalkatchem produced greater grain yield and shoot DM under low light intensity (Table 3.2). Under low light intensity, Wyalkatchem was highly responsive to the adequate P (Figure 3.2). Although wheat genotypes behave differently between light intensity treatments, RAC875 showed a slightly lower external P requirement (Figure 3.2) and greater relative grain yield (yield at low P/yield at adequate P) (Figure 3.3) than Wyalkatchem under both light treatments, indicating that RAC875 is more P efficient than Wyalkatchem. These results are consistent with our previous studies

under growth room conditions (Chapter 2). Indeed, the results in Chapter 2 showed that $PUE_{10/30}$ in RAC875 was 11.7% greater than that in Wyalkatchem and in this experiment, this difference was 3.4% and 4.5% (Figure 3.3) under low light and high light intensity, respectively. Thus, PUE values were repeatable between experiments under growth room conditions and growth room conditions could be used for screening P efficient wheat genotypes.

P deficiency affected photosynthetic parameters. Lower P supply led to a reduction in P_n and an increase in C_i (Table 3.4, Figure 3.4 A & C). Similar findings have been observed in maize (Usuda and Shimogawara, 1991) and in *Brachiaria brizantha* (Martins et al., 2015). Zulu et al. (1991) reported a decline in P_n in wheat under low P. Low P decreased the P concentration in flag leaf (Figure 3.5) and would therefore result in a reduced P_n. As reported in spinach, P starvation affected the regeneration of ribulose-1,5-biphosphate (RuBP) and the activity of ribulose-1,5-biphosphate carboxylase (RubisCO) that caused a decline in P_n (Brooks et al., 1988; Brooks, 1986). However, studies in maize have shown that P_n was affected under low P due to decreased RuBP regeneration rather than an effect on the activity of RubisCO (Rao and Terry, 1989; Rao et al., 1993). Lin et al. (2009) reported that P deficiency in tea (*Camellia sinensis* L.) decreased the efficiency of photosynthetic electron transport, therefore reducing ATP production which decreases the regeneration of RuBP. In contrast to P_n, C_i increased under low P. This could be due to the inefficient use of CO_2 by plants under low P. P supply, however, had no effect on Cond. This would indicate that P supply did not restrict the diffusion rate of H_2O. In contrast, low P reduced Cond in rice (Li et al., 2006c) and in *Brachiaria brizantha* (Martins et al., 2015). This

difference between results could be attributed to species variation.

Under low light intensity, Wyalkatchem produced greater grain yield and shoot DM than RAC875 (Table 3.2). Furthermore, Wyalkatchem also showed higher P_n than RAC875 under low light intensity (Table 3.5, Figure 3.4 A). It appears that Wyalkatchem was more tolerant to low light intensity than RAC875.

Wyalkatchem showed greater Cond and C_i than RAC875 and P_n was positively correlated with Cond under both light conditions (Table 3.6). Thus, higher Cond could result in greater P_n in Wyalkatchem. A correlation between P_n and Cond has also been observed in a cotton genotype (*Gossypium hirsutum* L. *cv* Deltapine 61) under different light conditions (Radin et al., 1988).

Redistribution of P from old leaves to sink tissues during senescence is important since it can enhance PUE and minimize P fertilization (Stigter and Plaxton, 2015). Reduced P concentration in senescing leaves was observed in this study, suggesting that P was remobilized from aged leaves to developing tissues. During senescence, remobilization of P was also observed in soybean (Crafts-Brandner, 1992) and in other plants including wheat (Maillard et al., 2015). Interestingly, RAC875 had lower P concentration in the oldest leaf (F-2) than Wyalkatchem which may indicate a greater degree of remobilization from this tissue, while no significant differences in P concentration were observed in younger leaves between two genotypes.

Conclusions

Overall, low light intensity reduced grain yield, shoot dry matter and photosynthetic rate in the two wheat genotypes, but increased C_i, flag leaf length and maximal leaf

width. Two wheat genotypes responded differently to different light intensities in which Wyalkatchem produced greater grain yield and shoot dry matter than RAC875 under low light intensity, but not under high light intensity. Wyalkatchem also had greater P_n, C_i and Cond than RAC875. However, under both growth conditions, RAC875 showed a slightly lower external P requirement and greater relative grain yield ratio which represents PUE. This indicates that light intensity did not affect PUE of the two wheat genotypes and RAC875 was more P efficient than Wyalkatchem under both light conditions.

Supplemental tables and figures

Table S 3.1. Tukey comparisons for grain yield, shoot dry matter (DM), tiller number, flag leaf length, maximum flag leaf width, photosynthetic rate and C_i between P supply (three-way ANOVA analysis: Genotype x Light intensity x P supply)

Parameter	P supply (mg P kg^{-1} soil)		Mean difference	SE	Sig.
Grain yield	5	10	-5.838706*	.4260472	.000
	5	30	-15.665473*	.4260472	.000
	10	30	-9.826767*	.4328644	.000
Shoot dry matter	5	10	-11.3730*	.96513	.000
	5	30	-28.7613*	.96513	.000
	10	30	-17.3883*	.98057	.000
Tiller number	5	10	-2.904*	.3559	.000
	5	30	-7.371*	.3559	.000
	10	30	-4.467*	.3616	.000
Flag leaf length	5	10	-1.705*	.6276	.027
	5	30	-2.078*	.6276	.006
	10	30	-.373	.6376	.829
Maximum flag leaf width	5	10	-.2107*	.04324	.000
	5	30	-.2627*	.04324	.000
	10	30	-.0520	.04393	.471
P_n	5	10	-.3431312	.57622294	.823
	5	30	-1.7763386*	.57622294	.011
	10	30	-1.4332073	.58544309	.050
Ci	5	10	.9468175	7.70110786	.992
	5	30	25.8781274*	7.70110786	.005
	10	30	24.9313099*	7.82433341	.008

*Significant difference

Table S 3.2. Student's t-test comparisons for grain yield, shoot dry matter, tiller number and flag leaf length between different light intensities (285 and 624 μmol m^{-2} s^{-1}) within the same P supply

Parameter	P supply (mg P kg^{-1} soil)	Sig.	Mean difference	SE
Grain yield	5	.594	-.1683500	.3087664
	10	.000	-2.9917357	.6059355
	30	.001	-4.2770959	.9782116
Shoot DM	5	.604	-.42505	.80157
	10	.000	-7.24370	1.48724
	30	.001	-8.71573	2.11153
Tiller number	5	.642	-.1250	.2631
	10	.014	-1.8036	.6353
	30	.017	-2.5536	.9368
Flag leaf length	5	.010	3.2125	1.0712
	10	.000	8.0661	.8384
	30	.000	5.1357	1.0696

Table S 3.3. Tukey comparisons for flag leaf length between P supply at each light intensity

Parameter	Light intensity	P supply (mg P kg^{-1} soil)		Mean difference	SE	Sig.
Flag leaf length	Low	5	10	-3.862*	.9454	.002
		5	30	-3.211*	.9786	.012
		10	30	.652	.9786	.786
	High	5	10	.991	.8221	.466
		5	30	-1.287	.7943	.264
		10	30	-2.279*	.8221	.033

*Significant difference

Table S 3.4. Tukey comparisons for P concentration between different parts of plant (three-way ANOVA analysis: Genotype x Parts of plant x P supply)

Parameter	Parts of plant		Mean difference	SE	Sig.
P concentration	F	F-1	170.6667*	42.71352	.001
	F	F-2	600.0000*	42.71352	.000
	F	Stem	254.6667*	42.71352	.000
	F-1	F-2	429.3333*	42.71352	.000
	F-1	Stem	84.0000	42.71352	.208
	F-2	Stem	-345.3333*	42.71352	.000

*Significantly different

CHAPTER 4

Variation in root architecture and morphology of two wheat genotypes with contrasting phosphorus use efficiency

Abstract

Root system architecture (RSA) and morphology (RSA/morphology) are important for improvement of phosphorus use efficiency (PUE). This research aimed to measure RSA and morphological traits of two wheat genotypes, RAC875 and Wyalkatchem differing in PUE to elucidate root traits associated with PUE. A simple soil-based cultivation was developed to obtain a root system without destruction for RSA analysis. RAC875 produced greater shoot dry matter at 24, 27, 48 DAS and maturity, and higher grain yield at maturity, while no significant differences in these parameters were found between the two wheat genotypes under adequate P. RAC875 had greater P uptake and root efficiency (mg shoot P uptake per g root dry matter) than Wyalkatchem at maturity. Root to shoot ratio was also smaller in RAC875. P supply affected most RSA traits with low P leading to reductions in convex hull area, root surface area, root volume, total root length, root tip number, spatial root distribution (*Y*). Shoot DM was correlated with convex hull area, root surface area, root volume and total root length at 24 DAS. Under low P supply, RAC875 produced greater convex hull area than Wyalkatchem, while Wyalkatchem had significantly smaller convex area than RAC875 under adequate P. Wyalkatchem produced longer root hairs than RAC875, whereas RAC875 had denser root hairs than Wyalkatchem. Root hair

density also behaves differently at different P treatments. At low P supply, RAC875 had greater root hair density than Wyalkatchem but no difference was observed at adequate P. Greater convex hull area and root hair density was associated with greater shoot biomass and higher grain yield in RAC875 under low P supply. These root characteristics appear to imply that a smaller root system (lower root dry matter at maturity) as in RAC875 can support higher biomass and yield production under P deficiency. Thus, it seems that convex hull area and root hair density could be potential phenotypic indicators of efficiency.

4.1 Introduction

Roots play important roles in mineral and water acquisition from soils and can be targeted for improved crop productivity (Meister et al., 2014). The characterization of root system architecture (RSA) and morphology (RSA/morphology) and the elucidation of a correlation between RSA/morphology and function are required for the development of improved crops. Root morphology refers to the features of a single root axis as an organ, including root hairs, root diameter and cortical senescence, while RSA are concerned with the whole root system or a large subset of the root system and may describe as topological or geometric measures of the root shape (Lynch, 1995; Bucksch et al., 2014). Although a number of platforms have been developed for root phenotyping, genetic studies on root traits are hindered due to their complexity, underground location (Paez-Garcia et al., 2015; Kuijken et al., 2015) and interactions with the environment (Topp et al., 2016). Therefore, the exploration of unrevealed root traits for improvement of crop yield would make a contribution to keeping pace with the increase in world food demand (Godfray et al., 2010; Gregory and George, 2011). Desired improvements to crop root systems are related to enhanced nutrient efficiency, including improved phosphorus use efficiency (PUE), which is currently of great interest (Li et al., 2016; Gahoonia and Nielsen, 2004b; van de Wiel et al., 2016).

Phosphorus (P) limitation occurs in the majority of terrestrial ecosystems and reduces crop productivity (Lynch, 2011; Shenoy and Kalagudi, 2005). This shortage can be overcome through the application of P fertilizers (Shepherd et al., 2016), however, this is only a part of the solution because P fertilizers are non-renewable, potentially harmful to the environment when in oversupply and costly (Roberts and Johnston,

2015; Jones et al., 1989; Liu, 2015). Therefore, the development of crops that harbor an enhanced ability to acquire P as well as to utilize P more efficiently, is an important strategy to improve agricultural productivity (Vance et al., 2003; Lynch, 2007; Lambers et al., 2006; Wang et al., 2010).

In response to P deficiency, plants may display a variety of adaptation mechanisms including modifications in RSA/morphology (Zhu et al., 2006; Niu et al., 2012). Plants can adjust their root systems to P stress via stimulation of root and lateral root growth (Zhu et al., 2005a; Gaume et al., 2001), enhancement of root hair development (Bates and Lynch, 1996; Foehse and Jungk, 1983) and formation of cluster root formation (Abdolzadeh et al., 2010; Wasaki et al., 2003), which improves P acquisition in plants.

Genotypic variation in RSA/morphology under P starvation has been observed in crops. For example, maize genotypes with shallow root systems show greater growth and P accumulation than deep-rooted genotypes under low P conditions (Zhu et al., 2005b). Bean genotypes with longer root hairs and shallow roots produced significantly greater biomass than short-haired, deep-rooted genotypes (Miguel et al., 2015). Root traits including root surface area, root volume and root length were moderately heritable in maize under low P supply (Zhang et al., 2014). Once heritable traits associated with PUE are identified, they can be used to generate P efficient crops through plant breading or genetic modifications.

Current efforts to study the structure of crop root systems has already resulted in a number of root phenotyping platforms that are able to elucidate RSA under various conditions, including laboratory conditions, greenhouse conditions and field conditions (Paez-Garcia et al., 2015). RSA phenotyping requires a growth system, root

imaging system and software tools. A variety of software tools have been developed for RSA characterization including manual, semi-automatic and automatic programmes (Lobet et al., 2015). Root images can be obtained with or without destruction of root systems depending on the cultivation method and imaging techniques. For example, under gel-based growth in glass tubes, roots are visualized and are able to be captured directly without root destruction (Iyer-Pascuzzi et al., 2010), while roots grown in soils need to be extracted from soils for root imaging (da Silva et al., 2016). Advanced techniques such as X-ray (Mooney et al., 2012) or magnetic resonance imaging (MRI) (van Dusschoten et al., 2016) can be applied for imaging in soils without harming the root system. Each method has its own advantages as well as disadvantages. Non-destructive root imaging using gel-based systems can provide intact root system and are able to be applied for large scale screening, however, roots behaving in gel may not be similar to roots behaving in soils. Whilst, some root traits such as root angle and convex hull area are not able to be measured with destructive root imaging methods. Although this limitation can be overcome by using X-ray or MRI imaging technologies, these are expensive approaches. Glass bead-based cultivation in designed rhizoboxes is another approach to retain 2D (two-dimensional) RSA in rice (Courtois et al., 2013). This method has potential since glass beads are more similar to soils than gel in gel-based growth and rhizoboxes can keep root systems in their places for imaging. However, the development of a simple soil-based cultivation that can obtain root systems without destruction is an advanced solution for RSA analysis.

Wheat (*Triticum aestivum* L.) is one of the most important staple food crops (Shewry,

2009), therefore understanding wheat roots for improvements of yield as well as to cope with P limitation is vital. Some studies on wheat roots have been documented (Edwards et al., 2015; Petrarulo et al., 2015; da Silva et al., 2016; Ren et al., 2012), however these studies has some limitations. For example, wheat grown in hydroponic conditions (Ren et al., 2012; Petrarulo et al., 2015) that are far different from soil conditions, showed limited root traits measured using the growth pouch method and the clear pot method at the seedling stage (Edwards et al., 2015), or destructive root imaging used (da Silva et al., 2016). Therefore, in this study, a simple method was developed using designed rhizoboxes to obtain 2D root systems for RSA characterization in wheat.

RAC875 and Wyalkatchem are two wheat genotypes with contrasting PUE and root efficiency. RAC875 produced greater grain yield under low P than Wyalkatchem. Also, root efficiency (defined as P uptake per unit of root dry matter) in RAC875 was higher than that in Wyalkatchem (Chapter 2). Thus, RAC875 would have favourable root characteristics for improvements of its grain yield under low P. This research also aimed to examine variation in RSA and morphology of these two wheat genotypes. Three experiments were carried out: 1. RSA characterization using the designed rootboxes; 2. root hair characterization; and 3. responses to P of the two wheat genotypes at different growth stages in a pot experiment.

4.2 Materials and methods

4.2.1 Materials

Two selected wheat genotypes, RAC875 (P efficient) and Wyalkatchem (P inefficient)

were used for the experiments.

4.2.2 Experiment 1. Responses to P of two wheat genotypes at different growth stages in a pot experiment

This experiment aimed to examine responses to P between two wheat genotypes at different growth stages in a pot experiment. Plants were grown in pots (round-shaped pots with the dimensions: 18.5-cm high x 17.5-cm diameter at the top x 16.0-cm diameter at the bottom) filled with 4.2 kg soil at two P levels; 10 and 30 mg P kg^{-1} soil. Basal nutrients (expressed in mg kg^{-1} of soil) consisting of $Ca(NO_3)_2.4H_2O$ (918), K_2SO_4 (113.6), $MgSO_4.7H_2O$ (140), $FeSO_4.7H_2O$ (1.4), $Na_2MoO_4.2H_2O$ (0.61), $CuSO_4.5H_2O$ (2.25), $MnSO_4.4H_2O$ (3.68), $ZnSO_4.7H_2O$ (6.6), H_3BO_3 (0.28) were added and mixed into the soil by massaging the soil through the outside of the bag. Phosphorus (P) (in the form of KH_2PO_4) was then added to the bags at two different levels, 10 and 30 mg P kg^{-1} soil. P was then mixed well into the soil and the bags were placed in the pots.

Wheat grains were sterilized in 2% hypochlorite for 10 min and were then rinsed with Milli-Q water. Grains were then placed in petri disks lined with moisturized filter papers that were kept in a dark place at room temperature for three days. Five germinated grains were sown into each pot and the experiment was conducted with four replicates in a growth room (temperature: 10 ^oC night time, 20 ^oC day time; 13 h light period with a light intensity of 700 µmol m^{-2} s^{-1} at the leaf level. The sort of light was the combination of fluorescent and incandescent. Two plants were harvested at 27 DAS and one plant was harvested at 48 DAS. Two plants were grown up to maturity. At maturity, spikelets were separated from shoots and stems were detached from roots

at the crown level. Shoots and stems were washed and rinsed with Milli-Q water and were then dried for 48 h at 85 ^{o}C for dry matter measurements. Spikelets were dried at 37 ^{o}C for five days and grains were collected using a Haldrup thresher and the relative grain yield was then measured. Plant roots were washed to remove excess soil and the volume of roots was measured by measuring the volume of displaced water that roots occupied in a volumetric flask. The dry matter of roots was also measured after 48 h drying at 85 ^{o}C.

4.2.3 Experiment 2. Root system architecture characterization

4.2.3.1 Design of rhizoboxes

The rhizobox design was modified from the rhizobox designed by Courtois et al. (2013). A rhizobox has three main parts including a plastic box (L x W x D: 29.5 x 20 x 4 cm) with holes for watering, a foam base with a grid of toothpicks that hold the root system in place when washing soils during harvest, a transparent plastic sheet with a grid of holes that can insert in the foam base through a grid of tooth picks (Figure 4.1). Once soils were removed and the root system was washed, the transparent plastic sheet with the root system on it was removed from the foam base on which the toothpicks are attached. The root system was dried by blotting with tissue papers and was imaged for RSA analysis.

4.2.3.2 Soil preparation

1.2 kg of double-washed sandy soil was placed in a plastic bag with added basal nutrients (expressed in mg kg^{-1} of soil) consisting of $Ca(NO_3)_2.4H_2O$ (918), K_2SO_4 (113.6), $MgSO_4.7H_2O$ (140), $FeSO_4.7H_2O$ (1.4), $Na_2MoO_4.2H_2O$ (0.61), $CuSO_4.5H_2O$

(2.25), $MnSO_4.4H_2O$ (3.68), $ZnSO_4.7H_2O$ (6.6), H_3BO_3 (0.28). Phosphorus (P) (in the form of KH_2PO_4) was added at the level of 10 or 30 mg P kg^{-1} soil. Nutrients were well mixed into the soil. The prepared soil was then filled into a rhizobox.

4.2.3.3 Sowing, harvest, imaging and root architectural analysis

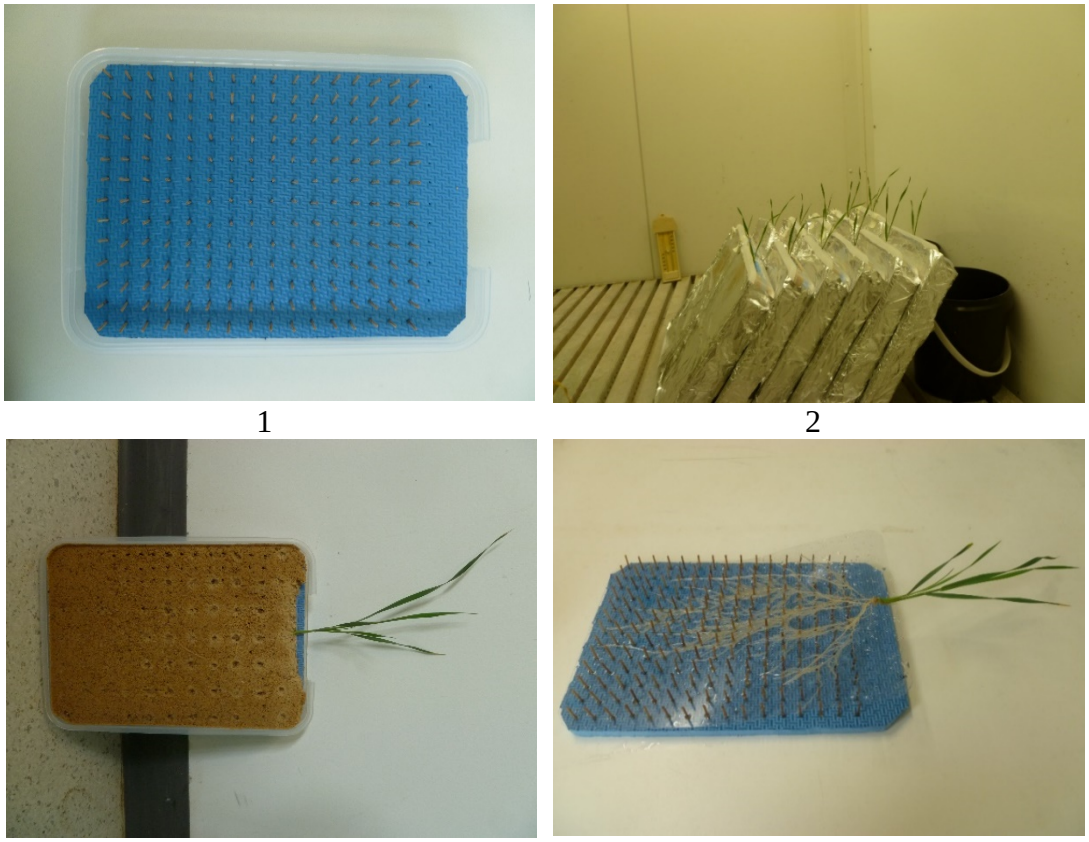
Wheat grains were sterilized in 2% hypochlorite for 10 min and were then rinsed with Milli-Q water. The grains were germinated on moistened, filter paper lined petri dishes that were kept in the dark at room temperature for two days. One germinated grain was sown into each rhizobox at two P levels; 10 and 30 mg P kg^{-1} soil. The experiment was carried out in triplicate in the growth room with the light intensity of 500 µmol m^{-2} s^{-1} at the leaf level. The sort of light was the combination of fluorescent and incandescent. The growth room temperature was 20 ^oC/10 ^oC (day/night) and the length of daylight was 13 h. Rhizoboxes were diagonally placed at a 60 ^{o} angle and plants were regularly watered three times a week to 8% of the soil weight.

Plants were harvested at 24 days after sowing (DAS). Shoots were detached from roots at the crown level. The foam holding the grid of toothpicks with the root systems in place were removed from the rhizobox and the soil was gently washed out in a tank of water. The root system on the foam was then washed under a gentle shower of water and the transparent sheet with the root system was removed from the foam. The root systems were dried on tissue paper and then scanned (8-bit grey scale, 400 dpi) on an Epson Perfection V700 Photo for RSA analysis. RSA traits were measured using two

software tools, GiA Roots (Galkovskyi et al., 2012) and DIRT (Bucksch et al., 2014). GiA Roots was used to measure convex hull area (the smallest area that encloses the

whole root system), root surface area, total root length, root volume, root diameter and DIRT was used to measure root tip number, seminal root diameter, root density, root angle, accumulated width over the depth at x% (D) and slope of the graph of D values (DS) used DIRT.

Shoot dry matter was measured after three days of freeze drying and root dry matter was measured after 48 h drying at 85 ºC.



1 2 3 4

Figure 4.1. Growth and harvest for root system architecture analysis using rhizoboxes. Plants were harvested at 24 DAS.

4.2.4 Experiment 3. Root hair characterization

Wheat grains were germinated as described in Experiment 2. Germinated grains were

grown in transparent plastic rootboxes filled with 600 g of sandy soil at two P levels of 10 and 30 mg P kg^{-1} soil with basal nutrients as described in Experiment 1. The experiment was carried out in four replicates in the growth room with the light intensity of 650 µmol m^{-2} s^{-1} at the leaf level. The sort of light was the combination of fluorescent and incandescent. The growth room temperature was 20 ^{o}C/10 ^{o}C (day/night) and the length of daylight was 13 h). Rootboxes were diagonally placed at a 45^{o} angle and the soil were regularly moistened by watering three times a week to 10% of the soil weight. The rootbox size was 24-cm length x 24-cm width x 2.5-cm depth. Root hair length and root hair density on the seminal roots were measured at 5, 7 and 10 DAS, using a microscope (LEICA MZ16, x12.5 and x20 magnifications) with a camera attachment (LEICA DFC280) and LAS v3.6 software. Images of roots and root hairs were observed through transparent plastic rootboxes and captured within 2-4 cm from the root tip. After that, LAS v3.5 software was used for gaining measurements. Eight measurements from each replicate were taken for root hair length measurement. The half-mean distance (r_s) between the root hair was measured by counting the number of root hairs per 0.5 mm of root length and four measurements were taken for each replicate. The root hair density (RHD) was calculated as $RHD=(\pi r_s^2)^{-1}$ (Vandamme et al., 2013).

4.2.5 Measurements

The P concentration in shoot, grain and straw was determined by ICP-MS (Agilent Technologies, Model 7500cx) at Flinders University using the digestion method (proposed closed-tube method) of Wheal et al. (2011). P efficiency of wheat genotypes was evaluated by four criteria: shoot biomass and grain yield at low P; ratio of growth

at low P to growth at adequate P; P acquisition efficiency (PAE, %), measured as the amount of shoot P uptake divided by the amount of P supplied in the soil per pot; and P utilisation efficiency (PUtE), $PUtE_{SM}$ (shoot matter) and $PUtE_{GY}$ (grain yield), calculated as shoot dry matter divided by shoot P uptake and calculated as grain yield divided by shoot P uptake, respectively (Osborne and Rengel, 2002a).

4.2.6 Statistical analysis

Statistical analyses were conducted by using IBM SPSS v23. The normality of data was tested using Kolmogorov-Smirnov and Shapiro-Wilk test ($P<0.05$). P uptake and root to shoot ratio were not normal distributed and were transformed using $\log_{10}$. Plant indices and root architectural traits were analysed by two-way ANOVA (Genotype x P supply). Root hair features were analysed by three-way ANOVA (Genotype x Growth stage x P supply). Mean comparisons between genotypes at each P treatment were performed by independent t-test ($P<0.05$). The particular sets of variables was subjected to Pearson's correlation analysis (Field, 2013).

4.3 Results

4.3.1 Experiment 1. Responses to P of two wheat genotypes at different growth stages in a pot experiment

Results in Table S 4.1 showed that shoot DM at 27 DAS slightly increased with the addition of P but significant ($P<0.001$) responses to P occurred at 48 DAS and at maturity (Table S 4.1). RAC875 produced significantly ($P<0.05$) greater shoot DM at 27 DAS, 48 DAS and maturity than Wyalkatchem (Table S 4.1). No significant G x P

interactions for these parameters were observed. Under low P, RAC875 had significantly ($P<0.05$) higher shoot DM at all growth stages and its grain yield was also higher than Wyalkatchem, while no significant variations in these parameters, between the two genotypes were found under adequate P supply (Figure 4.2, Figure 4.3). PUE, calculated by the ratio of shoot DM (or grain yield) under low P to that under adequate P, in RAC875 were about 11%, 3% and 15% higher for shoot DM at 27, 48 DAS and at maturity respectively, and 17% greater for grain yield than those in Wyalkatchem (Table S 4.3).

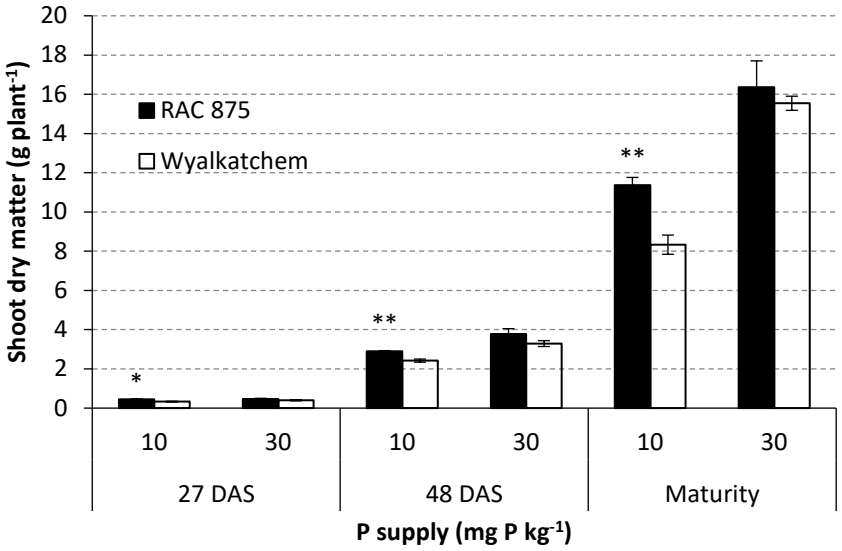


Figure 4.2. Shoot dry matter at different growth stages of two wheat genotypes RAC875 and Wyalkatchem grown under growth room conditions in a pot experiment. Data were the means of four replicates with standard errors. *, ** showed significantly different between genotypes within the same P supply at each growth stage at $P<0.05$, $P<0.01$, respectively.

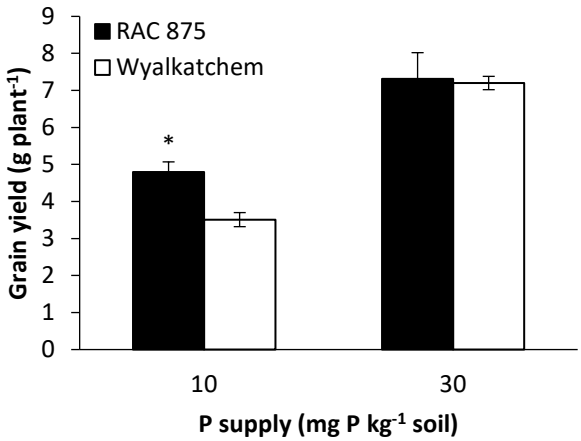


Figure 4.3. Grain yield of two wheat genotypes RAC875 and Wyalkatchem grown under growth room conditions in a pot experiment. Data were the means of four replicates with standard errors. * showed significantly different between genotypes at each P supply at $P<0.05$.

Similar to the results at 24 DAS from Experiment 1, at maturity RAC875 had significantly ($P<0.01$) smaller root to shoot ratio than Wyalkatchem (Table 4.1). At maturity, P uptake and PAE in RAC875 were significantly ($P<0.01$) higher than those in Wyalkatchem (Table S 4.2), while no significant differences were observed in $PUtE_{GY}$ and $PUtE_{SM}$. RAC875 had higher root efficiency than Wyalkatchem and under low P, root efficiency in RAC875 almost doubled that in Wyalkatchem (Table 4.1).

Table 4.1. Variation in root to shoot ratio, P uptake and root efficiency at maturity of two wheat genotypes grown under the growth room conditions in a pot experiment

P supply (mg P kg^{-1} soil)	Genotype	Root to shoot ratio (g g^{-1})	P uptake (mg $plant^{-1}$)	Root efficiency (mg shoot P uptake per g root DM)
10	RAC875	0.15^{a**}	6.8^{a**}	4.0^{a**}
	Wyalkatchem	0.32^{b}	5.2^{b}	2.1^{b}
30	RAC875	0.19^{a**}	21.5	7.7^{a*}
	Wyalkatchem	0.27^{b}	21.0	5.0^{b}
F Ratio				
Genotype (G)		29.919^{**}	10.324^{*}	24.537^{***}
P treatment (P)		0.031 ns	815.297^{***}	49.666^{***}
G × P		2.723 ns	2.242 ns	0.692 ns

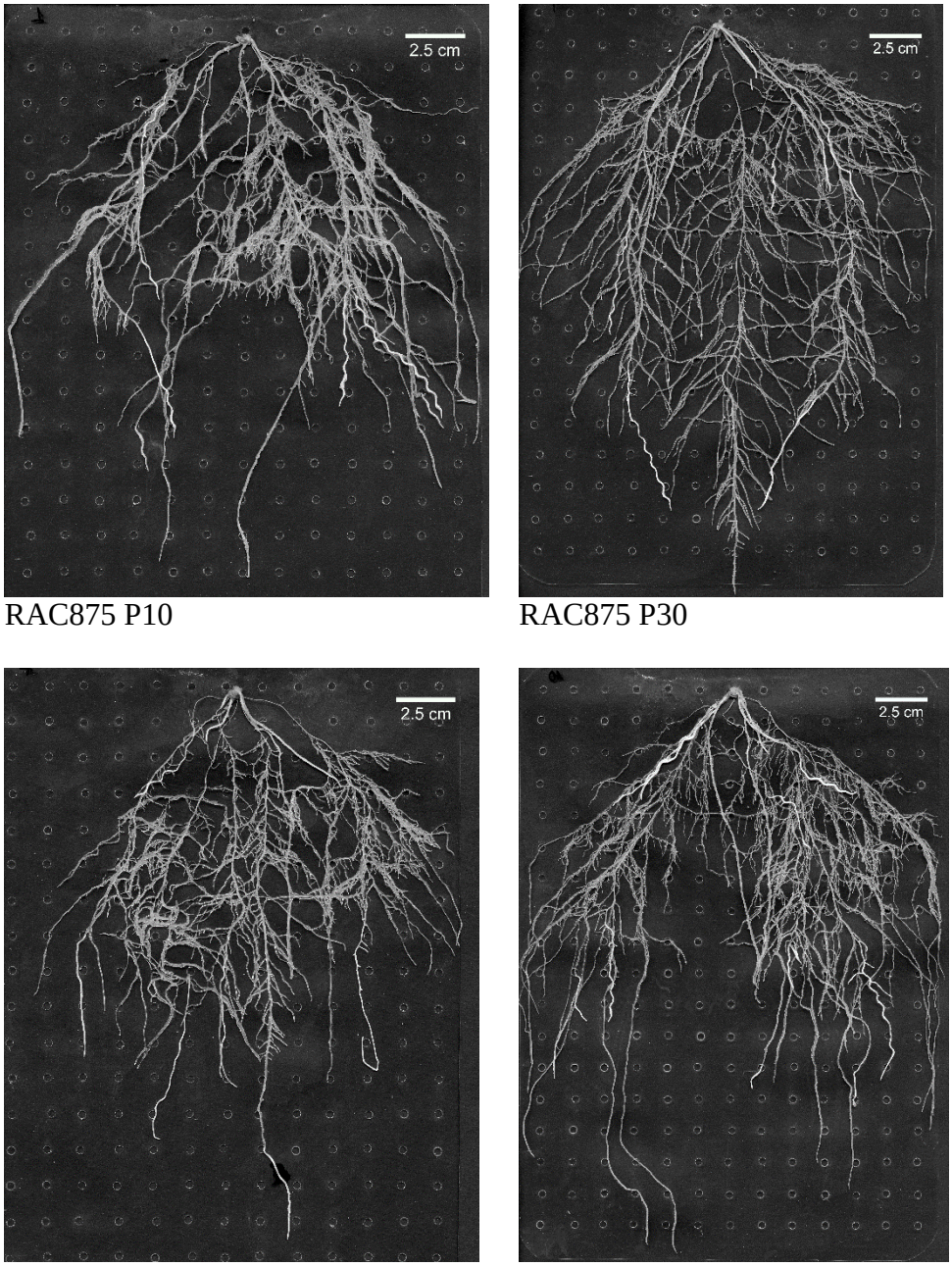
Data represent the means of four replicates. Analysis of variance performed on original data except for P uptake transformed using log10. *F* ratios are from two-way ANOVA analysis. *, ** and *** significant at P<0.05, P<0.01 and P<0.001, respectively; ns: not significant.

Different letters showed significant differences between genotypes at each P supply, *, ** following letters: significant at P<0.05, P<0.01, respectively.

4.3.2 Experiment 2. Root system architectural characterization

4.3.2.1 Root images

In the experiment for RSA analysis, sandy soil was gently, and easily washed out and the root system was held in place by the grid of toothpicks on the foam base (Figure 4.1). It was difficult to analyze the root system if it was captured altogether with the grid of toothpicks on the foam base. Therefore, the transparent sheet was inserted through the grid of toothpicks onto the foam base in order to obtain the root system only. Indeed, when the transparent sheet was removed from the foam base, the root system held well on to it. The root system was imaged with black background using a scanner (Figure 4.4).



RAC875 P10 RAC875 P30

Wyalkatchem P10 Wyalkatchem P30

Figure 4.4. Root structure of RAC875 and Wyalkatchem grown in sandy soil with designed rhizoboxes at two P treatments (P10 and P30: 10 and 30 mg P kg^{-1} soil). Plants were harvested at 24 DAS. Photos were taken by a scanner and one representative photo of each genotype was selected.

4.3.2.2 Responses to P of two wheat genotypes at 24 DAS

Shoot dry matter (DM) at 24 DAS significantly responded to P supply. Shoot DM at adequate P (30 mg P kg^{-1}) was about 74% greater than that at low P (10 mg P kg^{-1} soil) ($P<0.01$) (Figure 4.5 A, Table S 4.4). Under low P, shoot DM in RAC875 was 56% higher than that in Wyalkatchem ($P=0.07$), while shoot DM was not different between these two wheat genotypes at adequate P (Figure 4.5 A). Therefore, RAC875 showed 55% PUE than Wyalkatchem (PUE refers to the ratio of shoot DM at low P to shoot DM at adequate P).

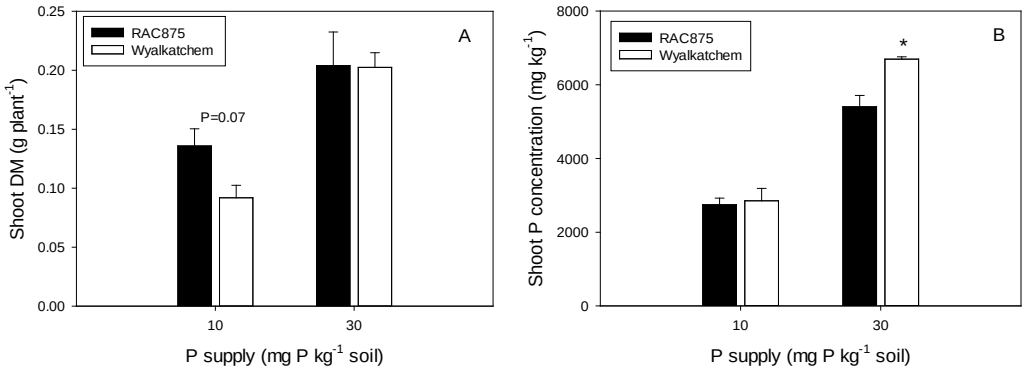


Figure 4.5. Shoot DM (A) and shoot P concentration (B) of two wheat genotypes RAC875 and Wyalkatchem grown in rhizoboxes at 24 DAS. Data were the means of three replicates with standard errors. *, ** significant within the same P supply at $P<0.05$, $P<0.01$, respectively.

No significant response to P supply was observed in root DM and no significant difference was found between the two wheat genotypes. The root to shoot ratio was significantly greater under low P when compared to adequate P ($P<0.01$) and the ratio was considerably lower in RAC875 when compared to Wyalkatchem ($P<0.05$) (Table S 4.4). No significant genotype (G) x phosphorus supply (P) occurred in the root to

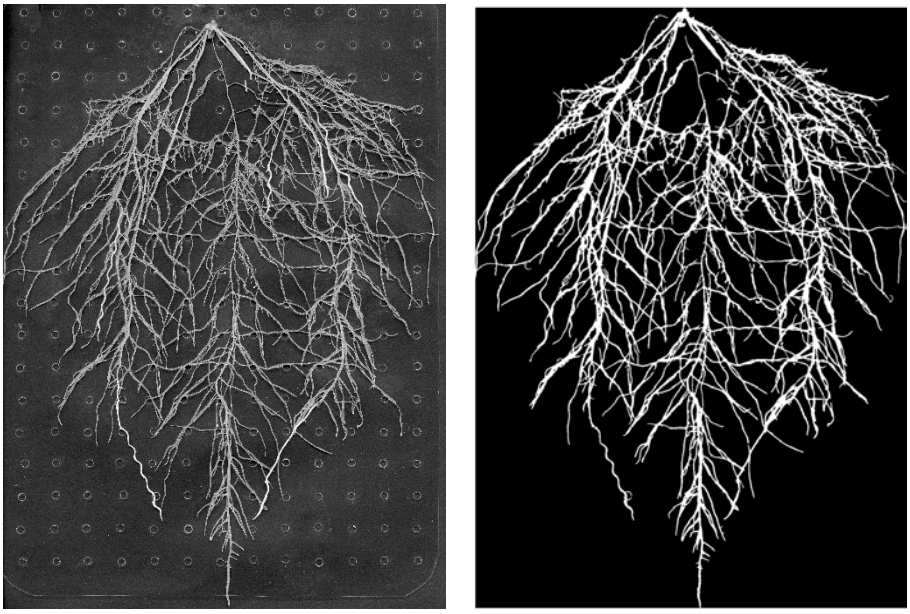
shoot ratio (Table S 4.4).

A significant G x P interaction in shoot P concentration was observed ($P<0.05$) (Figure 4.5 B, Table S 4.4) and this would indicate that these wheat genotypes behaved differently at different P levels. Indeed, shoot P concentration in Wyalkatchem was slightly higher than that in RAC875 at low P, while Wyalkatchem showed significantly ($P<0.05$) higher shoot P concentration than RAC875 at adequate P (Figure 4.5 B). In general, low P strongly reduced P concentration ($P<0.001$).

PUtE decreased by increasing P level ($P<0.001$) and RAC875 had significantly greater PUtE than Wyalkatchem (Table S 4.4). No significant differences in P uptake, PAE and root efficiency were observed between the two wheat genotypes, but RAC875 showed relatively higher in these parameters than Wyalkatchem under low P.

4.3.2.3 RSA at 24 DAS

Global RSA traits were measured using GiA Roots (Galkovskyi et al., 2012) and other RSA traits were measured using the DIRT software program (Bucksch et al., 2014) (Figure 4.6). The results showed that P supply did not affect root angle, average root diameter, seminal root diameter, spatial root density (*X*) ($RDISTR_x$) and average density (Table 4.2). However, under low P supply, a significant reduction in convex hull area, total root length, root surface area, root volume, root tip number, medium root with, and the absolute value of spatial root distribution (*Y*) ($RDISTR_y$) was observed (Table 4.2).



A B

Figure 4.6. Original image (A) and the image processed by DIRT (B).

Under low P supply, RAC875 showed a moderate decrease in convex hull area (11.2%), total root length (14.5%), root surface area (16%), root volume (17.2%), root tip number (22.3%), medium root width (14.8%), and showed no decline in the absolute value of spatial root distribution (*Y*). In contrast, Wyalkatchem had a marked reduction in convex hull area (43.5%), total root length (47%), root surface area (47.2%), root volume (48.5%), root tip number (41.4%), medium root with (44.8%), and showed no decrease in the absolute value of spatial root distribution (*Y*) (21.3%) (Table 4.2, Figure 4.7). There were no significant variations in RSA traits between the two genotypes, however the mean ratios between low P and adequate P for total root length, root surface area, root volume, root tip number and medium root width were 32.5%, 31.2%, 31.3%, 30% and 19% respectively, greater in RAC875 than in Wyalkatchem (Table S 4.6).

Table 4.2. Total root length, root surface area**,** root volume, root tip number, root top angle, medium root width, seminal root diameter and average root diameter of two wheat genotypes grown under growth room conditions at 24 DAS at different P treatments

P supply (mg P kg^{-1} soil)	Genotype	Total root length (m $plant^{-1}$)	Root surface area (cm^2 $plant^{-1}$)	Root volume (cm^{-3} $plant^{-1}$)	Root tip number (tip $plant^{-1}$)	Root top angle	Medium root width (cm)	Seminal root diameter (mm)	Average root diameter (mm)
10	RAC875	18.8	171.6	1.64	490	67	9.8	0.67^{a*}	0.29
	Wyalkatchem	15.1	140.1	1.34	445	67	9.0	0.57^{b}	0.29
30	RAC875	22.0	204.4	1.98	631	68	11.5	0.60	0.30
	Wyalkatchem	28.5	265.3	2.60	759	67	16.3	0.61	0.30
F Ratio									
Genotype (G)		0.209 ns	0.297 ns	0.409 ns	0.319 ns	0.072	1.195 ns	6.360^{*}	0.182 ns
P treatment (P)		7.430^{*}	8.577^{*}	9.967^{*}	9.401^{*}	0.632	6.156^{*}	0.676 ns	2.212 ns
G × P		2.765 ns	2.942 ns	3.320 ns	1.353 ns	0.045	2.431 ns	8.018^{*}	0.393 ns

Results represent the means of three replicates. *F* ratio are from two-way ANOVA analysis. *, *** significant at P<0.05 and P<0.001, respectively; ns: not significant.

Different letters showed significant differences between genotypes at each P supply; *, ** following letters: significant at P<0.05, P<0.01, respectively.

Table 4.2. (continue) Spatial root distribution of two wheat genotypes and average density of two wheat genotypes grown under growth room conditions at 24 DAS at different P treatments

P supply (mg P kg^{-1} soil)	Genotype	Spatial root distribution[1] (X component)	Spatial root distribution (Y component)	Average root[2] density
10	RAC875	-0.4	-19.0	0.42
	Wyalkatchem	-0.3	-15.9	0.53
30	RAC875	-0.4	-18.7[a*]	0.51
	Wyalkatchem	-1.0	-20.2[b]	0.34
F Ratio				
Genotype (G)		0.374 ns	1.008 ns	0.051 ns
P treatment (P)		0.699 ns	6.240[*]	0.042 ns
G × P		0.619 ns	8.122[*]	6.448[*]

Data represent the means of three replicates. *F* ratios are from two-way ANOVA analysis. * significant at P<0.05; ns: not significant.

[1]Spatial root distribution: displacement of the center of mass between the bounding box of the RTP skeleton and the RTP skeleton excluding the central path (Bucksch et al., 2014).

[2]Ratio between foreground and background pixels of the extracted root (Bucksch et al., 2014).

Different letters showed significant differences between genotypes at each P supply; * following letters: significant at P<0.05.

Significant G x P interactions for convex hull area and seminal root diameter were observed. Under low P supply, convex hull area in RAC875 was 16.1% greater than that in Wyalkatchem ($P<0.05$), while 19.4% lower under adequate P ($P<0.01$) (Figure 4.7). Also, under low P supply, RAC875 had considerably greater seminal root diameter than Wyalkatchem ($P<0.05$), whereas no significant variation in this parameter was observed between the two genotypes under adequate P supply (Table 4.2).

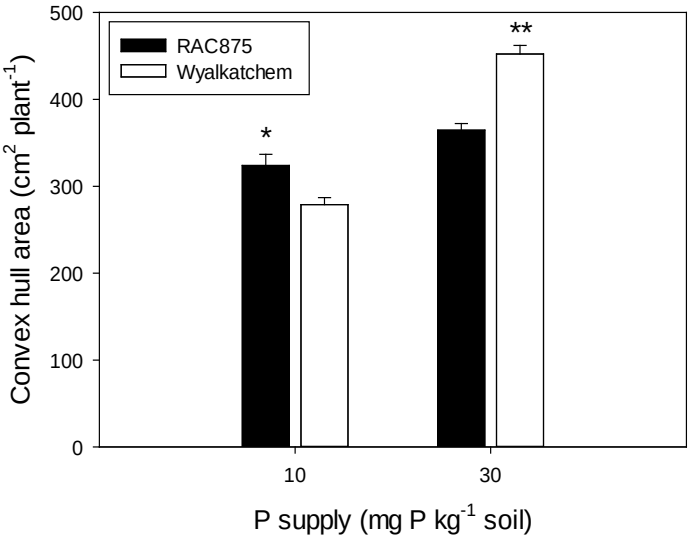


Figure 4.7. Convex hull area of two wheat genotypes RAC875 and Wyalkatchem grown in rhizoboxes at 24 DAS. Data were the means of three replicates with standard errors. *, ** significant within the same P supply at $P<0.05$, $P<0.01$, respectively.

Under low P supply, no genotypic variations in specific accumulated width (D) over depth and specific slope of the graph of D values (DS) were observed (Figure S 4.1 A, Figure S 4.2 A), except for D90 where RAC875 showed greater ($P<0.05$) value (D90=0.8) than Wyalkatchem (D90=0.7). Wyalkatchem showed higher D values but

lower DS values than RAC875 under adequate P (Figure S 4.1 B, Figure S 4.2 B).

Under low P supply, a significant reduction occurred in shoot P uptake per unit of root trait (including convex hull area, root surface area, root volume, total root length and root tip number), however significant differences in these parameters were not found between genotypes. Under low P, shoot P uptake per unit of average root density in RAC875 was significantly higher than that in Wyalkatchem ($P<0.05$) but no variation in this parameter under adequate P was observed between the two wheat genotypes (Table S 4.5). Root surface area was positively correlated with root volume, total root length and root tip number under both levels of P supply (Table 4.3). Root volume was positively correlated with total root length under both P treatments. A positive correlation for root volume and root tip number was also observed under adequate P but not under low P supply (Table 4.3).

The results in Table 4.3 also showed the relationship between shoot DM and shoot P uptake with RSA traits. Under low P supply, shoot DM was positively correlated with convex hull area ($r=0.875^{*}$), root surface area ($r=0.897^{*}$), total root length ($r=0.898^{*}$) and seminal root diameter ($r=0.844^{*}$) but these significant correlations were not evident under adequate P supply. Under low P, shoot DM was not significantly correlated with root tip number, while a strong correlation between shoot DM and root tip number was found under adequate P ($r=0.826^{*}$). Shoot DM was positively correlated with P uptake at both P treatments ($r=0.937^{**}$, $r=0.871^{*}$ for low and adequate P, respectively). Shoot P uptake was positively associated with root surface area, root volume and total root length under both P treatments. Shoot P uptake was also positively correlated with root tip number under adequate P but not under low P.

Table 4.3. Pearson correlation coefficients between shoot DM, shoot P uptake, convex hull area, root surface area, root volume, total root length, seminal root diameter, root tip number, root top angle and average density of two wheat genotypes (24 DAS) at two P treatments: 10 mg P kg^{-1} soil (above on the right of the table) and 30 mg P kg^{-1} soil (below on the left of the table). *, ** significant at P<0.05, P<0.01, respectively.

	Shoot DM (g $plant^{-1}$)	Shoot P uptake (mg $shoot^{-1}$)	Convex hull area (cm^2 $plant^{-1}$)	Root surface area (cm^2 $plant^{-1}$)	Root volume (cm^{-3} $plant^{-1}$)	Total root length (m $plant^{-1}$)	Seminal root diameter (mm)	Root tip number (tip $plant^{-1}$)	Root top angle	Average density
Shoot DM (g $plant^{-1}$)	1	0.937**	0.875*	0.897*	0.909*	0.898*	0.844*	0.604	0.301	-0.565
Shoot P uptake (mg $shoot^{-1}$)	0.871*	1	0.797	0.966**	0.981**	0.956**	0.626	0.710	0.313	-0.283
Convex hull area (cm^2 $plant^{-1}$)	0.153	0.592	1	0.787	0.803	0.779	0.720	0.418	0.626	-0.448
Root surface area (cm^2 $plant^{-1}$)	0.741	0.892*	0.667	1	0.997**	0.999**	0.634	0.840*	0.292	-0.149
Root volume (cm^{-3} $plant^{-1}$)	0.704	0.875*	0.697	0.998**	1	0.992**	0.621	0.804	0.324	-0.171
Total root length (m $plant^{-1}$)	0.766	0.901*	0.646	0.999**	0.994**	1	0.659	0.855*	0.261	-0.162
Seminal root diameter (mm)	0.261	0.251	0.329	0.314	0.331	0.311	1	0.479	0.121	-0.751
Root tip number (tip $plant^{-1}$)	0.826*	0.937**	0.650	0.925**	0.917**	0.933**	0.532	1	0.050	-0.165
Root top angle	0.740	0.561	0.111	0.444	0.419	0.471	0.748	0.710	1	0.010
Average density	0.023	-0.283	-0.527	-0.065	-0.060	-0.073	0.223	-0.164	-0.056	1

4.3.3 Experiment 3. Root hair length and density

For estimation of root hairs, a preliminary trial showed that root hairs of wheat were sparse and delicate and they were mostly lost or were aggregated when they were harvested from a pot experiment. Thus, growing wheat in transparent rootboxes for root hair measurements is a potential approach. The results illustrated that root hairs were well observed and captured (Figure 4.8) through the transparent rootboxes under a microscope attached with a camera.

Growth stage and genotype affected root hair length (RHL) and root hair density (RHD) (Table 4.4). RHL and RHD significantly increased from 5 DAS to 7 DAS ($P<0.001$) and remained stable until 10 DAS (Table 4.4, Figure 4.9 A & B). Wyalkatchem produced significantly ($P<0.001$) greater RHL than RAC875, while RAC875 had significantly ($P<0.001$) higher RHD than Wyalkatchem. No significant genotype (G) x growth state (GS) interactions for RLH and RHD were observed (Table 4.4).

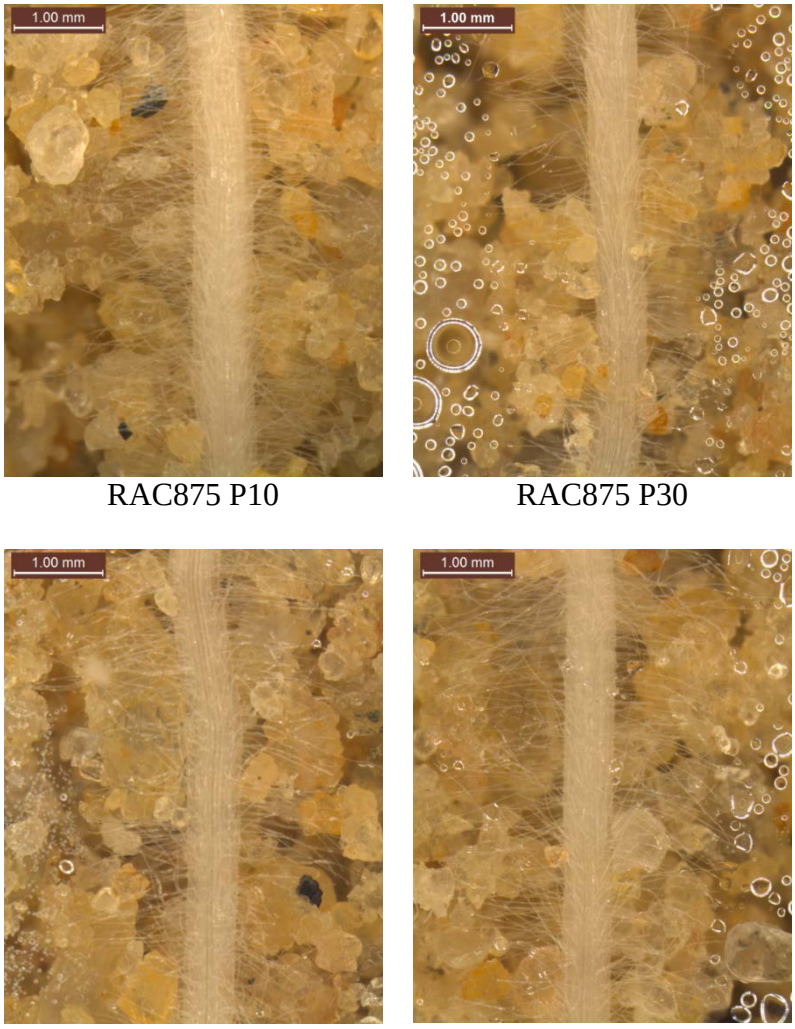


Figure 4.8. Root hairs of RAC875 and Wyalkatchem grown in sandy soil using plastic transparent rootboxes at two P treatments (P10 and P30: 10 and 30 mg P kg^{-1} soil). Root hair images were taken on seminal roots within 2-4 cm from the root tips at 7 DAS. Photos were taken under microscope (x20 magnification) attached with a camera and one representative photo of each genotype was selected.

Table 4.4. The effect of genotype, P supply and growth stage on root hair length (RHL) and root hair density (RHD) of two wheat genotype RAC875 and Wyalkatchem. Plants were grown at two P treatments (10 mg P kg^{-1} soil) and root features were measured at three growth stages (5, 7 and 10 days after sowing).

Effect	Root hair length (mm)		Root hair density (root hair mm^{-2})	
	df	*F* ratio	df	*F* ratio
Genotype (G)	1	15.073***	1	8.925**
P supply (P)	1	0.445 ns	1	9.937**
Growth stage (GS)	2	38.611***	2	4.360*
G × P	1	0.058 ns	1	11.009**
G × GS	2	1.457 ns	2	0.010 ns
P × GS	2	1.765 ns	2	0.241 ns
G × P × GS	2	2.174 ns	2	0.224 ns
Error	33		33	
Total	45		45	

Analysis of variance performed on original data of RHL and lg_{10} of RHD. *F* ratios are from three-way ANOVA analysis. *, ** and *** significant at P<0.05, P<0.01 and P<0.001, respectively; ns: not significant.

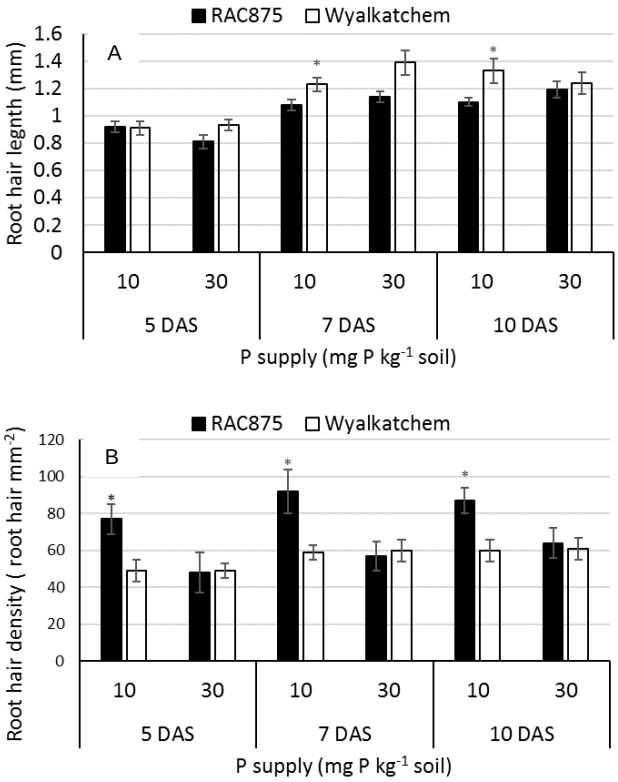


Figure 4.9. Variation in root hair length (A) and density (B) of two wheat genotypes RAC875 and Wyalkatchem under different P supply at different growth stages. Results were the means of four replicates with standard errors. * showed significantly different between genotypes within the same P supply at each growth stage at P<0.05.

P supply did not affect RHL but had an effect on RHD. Wheat produced significantly ($P<0.001$) greater RHD under low P (Table 4.4, Figure 4.9 B). A significant interaction between genotype (G) x P supply (P) was observed for RHD, indicating that the two wheat genotypes behave differently at each P treatment (Table 4.4). Indeed, under low P supply, RAC875 produced significantly ($P<0.001$) more dense root hairs when compared with the adequate P treatment (Figure 4.9 B), whereas there was no significant variation in RHD in Wyalkatchem under different P treatments.

4.4 Discussion

4.4.1 Responses to P of two wheat genotypes

The two genotypes of wheat chosen for this study were previously characterized for their differences in PUE (Chapter 2). In this study, root traits were measured after growing plants in rhizoboxes. In this plant growth system, P supply significantly affected shoot DM at 24 DAS (Figure 4.5 A, Table S 4.4), while in the pot experiment a slight increase in shoot DM at 27 DAS occurred with increasing P level and significant responses to P were observed at 48 DAS and maturity (Figure 4.2, Table S 4.1). Under low P supply, RAC875 produced significantly greater shoot DM at all growth stages as well as higher grain yield, when compared to Wyalkatchem (Figure 4.2, Figure 4.3). Greater shoot DM under low P was evident in both the pot experiment and rhizobox experiment (Figure 4.5 A). High yield production in RAC875 grown at low P supply, was also observed in field experiments (McDonald et al., 2015). The consistency occurring between various growth conditions indicates that rhizoboxes can be used for characterizing RSA traits. Also, the results indicate that RAC875 had

greater PUE than Wyalkatchem, therefore, these two wheat genotypes are useful for elucidating mechanisms of PUE.

Wyalkatchem had a significantly higher shoot P concentration when compared to RAC875, although it produced less biomass at 24 DAS (Table S 4.4). This would indicate that Wyalkatchem requires more P for their normal growth in comparison to RAC875. Furthermore, RAC875 appears to utilize P more efficiently than Wyalkatchem. Indeed, PUtE in RAC875 was significantly higher ($P<0.05$) than that in Wyalkatchem (Table S 4.4). However, $PUtE_{SM}$ and $PUtE_{GY}$ were not significantly different between the two wheat genotypes at maturity. At maturity and under low P supply, P uptake in RAC875 was 30.7% greater than that in Wyalkatchem (Table 4.1) and this value in RAC875 was relatively higher than that in Wyalkatchem at 24 DAS (Table S 4.4).

In contrast to a previous study in coffee plants, where high root to shoot ratio was positively associated with efficiency in P uptake (Neto et al., 2016), in this study, root to shoot ratio in RAC875 was significantly smaller than that in Wyalkatchem at 24 DAS (Table S 4.4) and at maturity (Table S 4.1). RAC875 also has a smaller root biomass than Wyalkatchem at maturity (Table S 4.1). In agreement with these findings, recently, da Silva et al. (2016) also reported that P efficient wheat genotypes possessed smaller root systems than P inefficient genotypes. It appears that plants with smaller root systems require less energy for root growth, therefore they can sustain biomass under limited P nutrition. However, a question arises is how the smaller root system can maintain shoot biomass under P deficiency, suggesting that RAC875 may have specific efficiency mechanisms for P acquisition.

4.4.2 RSA characteristics and PUE

At 24 DAS, there was no significant variation in root DM under different P treatments, yet at this growth stage, a decrease in P supply led to a significant reduction in convex hull area, total root length, root surface area, root volume, root tip number, medium root width, and the absolute value of spatial root distribution (*Y*) (Table 4.2, Figure 4.7). These results would indicate that these root architectural traits respond to P supply earlier than root DM. Under low P, a decrease in total root length, root surface area and root tip number was observed in barley (Wang et al., 2015), however enhanced root length under low P was observed in wheat grown in a hydroponic experiment (Horst et al., 1993), and in barley grown in a field experiment (Steingrobe et al., 2001). This variation in results could be due to differences in harvest stages and in methodologies (i.e. this study used a soil based cultivation in rhizoboxes and the plants were harvested at 24 DAS, while Horst et al examined root traits at 14 DAS in a hydroponic experiment; Steingrobe et al harvested only the 0-30 layer in the field but not the whole root system).

Although previous studies showed that topsoil foraging is advantageous for phosphorus acquisition in common bean and maize under low P (Zhu et al., 2005b; Lynch and Brown, 2001), root top angle did not vary between the two wheat genotypes (Table 4.2). Thus, RAC875 could have different mechanisms of PUE. Indeed, under low P, RAC875 had significantly larger convex hull area (the smallest area that encloses the whole root system) than Wyalkatchem, while this parameter in RAC875 was smaller than that in Wyalkatchem under adequate P (Figure 4.7). Therefore, the mechanism of PUE seems to be interesting in this case. Under low P, RAC875 had an

expanded, larger root area to acquire P more efficiently but did not need to stretch out the root under adequate P (Figure 4.4). In contrast, the root system of Wyalkatchem seems to grow well under adequate P, while their root expansion appeared to shrink under low P. Furthermore, the convex hull area was positively correlated with shoot DM under low P (Table 4.3), thus convex hull area appears to be a potential indicator for screening P efficient wheat under low P.

Under low P, shoot DM was positively correlated with root surface area, total root length and root volume at 24 DAS. Shoot P uptake also had a strong relationship with root surface area, total root length and root volume (Table 4.3). These results were consistent with those reported in soybean (Liang et al., 2010), which suggests that root surface area, total root length and root volume would be advantageous characteristics of P efficient crops.

4.4.3 Root hair development and PUE

Root hairs are important traits for improvement of PUE (Haling et al., 2013; Gahoonia et al., 1997; Gahoonia and Nielsen, 2003). Although most studies demonstrate that root hairs become longer under low P; such as in *Arabidopsis thaliana* (Bates and Lynch, 2000a), maize (Zhu et al., 2010) and rice (Vejchasarn et al., 2016), this study showed that P supply did not affect RHL (Table 4.4). This difference might be attributed to genetic differences between plants. A recent study even indicated that an increase in P supply resulted in improved RHL in wheat (Yuan et al., 2016). Obviously, variation in observed results between research groups would indicate that a large number of genotypes should be used for evaluating if plants adapt to low P by promoting RHL.

In the study presented here, RAC875 is a P efficient genotype yet produces shorter root hairs than the P inefficient genotype, Wyalkatchem (Table 4.4, Figure 4.9 A). In contrast, Gahoonia and Nielsen (2004a) reported that barley genotypes with long root hairs improved grain yield in comparison with short root hair genotypes. However, Brown et al. (2012) pointed out that RHL is not important for grain yield but for shoot P accumulation. This seems to agree with our research in which Wyalkatchem had longer root hairs and significantly greater shoot P concentration than RAC875. Recently, quantitative trait loci (QTLs) for RHL have been identified in wheat and they co-locate with loci for yield components (Horn et al., 2016). Thus, a population study should be investigated to identify if RHL is important for grain yield under low P. Growth conditions need to be considered since they can affect results. For example, genotypic variation in rhizosheath size (that correlates with root hair length) in wheat obviously occurred only when low soil pH and Al^{3+} was present (James et al., 2016).

Contrary to RHL, in this study, RHD increased under low P (Table 4.4, Figure 4.9 B) and this result agrees with a number of studies (Bates and Lynch, 2000a; Ma et al., 2001; Hill et al., 2010; Hu et al., 2010). However, two wheat genotypes behave differently at each P treatment. Under low P, RHD increased in RAC875 but not in Wyalkatchem. Also, RAC875 produced significantly greater RHD than Wyalkatchem under low P but not under adequate P, indicating that more dense root hairs could contribute to greater shoot DM and yield in RAC875 under low P.

4.4.4 Which root features could be associated with PUE in RAC875?

A previous study (Chapter 2) showed that root efficiency (mg P uptake per g root dry matter) was positively correlated with grain yield under low P. In this study, RAC875

was shown to have greater root efficiency than Wyalkatchem and produce higher yield under low P than Wyalkatchem. Thus, root efficiency is important for PUE. Under low P supply, convex hull area in RAC875 was larger than that in Wyalkatchem (Figure 4.7). Also, under low P, RAC875 had greater root hair density than Wyalkatchem (Figure 4.9 B). Keyes et al. (2013) reports that roots and root hairs equally contribute for P uptake, in which root hairs are more important for localized P acquisition. Therefore, it appears that the enlargement of a root system under low P and the development of more root hairs under low P, ensures that the P efficient genotype with a small root system (in terms of root dry matter) can support higher yield production.

Conclusions

In summary, larger convex hull area and denser root hair length could lead to great production in shoot DM and grain yield in RAC875 under low P. These root characteristics would ensure that a small root system (small root DM at maturity) as in RAC875 can support great biomass and yield production. Thus, it seems that small but efficient root systems would be a beneficial indicator for screening P efficient crops.

Supplemental tables and figures

Table S 4.1. Shoot DM at 27 DAS, 48 DAS and maturity, and grain yield and root DM at maturity of two wheat genotypes grown under growth room conditions in a pot experiment at different P treatments

P supply (mg P kg^{-1} soil)	Genotype	Shoot DM at 27 DAS (g $plant^{-1}$)	Shoot DM at 48 DAS (g $plant^{-1}$)	Shoot DM at maturity (g $plant^{-1}$)	Grain yield (g $plant^{-1}$)	Root DM at maturity (g $plant^{-1}$)
10	RAC875	0.44^{a*}	2.9^{a**}	11.4^{a**}	4.8^{a**}	1.7^{a*}
	Wyalkatchem	0.33^{b}	2.4^{b}	8.3^{b}	3.5^{b}	2.6^{b}
30	RAC875	0.47	3.8	16.4	7.3	3.0^{a**}
	Wyalkatchem	0.40	3.3	15.5	7.2	4.2^{b}
F Ratio						
Genotype (G)		11.034^{**}	9.136^{*}	7.971^{*}	3.054 ns	26.262^{***}
P treatment (P)		2.762 ns	30.733^{**}	79.443^{***}	59.203^{***}	52.558^{***}
G × P		0.581 ns	0.085 ns	2.613 ns	0.171 ns	0.760 ns

Data represent the means of four replicates. *F* ratios are from two-way ANOVA analysis. *, ** and *** significant at P<0.05, P<0.01 and P<0.001, respectively; ns: not significant.

Different letters showed significant differences between genotypes at each P supply; *, ** following letters: significant at P<0.05, P<0.01, respectively. Statistical analysis was performed by independent Student's t-test.

Table S 4.2. Shoot P concentration, shoot P uptake, PAE, $PUtE_{GY}$, $PUtE_{SM}$ and root efficiency at maturity of two wheat genotypes grown under growth room conditions in a pot experiment at different P treatments

P supply (mg P kg^{-1} soil)	Genotype	Shoot P concentration (mg kg^{-1})	PAE (shoot P uptake P supplied^{-1}, %)	$PUtE_{GY}$ (mg grain yield mg shoot P uptake^{-1})	$PUtE_{SM}$ (mg shoot DM mg shoot P uptake^{-1})
10	RAC875	598	16.2[a]**	706	837
	Wyalkatchem	624	12.4[b]	674	799
30	RAC875	1392	17.0	321	361
	Wyalkatchem	1352	16.6	343	370
F Ratio					
Genotype (G)		0.063 ns	7.402*	0.100 ns	0.829 ns
P treatment (P)		777.260***	9.472*	710.523***	851.780***
G × P		1.447 ns	0.248 ns	3.923 ns	2.242 ns

Data represents the means of four replicates. Analysis of variance performed on original data except for P uptake transformed using log10. *F* ratios are from two-way ANOVA analysis. *, ** and *** significant at P<0.05, P<0.01 and P<0.001, respectively; ns: not significant.

Different letters showed significant differences between genotypes at each P supply, *, ** following letters: significant at P<0.05, P<0.01, respectively. Statistical analysis was performed by independent Student's t-test.

Table S 4.3. PUE of two wheat genotypes at different growth stages in the pot experiment

Genotype	PUE* (%)			
	27 DAS	48 DAS	Maturity	
			PUE_{GY}	PUE_{SM}
RAC875	93.6	76.7	65.7	69.3
Wyalkatchem	82.5	73.6	48.8	53.6

* PUE: mean ratio between shoot DM (grain yield) at low P and at adequate P

GY: grain yield, SM: shoot matter

Table S 4.4. Shoot DM, root DM, root to shoot ratio, shoot P concentration, shoot P uptake, PAE, PUtE and root efficiency of two wheat genotypes grown under growth room conditions at 24 DAS at different P treatments

P supply (mg P kg^{-1} soil)	Genotype	Shoot DM (g $plant^{-1}$)	Root DM (g $plant^{-1}$)	Root to shoot ratio (g g^{-1})	Shoot P concentration (mg kg^{-1})	Shoot P uptake (mg $shoot^{-1}$)	PAE (shoot P uptake/P supplied, %)	PUtE (mg shoot DM mg^{-1} P uptake)	Root efficiency[1]
10	RAC875	0.14	0.11	0.82	2746	0.38	3.1	367	3.4
	Wyalkatchem	0.09	0.09	0.94	2853	0.27	2.2	319	3.1
30	RAC875	0.20	0.11	0.53	5403^{a*}	1.12	3.1	186	10.3
	Wyalkatchem	0.20	0.14	0.70	6697^{b}	1.36	3.8	149	9.9
F Ratio									
Genotype (G)		1.607 ns	0.008 ns	7.075*	8.040*	0.289 ns	0.069 ns	8.829*	0.206 ns
P treatment (P)		24.811***	3.378 ns	23.724**	173.314***	58.325***	2.601 ns	150.383***	70.730***
G × P		1.406 ns	4.694 ns	0.744 ns	5.776*	2.072 ns	2.842 ns	0.149 ns	0.021 ns

Results represent means of three replicates. Analysis of variance performed on original data except for root to shoot ratio transformed using log10. *F* ratios are from two-way ANOVA analysis. *, ** and *** significant at P<0.05, P<0.01 and P<0.001, respectively; ns: not significant.

[1]Root efficiency: mg shoot P uptake per g dry weight of root dry matter.

Different letters showed significant differences between genotypes at each P supply; * following letters: significant at P<0.05. Statistical analysis was performed by independent Student's t-test.

Table S 4.5. Shoot P uptake per unit of convex hull area (CHA), root surface area (RSurA), root volume, total root length and root tip number of two wheat genotypes grown under the growth room conditions at 24 DAS at different P treatments

P supply (mg P kg^{-1} soil)	Genotype	Shoot P uptake CHA^{-1} (µg cm^{-2})	Shoot P uptake RSA^{-1} (µg cm^{-2})	Shoot P uptake root $volume^{-1}$ (mg cm^{-3})	Shoot P uptake total root $length^{-1}$ (µg m^{-1})	Shoot P uptake root tip^{-1} (µg tip^{-1})	Shoot P uptake average root $density^{-1(*)}$
10	RAC875	1.16	2.17	0.23	19.9	0.77	1.00^{a*}
	Wyalkatchem	1.03	1.89	0.20	17.4	0.60	0.57^{b}
30	RAC875	3.05	5.46	0.56	51.0	1.76	2.70
	Wyalkatchem	3.00	5.19	0.53	48.5	1.80	3.26
F Ratio							
Genotype (G)		0.094 ns	0.987 ns	1.105 ns	0.925 ns	0.448 ns	0.266 ns
P treatment (P)		41.916^{***}	134.490^{***}	121.728^{***}	139.519^{***}	134.529^{***}	269.137^{***}
G × P		0.021 ns	0.002 ns	0.000 ns	0.000 ns	1.231 ns	13.766^{**}

CHA: Convex hull area, RSurA: Root surface area.

Data represent the means of three replicates. *F* ratios are from two-way ANOVA analysis. *, *** significant at P<0.05 and P<0.001, respectively; ns: not significant.

[*]Unit of mg P per average root density. Average root density is ratio between foreground and background pixels of the extracted root (Bucksch et al., 2014).

Different letters showed significant differences between genotypes at each P supply; * following letters: significant at P<0.05. Statistical analysis was performed by independent Student's t-test.

Table S 4.6. Mean ratio of shoot DM, shoot P uptake, convex hull area, root surface area, root volume, root tip number, total root length, medium root width and average density between P supply of 10 to 30 mg P kg^{-1} soil

Parameter	Genotype	Mean ratio between P supply of 10 to 30 mg P kg^{-1} soil (%)
Shoot DM	RAC875	70.0
	Wyalkatchem	45.0
P uptake	RAC875	33.9
	Wyalkatchem	19.9
Convex hull area	RAC875	88.8
	Wyalkatchem	56.6
Root surface area	RAC875	84.0
	Wyalkatchem	52.8
Root volume	RAC875	82.8
	Wyalkatchem	51.5
Root tip number	RAC875	77.6
	Wyalkatchem	58.6
Total root length	RAC875	85.5
	Wyalkatchem	53.0
Medium root width	RAC875	85.2
	Wyalkatchem	55.2
Average density	RAC875	82.4
	Wyalkatchem	155.9

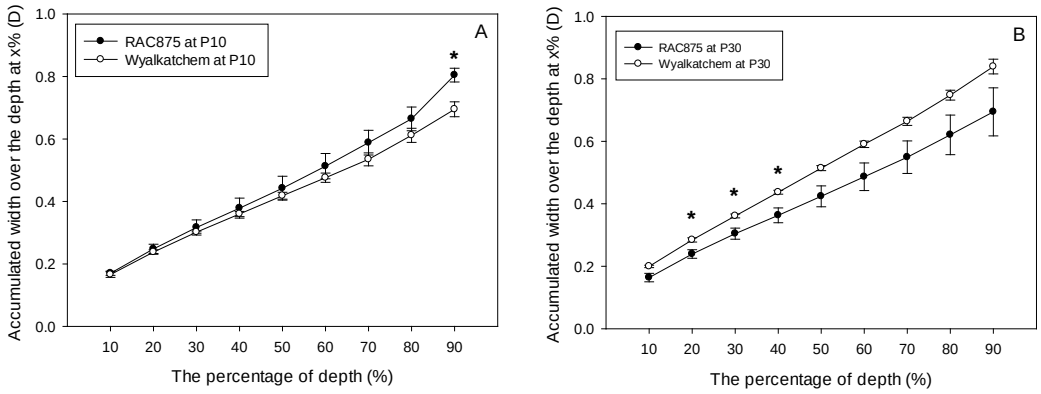


Figure S 4.1. Accumulated width over the depth at x% (D) of two wheat genotypes at low P supply (A) (10 mg P kg^{-1} soil (P10)) and adequate P supply (B) (30 mg P kg^{-1} soil (P30)). * significantly different between genotypes at specific depth ($P<0.05$).

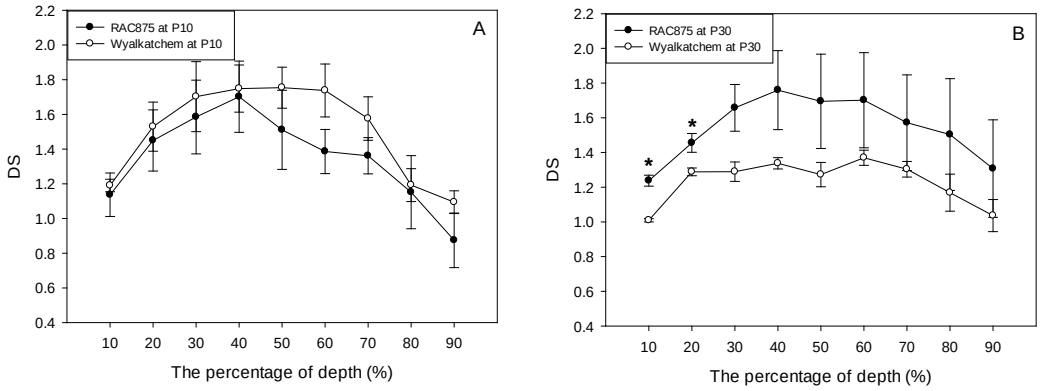


Figure S 4.2. The slope of the graph of D value (DS) of two wheat genotypes at low P supply (A) (10 mg P kg^{-1} soil (P10)) and adequate P supply (B) (30 mg P kg^{-1} soil (P30)). * significantly different between genotype at specific depth ($P<0.05$)

CHAPTER 5

Phosphorus use efficiency in RAC875 is associated with the accumulation of raffinose and maintenance of phosphorylated sugars

Abstract

Variation in metabolite responses is important for how a plant responds to phosphorus (P) deficiency and can lead to an exploration of the mechanism that a plant might use to tolerate deficiency. This study aimed to investigate metabolite profiles of a P efficient wheat (RAC875) and P inefficient wheat (Wyalkatchem) to explore metabolic mechanisms of P use efficiency (PUE) in RAC875. The results showed that at 28 days after sowing (DAS), under low P supply, RAC875 produced 42% more shoot biomass than Wyalkatchem, Wyalkatchem. RAC875 also had greater rhizosheath size than Wyalkatchem at low P supply. Metabolite responses in leaves and roots were observed and under low P the two wheat genotypes showed different responses. P deficiency increased the levels of raffinose and 1-ketose in roots of both wheat genotypes, while P deficiency showed no effect on raffinose and 1-ketose in leaves. RAC875 accumulated more raffinose in roots than Wyalkatchem. P deficiency had no significant impact on the levels of sucrose, maltose, glucose and fructose. Phosphorylated sugars (glucose-6-P and fructose-6-P) remained unchanged in RAC875, but declined in both leaves and roots of Wyalkatchem. Glycerol-3-P strongly declined in roots of both wheat genotypes in responses to low P and no significant changes occurred in organic acids (citrate, succinate and fumarate) of the TCA cycle

in both wheat genotypes under low P, whereas low P strongly increased the levels of shikimic acid and quinic acid in the leaves of RAC875. RAC875 exhibited lower levels of fumarate, malate, maleate and itaconate in roots than Wyalkatchem. Most of the amino acid levels were not affected by low P, however RAC875 showed greater accumulation of aspartate, glutamine and β-alanine in leaves than Wyalkatchem under low P supply. Greater accumulation of raffinose in roots and aspartate, glutamine and β-alanine in leaves may be associated with enhanced PUE in RAC875. Glucose-6-P and fructose-6-P are important for glycolysis, thus maintaining these metabolites would enable RAC875 to maintain carbohydrate metabolism and shoot biomass under P deficiency.

5.1 Introduction

Phosphorus (P) is a vital macronutrient for plant growth and development and plays a variety of cellular functions including structural roles (i.e. nucleic acids and phospholipids), energy transfer (i.e. ATP) and phosphorylated intermediates (i.e. glucose-6-P, fructose-6-P), and therefore it is involved in the regulation of metabolic pathways (Muller et al., 2015; Huang et al., 2008). However, available soil P is low in many agricultural soils and this reduces crop growth and productivity (Lynch, 2011). Besides, P fertilizer resources are limited and they are predicted to be depleted by 2050 (Vance et al., 2003), therefore the development of plants with improved phosphorus use efficiency (PUE) is critical to cope with this issue.

Plants respond to low P through various mechanisms including modifications in metabolic processes and alternation of metabolites (Jones et al., 2015). Plants modify metabolic pathways to enhance internal P efficiency as well as P acquisition (Vance et al., 2003). For example, phospholipids in membranes can be replaced by sulfolipids and galactolipids in response to P starvation (Veneklaas et al., 2012). Under P deficiency, instead of using P_i, plants can activate metabolic bypass enzymes that depend on pyrophosphate (PP_i) (Plaxton and Tran, 2011). There are PP_i-dependent glycolytic enzymes thereby enabling plants to maintain the carbon flux under P starvation (Plaxton and Podesta, 2006). This activation of alternative metabolic pathways could result in changes in metabolites and this has been reported under P deficiency where studies have shown that plants accumulate sugars under P starvation. For example, di- and trisaccharides (sucrose, maltose and raffinose) increased in barley (Huang et al., 2008) and maize (Ganie et al., 2015) under P deprivation. The

accumulation of sugars was also observed in bean roots (Rychter and Randall, 1994) and all cucumber tissues (Ciereszko et al., 2002) under low P. However, a study in lupin was able to show that fructose, glucose and sucrose in shoots reduced after 14 days of P deficiency and after 22 days of P deficiency no effect on sugar levels in shoots and roots was found (Muller et al., 2015).

Low P has also reported to lead to a decrease in phosphorylated sugars. For example, reductions in glucose-6-P, fructose-6-P and inositol-P was found in barley (Huang et al., 2008), maize (Ganie et al., 2015) and lupin (Muller et al., 2015). A decrease in phosphorylated sugars was also observed in other plants (Rychter and Randall, 1994; Warren, 2011). The accumulation of sugars and a decrease in phosphorylated sugars could be a plant adaptive mechanism that helps plants to use P more efficiently under low P supply. Alterations in amino acid and organic acid levels also occurred under low P. For example, glutamine and asparagine increased, while organic acids (i.e. α-ketoglutarate, succinate, fumarate and malate) also increased (Huang et al., 2008).

Although metabolites have been profiled in wheat under abiotic stress, such as drought and salt stress (Bowne et al., 2012), no study has been reported on metabolite profiles in wheat under P stress. Previous studies (Chapter 2 and Chapter 3) showed that two wheat genotypes, RAC875 and Wyalkatchem vary in PUE and metabolite profiling of these wheat genotypes under P starvation could elucidate mechanisms of PUE. Therefore, this study aimed to examine metabolite composition of roots and shoots of these two wheat genotypes under low and adequate P supply.

5.2 Materials and methods

5.2.1 Materials

Two selected wheat genotypes, RAC875 and Wyalkatchem were used for this experiment.

5.2.2 Experimental design and sampling

Plants were grown in square-shaped pots holding 1.1 kg of sandy soil. The pot size was 18.0-cm high x 8.5-cm dimension at the top x 6.5-cm dimension at the bottom. Two P levels were used; 10 and 30 mg P kg^{-1} soil. Basal nutrients consisting of $Ca(NO_3)_2.4H_2O$ (918), K_2SO_4 (113.6), $MgSO_4.7H_2O$ (140), $FeSO_4.7H_2O$ (1.4), $Na_2MoO_4.2H_2O$ (0.61), $CuSO_4.5H_2O$ (2.25), $MnSO_4.4H_2O$ (3.68), $ZnSO_4.7H_2O$ (6.6), H_3BO_3 (0.28) and phosphorus (KH_2PO_4) were applied to the 1.1 kg of soil and massaged in prior to sowing.

Wheat rains were sterilized in 2% hypochlorite for 10 min and were then rinsed with Milli-Q water. The grains were then germinated on moistened, filter paper lined petri dishes that were kept in the dark at room temperature for two days. Three germinated grains were sown into each pot and the experiment was carried out with four replicates. The experiment was housed in a growth room with a light intensity of 660 µmol m^{-2} s^{-1} at the leaf level. The sort of light was the combination of fluorescent and incandescent. The growth room temperature was 20 ^oC/10 ^oC (day/night) and the length of day-light was 13 hours. Plants were watered three times a week to 10% of soil weight. Two plants were thinned from each pot at 7 and 15 DAS and one plant

was grown up to 28 DAS when they were then harvested. The two youngest leaves from the primary tillers and older leaves were sectioned and stems were detached from the roots at the crown levels. All leaves and stems were rinsed Milli-Q water and then put in 50 mL tubes. Tubes with leaves and stems were immediately plunged into liquid N and then stored at -85 ^{o}C.

Leaves and roots were sampled on the same day (in the morning). Once the shoots were removed, the soil containing the roots was then poured out into a tray and the roots were gently taken without dislodging and the soil core was gently removed. The roots with soil bound to the root were weighed for rhizosheath (Figure 5.1 A) measurements. After that, the roots were washed then rinsed with milli-Q water and dried using tissue papers and weighed to obtain fresh weights (Figure 5.1 B). Roots were then immediately transferred into a 30 mL tube and plunged into liquid nitrogen and then stored at -80^{o} C. The rhizosheath weight is expressed as the difference between the root weight with rhizosheath soil and the fresh root weight (George et al., 2014). Rhizosheath is calculated as the ratio of roots with soil attached per unit of fresh root weight. Pots were watered to obtain similar moisture content before harvest to minimize any variation derived from moisture.

Leaves, stems and roots were then freeze-dried for 48 h for dry weight measurement and metabolite profiling.

A B

Figure 5.1. Rhizosheath (A) and fresh roots (B) of two wheat genotypes, RAC875 and Wyalkatchem grown at 10 and 30 mg P kg^{-1} soil (referred to as P10 and P30, respectively). From left to right, RAC875 at P10, Wyalkatchem at P10, RAC875 at P30 and Wyalkatchem at P30.

Statistical analysis

Statistical analyses were conducted by using IBM SPSS v23. The normality of data was tested using Kolmogorov-Smirnov and Shapiro-Wilk test ($P<0.05$). Plant indices were analysed using two-way ANOVA (Genotype x P supply). Mean comparisons between genotypes within each P treatment were performed by independent Student's t-test ($P<0.05$) (Field, 2013).

5.2.3 Metabolite profiling

Sample preparation and TMS derivatisation

Samples were extracted and analysed for metabolites by Metabolomics Australia at The University of Melbourne, a NCRIS initiative under Bioplatforms Australia Pty Ltd. Aliquots (15 mg) of freeze-dried shoot or root material were transferred to Cryo-mill tubes and accurate weights were recorded. Methanol (MeOH, 600 µL) containing the following internal standards, $^{13}C_6$-Sorbitol (0.02 mg mL^{-1}) and $^{13}C_5$-^{15}N-Valine (0.02 mg mL^{-1}), was added to the sample tubes. The samples were homogenized using

a Cryo-mill (Berting Technologies; program #2 (6800-3x30x30 at -10ºC)) and then incubated in a Thermomixer at 30 ºC with a mixing speed of 900 rpm for 15 min, followed by 5 min of centrifugation at 15,000 rpm (21,200 x *g*). The MeOH supernatant was transferred into a 1.5 mL Eppendorf tube and set aside. Water (600 µL) was added to the remaining sample pellet and vortexed before being centrifuged for 10 minutes at 15,000 rpm (21,200 x *g*). The supernatant was removed and combined with the MeOH supernatant. Aliquots of 50 µL were transferred to clean glass insert in Eppendorf tubes and dried *in vacuo* using a Rotational Vacuum Concentrator (RVC 2-33 CD plus, John Morris Scientific, Pty Ltd, Melbourne, Australia) set at ambient temperature.

Dried samples were prepared by the addition of 20 µL of Methoxyamine Hydrochloride (30 mg/ml in Pyridine) followed by shaking at 37 ºC for 2h. The sample was then derivatised with 20 µL of *N,O-bis* (Trimethylsilyl)trifluoroacetamide with Trimethylchlorosilane (BSTFA with 1% TMCS, Thermo Scientific) for 30 min at 37 °C. The sample was then left for 1 h before 1 µL was injected onto the GC column using a hot needle technique. Splitless and split (1:20) injections were done for each sample.

Analytical instrumentation

The GC-MS system used comprised of a Gerstel 2.5.2 autosampler, a 7890A Agilent gas chromatograph and a 5975C Agilent quadrupole mass spectrometer (Agilent, Santa Clara, USA). The mass spectrometer was tuned according to the manufacturer's recommendations using tris-(perfluorobutyl)-amine (CF43).

GC-MS method

Gas chromatography was performed on a 30 m Agilent J & W VF-5MS column with 0.25 µm film thickness and 0.25 mm internal diameter with a 10 m Integra guard column. The injection temperature (Inlet) was set at 250 °C, the MS transfer line at 280 °C, the ion source adjusted to 230 °C and the quadrupole at 150 ºC. He was used as the carrier gas at a flow rate of 1 mL min^{-1}.

The analysis of TMS samples was performed under the following temperature program; start at injection 70 °C, a hold for 1 minute, followed by a 7 °C min^{-1} oven temperature ramp to 325 °C and a final 6 min heating at 325 °C. Mass spectra were recorded at 2.66 $scans.s^{-1}$ with an *m/z* 50-600 scanning range.

Data processing and statistical analysis

Data were processed using the Agilent MassHunter Quantitative Analysis version B.07.00 software. Mass spectra of eluting TMS compounds were identified using the commercial mass spectra library NIST (http://www.nist.gov), the public domain mass spectra library of Max-Planck-Institute for Plant Physiology, Golm, Germany (http://csbdb.mpimp-golm.mpg.de/csbdb/dbma/msri.html) and the *in-house* Metabolomics Australia mass spectral library. Resulting relative response ratios normalized per mg dry weight for each analysed metabolite were prepared as described by Roessner et al. (2001). Differences between sample groups were validated using the Student's *t*-test (*p*-value < 0.05). Statistical analysis was done using Excel (Microsoft, www.microsoft.com).

5.3 Results

5.3.1 Responses to P at 28 DAS

At 28 DAS, plants produced significantly (P<0.01) greater shoot dry matter (DM) under adequate P supply (30 mg P kg^{-1} soil) when compared to that at low P supply (10 mg g P kg^{-1} soil) (Table 5.1, Figure 5.2 A). Under low P, RAC875 showed 42% higher (P<0.01) shoot DM than Wyalkatchem, while no significant difference occurred under adequate P (Figure 5.2 A). P supply did not significantly affect root DM and no significant variation was observed between the wheat genotypes (Table 5.1, Figure 5.2 C). However, Wyalkatchem had greater root to shoot ratio than RAC875 under adequate P (Table 5.1, Figure 5.2 D).

Table 5.1. Shoot DM, root DM, root to shoot ratio and rhizosheath size of two wheat genotypes grown under the growth room conditions at 28 DAS (two-anova analysis)

Effect	Shoot DM (g $plant^{-1}$)		Root DM (g $plant^{-1}$)		Root to shoot ratio (g g^{-1})		Rhizosheath size (g g^{-1})	
	df	*F* Ratio	df	*F* Ratio	df	*F* Ratio	df	*F* Ratio
Genotype (G)	1	17.668**	1	2.549 ns	1	14.654**	1	31.715***
P treatment (P)	1	12.967**	1	2.019 ns	1	11.186**	1	56.844***
G × P	1	0.551 ns	1	0.027 ns	1	0.004	1	11.321**
Error	12		12		12		12	
Total	16		16		16		16	

Results are from two-way ANOVA analysis. **, *** significant at P<0.01 and P<0.001, respectively; ns: not significant.

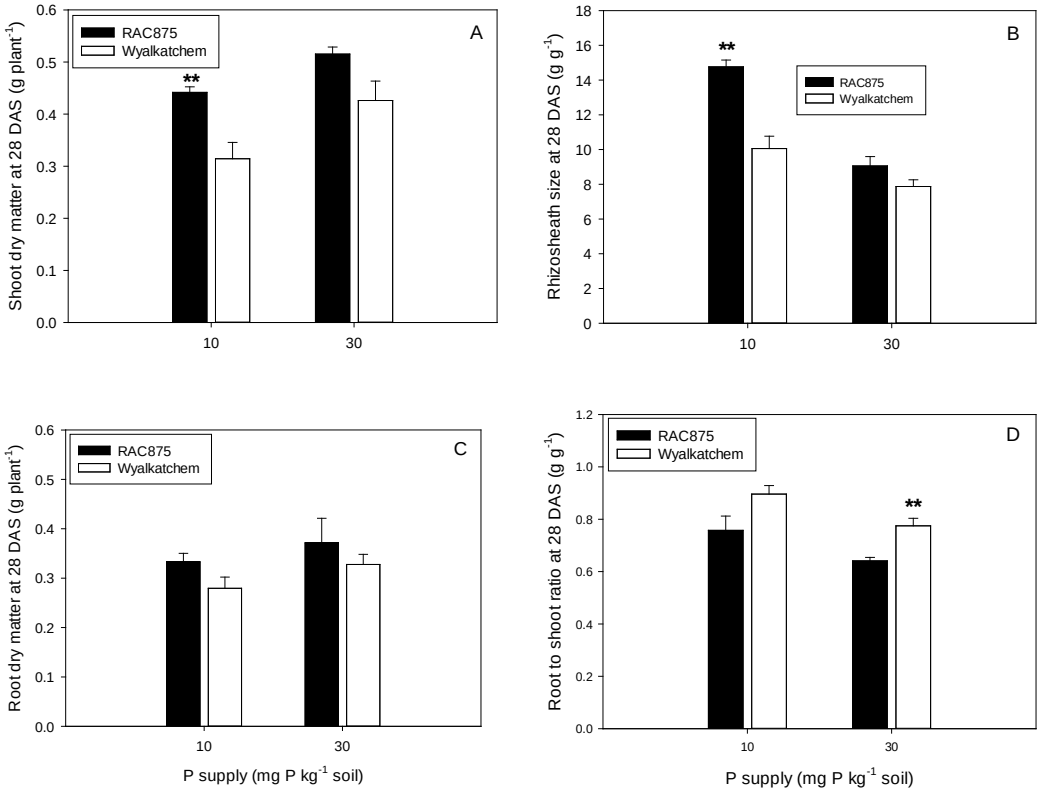


Figure 5.2. The effect of P supply on shoot dry matter (A), rhizosheath (B), root dry matter and root to shoot ratio (D) of two wheat genotype at 28 day after sowing (DAS) under growth room conditions. ** showed significant differences between genotypes within the same P supply (P<0.01).

Low P significantly (P<0.01) increased the root to shoot ratio (Table 5.1, Figure 5.2 D) and significantly (P<0.001) increased rhizosheath size (Table 5.1, Figure 5.2 B). RAC875 had a lower root to shoot ratio but greater rizhosheath size than Wyalkatchem. A significant (P<0.01) interaction between genotype (G) and P supply (P) for rhizosheath size was observed. RAC875 showed 46.5% larger (P<0.01) rizhosheath size than Wyalkatchem under low P, but not under adequate P.

Phosphorus use efficiency (PUE) was calculated as relative shoot DM between low and adequate P supply, by which RAC875 showed 12.5% greater PUE than Wyalkatchem.

5.3.2 Metabolic alterations under P deprivation at 28 DAS

This study used a comparative GC-MS based method to identify metabolic changes under low P compared with adequate P and differences in metabolites between two wheat genotypes with contrasting PUE in response to P starvation. A total of 79 and 84 metabolites were measured in the leaves and roots of the two wheat genotypes, respectively. Shoot-derived carbohydrates are important for plant growth since they are not only carbon sources, but they are also used to synthesize other compounds that are essential for plants, such as amino acids. Thus, it is interesting to investigate alterations of carbohydrates in response to P stress as well as variations between wheat genotypes. The effect of P supply on metabolite levels in leaves and roots of the two wheat genotypes were shown in Figure 5.3. Low P did not significantly affect levels of sucrose, maltose, raffinose, glucose and fructose in leaves of both wheat genotypes. However, xylose significantly increased (1.3-fold) in leaves of RAC875 and slightly increased (1.1-fold) in Wyalkatchem leaf tissues. Ribose significantly decreased (0.8-fold) in leaves of RAC875 but not in Wyalkatchem leaves. Under low P, the concentration of leaf galactinol, a sugar of raffinose oligosaccharides (RFOs) (Pluskota et al., 2015), increased in RAC875 (1.3-fold) while a slight decrease was observed in Wyalkatchem. Similar to leaves, low P led to no significant changes in sucrose, maltose, glucose and fructose within the roots, except raffinose, which significantly ($P<0.05$) increased in both RAC875 (2.8-fold) and Wyalkatchem (2.0-fold) under P deficiency. The level of 1-ketose in roots also increased under low P in both wheat genotypes. Low P significantly enhanced arabinose, galactosylglycerol, manose and xylose levels in roots of Wyalkatchem but these sugar levels remained

unchanged in RAC875 between P treatments.

Under both levels of P supply, leaf sucrose, maltose, raffinose, glucose and fructose were not significantly different between the two wheat genotypes (Figure 5.4). Under low P supply, RAC875 showed a greater leaf xylose level (1.2-fold) but lower leaf galactonic acid level (0.7-fold) when compared to Wyalkatchem. The two wheat genotypes also did not show significant differences in sugar levels in roots except the levels of 1-ketose and raffinose, which were higher (each, 1.9-fold) in RAC875 when compared to Wyalkatchem (Figure 5.4).

Under low P, the levels of phosphorylated sugars glucose-6-P significantly reduced (0.5-fold) in Wyalkatchem roots and fructose-6-P significantly decreased in both leaves (0.6-fold) and roots (0.5-fold) of Wyalkatchem. Meanwhile, low P had no significant effect on these phosphorylated metabolites in RAC875 (Figure 5.3). There were no significant differences in these two phosphorylated sugars between two wheat genotypes under both low and adequate P (Figure 5.4). Under low P, glycerol-3-P level decreased 0.5-fold in roots of both RAC875 and Wyalkatchem (Figure 5.3). No genotypic variation occurred in glycerol-3-P under both P treatments (Figure 5.4).

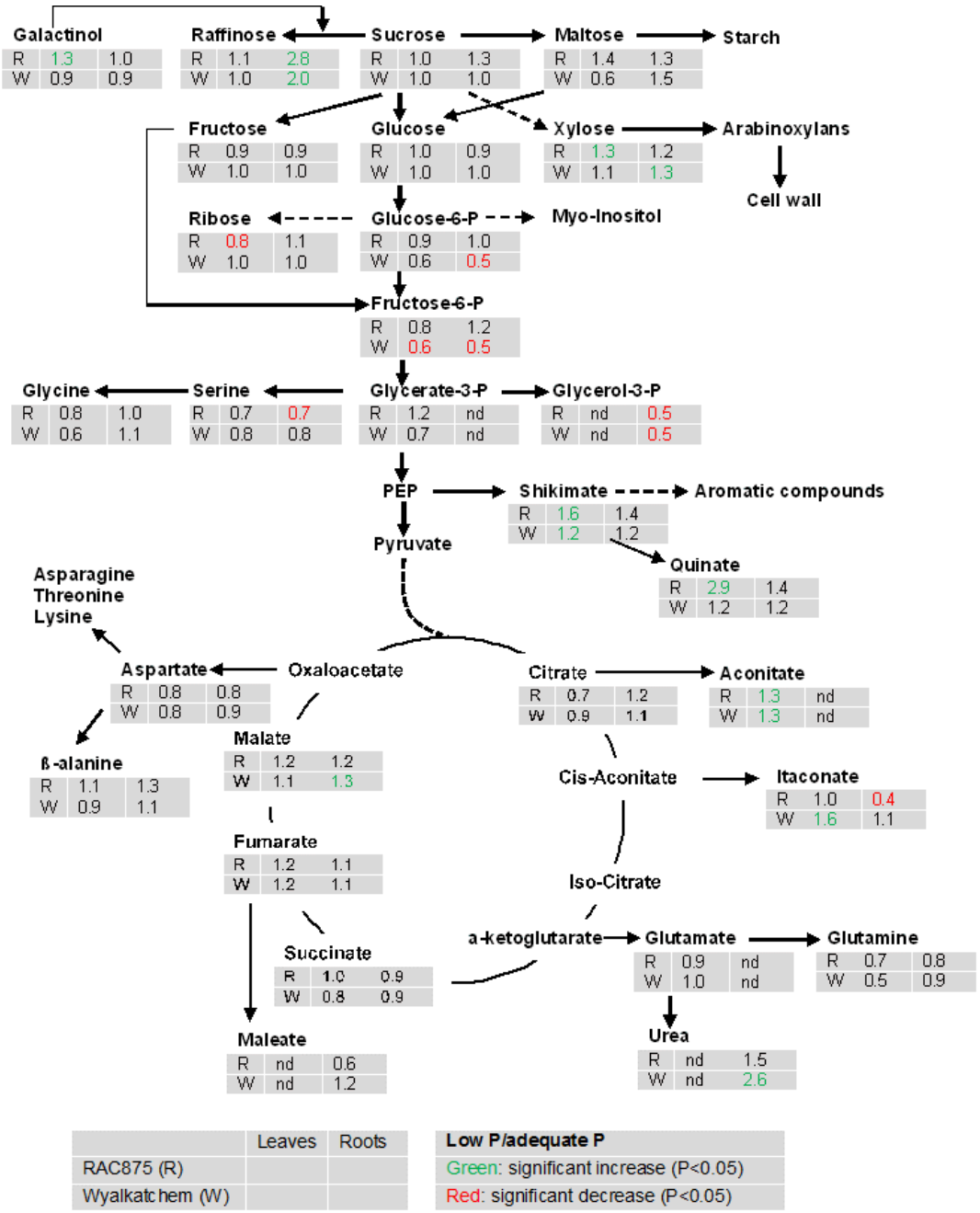


Figure 5.3. The effect of P supply on the levels of metabolites from leaves and roots of two wheat genotypes RAC875 (the upper row) and Wyalkatchem (the lower row) at 28 days after sowing (DAS). Plants were grown in sandy soils at low (10 mg P kg^{-1} soil) and adequate (30 mg P kg^{-1} soil) P supply. Relative ratios (low P/adequate P) in leaves (the first column) and in roots (the second column) are presented as means of four replicates. Significant increases ($P<0.05$) are indicated in green and significant decreases ($P<0.05$) are indicated in red; nd: not detectable.

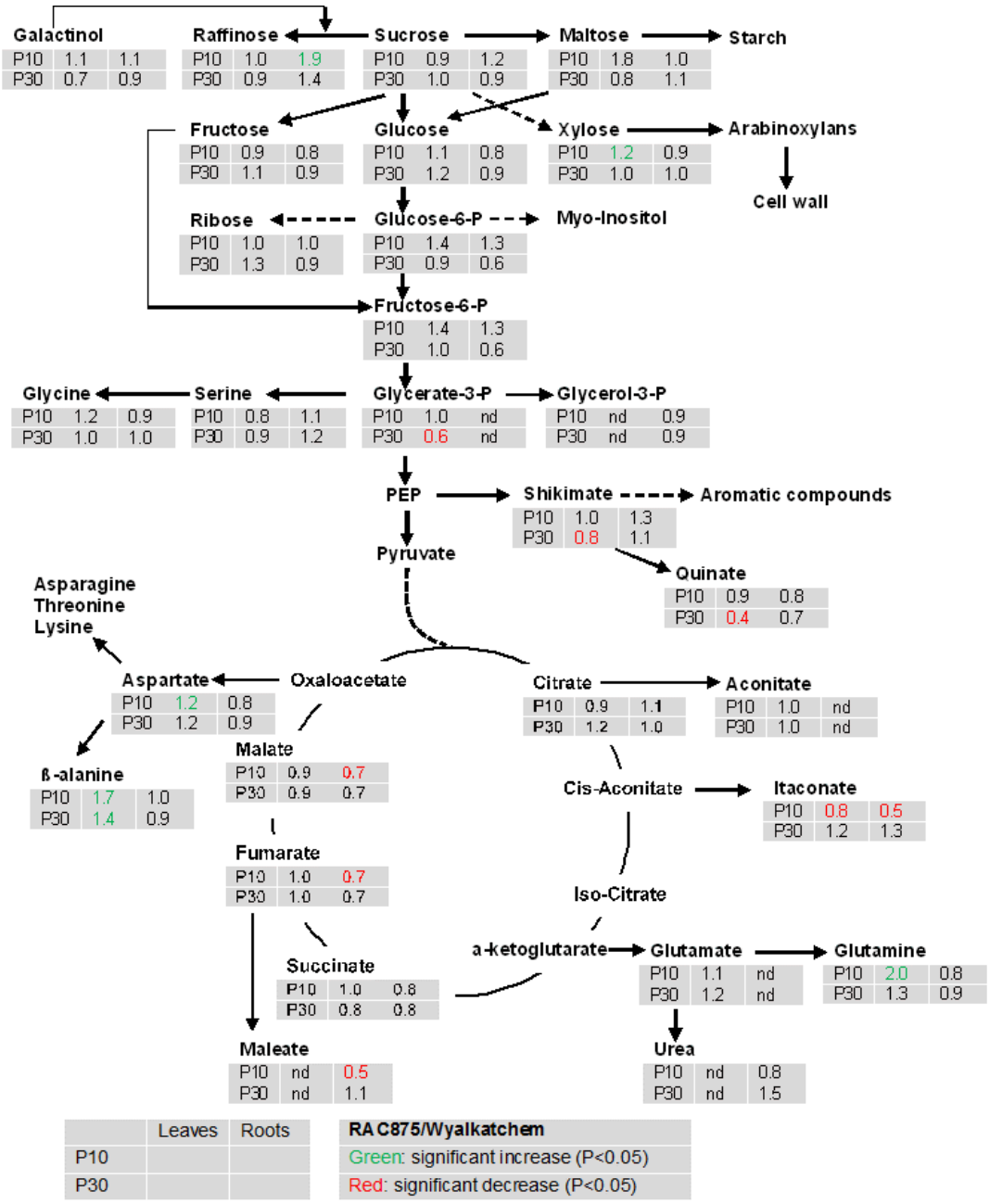


Figure 5.4. Variation in the levels of metabolites from leaves and roots between two wheat genotypes RAC875 and Wyalkatchem at 28 days after sowing (DAS). Plants were grown in sandy soils at low (10 mg P kg^{-1} soil – P10) and adequate (30 mg P kg^{-1} soil – P30) P supply. Relative ratios between RAC875 and Wyalkatchem in leaves (the first column) and roots (the second column) are presented as means of four replicates and relative ratios were compared at low P (P10) (the first row) and adequate P (P30) (the second row). Significant increases ($P<0.05$) are indicated in green and significant decreases ($P<0.05$) are indicated in red; nd: not detectable.

The levels of most organic acids of the tricarboxylic acid (TCA) cycle (citrate, succinate and fumarate) remained unchanged under low P in both leaves and roots of the two wheat genotypes (Figure 5.3). However, low P promoted higher levels of leaf aconitic acid (1.3-fold) in both RAC875 and Wyalkatchem. In leaves, low P increased levels of iso citric acid (1.2-fold) and malonic acid (1.6-fold) in RAC875 but no change in these organic acid profiles was found in leaves of Wyalkatchem (Table S 5.1). Itaconate enhanced 1.6-fold in Wyalkatchem leaves under low P. In roots, the levels of malic acid increased (1.3-fold) in Wyalkatchem under low P. P deficiency led to increases in galactonic acid (1.3-fold), gluconate (1.4-fold) and glyceric acid (1.4-fold) in roots of Wyalkatchem, while low P did not affect the levels of these organic acids in roots of RAC875 (Table S 5.2). Low P resulted in the enhancement of shikimic acid, a precursor for aromatic compounds in leaves of both RAC875 (1.6-fold) and Wyalkatchem (1.2-fold), while no effect on this organic acid was found in roots under low P (Figure 5.3).

Under low P, the levels of most organic acids in leaves including citrate, succinate, furmarate, malate and shikimic acid were not different between the two wheat genotypes, but RAC875 had lower levels of itaconate and sinaptic acids than Wyalkatchem (Figure 5.4, Table S 5.1, Table S 5.2). The levels of many organic acids were also lower in roots of RAC875 (i.e. fumaric 0.7-fold, galactonic 0.6, itaconate 0.5-fold, malic 0.7-fold) than in Wyalkatchem under P deficiency (Figure 5.4, Table S 5.2).

The levels of most amino acids remained unchanged under low P in both wheat genotypes, however, several amino acids in leaves reduced under low P (Figure 5.3,

Table S 5.1, Table S 5.2). For example, N-acetyl serine and pyroglutamic acid declined 0.6 and 0.5-fold respectively, in RAC875; alanine and putrescine decreased 0.7-fold in Wyalkatchem. In roots, P deficiency reduced levels of O-acetyl serine and threonine in Wyalkatchem and serine in RAC875. Under low P, the levels of several amino acids (aspartic acid 1.2-fold, beta-alanine 1.7, glutamine 2.0-fold) in RAC875 were greater than those in Wyalkatchem (Figure 5.4). However, no variations in amino acids were found between the two wheat genotypes under adequate P.

5.4 Discussion

5.4.1 P efficient wheat had greater rhizosheath size

Rhizosheath consists of soil that adheres firmly to roots of many species and is an indicative of root hair length (Delhaize et al., 2015). Under low P, RAC875 produced greater shoot biomass than Wyalkatchem and this genotype also possessed a larger rhizosheath (Figure 5.2 A & B). This result is consistent with a report from James et al. (2016), in which larger rhizosheath size was correlated with shoot biomass. Thus, larger rhizosheath size could contribute to great shoot biomass in RAC875. However, RAC875 showed shorter but denser root hairs than Wyalkatchem (Chapter 4). This means that rhizosheath size may not be the only indicator for root hair length but also for root hair density since the denser root hairs could retain soils bound to roots more firmly. Contrasting shoot biomass, rhizosheath size and differences in root hair features between the two wheat genotypes could make them have different metabolic mechanisms in response to P deficiency.

5.4.2 P deficiency enhances levels of several sugars and the P efficient wheat genotype (RAC875) accumulated greater raffinose

Although P deficiency resulted in increased levels of several sugars such as raffinose, galactinol, 1-ketose and xylose, sugar responses to low P were not all similar between leaves and roots, and between the two wheat genotypes. Indeed, P deficiency strongly increased raffinose in roots of both wheat genotypes, while no changes in this sugar was observed in leaves (Figure 5.3). Under low P, an increase in galactinol was found only in leaves of RAC875. Significant increases in xylose were only found in leaves of RAC875 and roots of Wyalkatchem. Low P led to the dramatic enhancement of 1-ketose in roots of both wheat genotypes, however this metabolite was undetectable in leaves. Opposite sugars responses in shoots and roots to drought stress have also been observed in the common C3 grasses *A. pratensis* and *H. lanatus* (Gargallo-Garriga et al., 2014).

Raffinose belongs to the raffinose family oligosaccharides (RFOs) functioning as stored carbohydrates and stress tolerance factors (Van den Ende, 2013). Therefore, the accumulation of raffinose would be an adaptive mechanism of plants in response to stress conditions. A dramatic increase of raffinose in roots under low P in this research is consistent with a study in tomato (Sung et al., 2015). Increased levels of raffinose under low P have also been documented in barley (Huang et al., 2008) and maize (Ganie et al., 2015). The accumulation of raffinose in plants has been found under stress conditions such as iron deficiency (Rellán-Álvarez et al., 2010), cold (Rohloff et al., 2012), drought (Peters et al., 2007) and salinity (Shelden et al., 2016). Interestingly, the results here reveal that the efficient genotype, RAC875 had greater

root raffinose accumulation than the inefficient genotype, Wyalkatchem under P deficiency (Figure 5.4). The higher production of raffinose in roots appears to be associated with P efficiency in RAC875.

Contrary to previously reported studies that sucrose and maltose increased under low P as in barley (Huang et al., 2008) and in maize (Ganie et al., 2015) or other plants (Ciereszko et al., 2002; Ciereszko and Barbachowska, 2000), this study shows that P supply had no significant impact on the levels of these sugars (Figure 5.3). This difference could be due to different cultivating methods and the harvest stages (i.e. in this study, plants were grown in sandy soil and harvested at 28 DAS, while Huang et al. (2008) and Ganie et al. (2015) used hydroponic cultivation and harvested at earlier stages). The level of deficiency could lead to different results. Indeed, in the experiment carried out by Huang et al. (2008), barley plants were grown under severe P deficiency. Genotypic variation may also result in differences in metabolic responses to low P. For example, sucrose levels significantly dropped in the leaves of a *Brachiaria* hybrid under low P but not in rice (Nanamori et al., 2004). Growth stages also lead to differences in sugar responses to low P. For example, Muller et al. (2015) were able to show that low P reduced the levels of sucrose, glucose and fructose in lupin shoot after 14 days of P deficiency, but no changes in these sugars were found after 22 days of P deficiency. Glucose and fructose levels were also found to stay unchanged under low P deprivation in this study.

5.4.3 Maintaining phosphorylated sugars in the P efficient wheat

Phosphorylated sugars, glucose-6-P and fructose-6-P strongly declined in both leaves and roots of the low P intolerant wheat, Wyalkatchem under P deficiency (Figure 5.3).

This result is consistent with previous studies. For example, a reduction in these metabolites was also observed in shoots and roots of barley (Huang et al., 2008) and of maize (Ganie et al., 2015). Low P also reduced the levels of phosphorylated sugars in bean roots (Rychter and Randall, 1994) and in leaves of *Eucalyptus globulus* (Warren, 2011). Glucose-6-P and fructose-6-P are important intermediates for glycolysis. Glycolysis is the first stage of carbohydrate metabolism, followed by the tricarboxylic acid cycle (TCA) in the cell. This process not only generates energy but also provides important intermediates for the biosynthesis of essential molecules (i.e. amino acids). Thus, the dramatic reduction in glucose-6-P and fructose-6-P would essentially lead to lower biomass production in Wyalkatchem under P deficiency.

In contrast to Wyalkatchem, low P had no effect on the levels of these phosphorylated sugars in the low P tolerant wheat, RAC875 (Figure 5.3). The maintenance of phosphorylated sugars in RAC875 under low P could maintain respiratory carbon flux to generate energy and carbon skeletons for key biochemical processes in plants, which supports RAC875 in producing a great biomass under low P supply. Besides, the relative ratio of inorganic P (P_i) in leaves between low P and adequate P was higher in RAC875 (0.8-fold) than in Wyalkatchem (0.5-fold) (Table S 5.1), meaning that under low P, RAC875 can maintain relatively high P_i levels. This enables RAC875 to maintain more efficient biochemical processes that requires P_i. The high ratio of P_i in leaves (low P/adequate P) of RAC875 is correlated with the ratio of shoot P concentration at 24 DAS (low P/adequate P) which was 0.5-fold for RAC875 and 0.4-fold for Wyalkatchem (Chapter 4, Figure 4.5 B).

When P supply is low, a dramatic drop in glycerol-3-P occurred in roots of both wheat

genotypes (Figure 5.3). This result agrees with a report in lupin (Muller et al., 2015). Glycerol-3-P is a structural component of phospholipids that can be replaced with sulfo- and galactolipids under P deprivation (Veneklaas et al., 2012; Plaxton and Tran, 2011; Lambers et al., 2012). Therefore, it would be interesting to identify whether variation in sulfo-and galactolipids under P deficiency is present between the two wheat genotypes.

5.4.4 Low organic acids of TCA cycle but high accumulation of shikimic acid and quinic acid in the P efficient wheat

The levels of most organic acids (i.e. citrate, succinate and fumarate) involved in the TCA cycle were not affected by P deficiency (Figure 5.3). This is similar to the results from *Eucalyptus globulus* (Warren, 2011). However, low P reduced the levels of succinate and fumarate in barley roots (Huang et al., 2008) and decreased succinate levels in maize leaves (Ganie et al., 2015), while low P enhanced the levels of citrate, succinate and fumarate in shoots and roots of lupin (Muller et al., 2015). Huang et al. (2008) suggests that decreased organic acid levels in barley roots under low P are related to the shortage of carbohydrate and the secretion of organic acids in response to the P starvation could reduce their levels. The drop in organic acids seems to be the main reason that hindered the TCA cycle in barley since plants was grown under severe P deficiency in the experiment carried out by Huang et al. (2008). Meanwhile, lupin forms cluster roots that produce high amounts of organic acids (Muller et al., 2015) and they can be secreted into the rhizosphere to respond to P starvation (Hocking and Jeffery, 2004; Cheng et al., 2014). In this study, no changes in citrate, succinate and fumarate under low P was observed, indicating that plants appear to maintain normal

level of carbohydrates for respiration. However, low P enhanced aconitate and itaconate in leaves of Wyalkatchem and increased maleate in roots of Wyalkatchem. Low P strongly reduced the level of itaconate in roots of RAC875 under low P supply.

Interestingly, under low P, RAC875, showed lower levels of fumarate, malate, maleate and itaconate in roots when compared to Wyalkatchem (Figure 5.4). This may indicate that RAC875 requires lower levels of carbohydrates to maintain its normal carbohydrate metabolism and that, while Wyalkatchem needs higher levels of carbohydrates for its normal cellular activities.

Contrary to the stability of organic acids in the TCA cycle between P treatments, shikimic acid and quinic acid dramatically increased in leaves of RAC875 in response to P deficiency (Figure 5.3). Low P did not affect quinic acid in both leaves and roots of Wyalkatchem, but shikimic acid increased in leaves of Wyalkatchem. Under low P, increased levels of shikimic acid were also observed in barley shoots (Huang et al., 2008) and in lupin shoots (Muller et al., 2015). Shikimic acid is important in the biosynthesis of aromantic compounds (Herrmann, 1995), while quinic acid is a side product of the shikimic pathway that may be used as a stored source for shikimic acid production (Marsh et al., 2009). Plants appear to produce secondary metabolites from shikimic acid to protect them from abiotic stress environments (Herms and Mattson, 1992; Rakhmankulova et al., 2003).

5.4.5 The P efficient wheat accumulated several amino acids to a higher level

In contrast to increased levels of amino acids in barley and lupin under low P (Huang et al., 2008; Muller et al., 2015), in this research, low P had no effect on most of the

amino acids and amines in both wheat genotypes (Figure 5.3, Table S 5.1, Table S 5.2). However, RAC875 showed significantly higher levels of aspartate, glutamine and β-alanine in leaves when compared to Wyalkatchem under low P supply. Aspartate is an important amino acid since it is a precursor for the biosynthesis of other amino acids (i.e. asparagine, lysine, methionine, isoleucine and threonine) and other essential cellular compounds (pyrimidine and NAD) (Jander and Joshi, 2009; Reitzer, 2004), while glutamine is considered as a hub for nitrogen metabolism and functions as an amino group donor for cellular processes (Sheppard, 2015). Thus, increased accumulation of aspartate and glutamine would enable RAC875 to maintain metabolic activities under P deficiency. β-alanine is known for the biosynthesis of β-alanine betaine (Duhazé et al., 2003) which has a protective role for plants being exposed to abiotic stress (Singh et al., 2015). Higher β-alanine in RAC875 contributes to greater β-alanine betaine production which may lead to RAC875 being more tolerant to P deficiency.

Conclusions

In general, changes in metabolites were observed in the wheat plants in response to P deficiency and differences in metabolites were found between the two wheat genotypes under P deficiency. Low P increased the levels of raffinose and 1-ketose in roots of both wheat genotypes. The efficient wheat, RAC875 accumulated higher raffinose in roots than the inefficient wheat, Wyalkatchem. RAC875 also maintains the levels of phosphorylated sugars (glucose-6-P and fructose-6-P) under low P, while P deficiency reduced these phosphorylated metabolites in both leaves and roots of Wyalkatchem. Organic acids of the TCA cycle (citrate, succinate and fumarate) had

no change under P deficiency, whereas at low P, shikimic acid and quinic acid increased in leaves of RAC875. RAC875 showed lower levels of fumarate, malate, maleate and itaconate in roots, when compared to Wyalkatchem under low P supply. P deficiency had no effect on most amino acids, however RAC875 accumulated greater aspartate, glutamine and β-alanine in leaves than Wyalkatchem under low P. Greater accumulation of raffinose in roots and aspartate, glutamine and β-alanine in leaves may contribute to more P efficient in RAC875. Maintaining levels of glucose-6-P and fructose-6-P would appear to maintain normal carbohydrate flux that is beneficial for the growth of RAC875 under P deficiency.

Supplemental tables

Table S 5.1. Fold-change in metabolite levels in leaves of efficient (RAC875) and inefficient (Wyalkatchem). Comparisons between low P supply and adequate P supply (low P/adequate P); and between RAC875 and Wyalkatchem (RAC875/Wyalkatchem) at each P supply. R: RAC875; W: Wyalkatchem; Low P: 10 mg P kg^{-1} soil (P10), adequate P: 30 mg P kg^{-1} soil (P30); sem: standard error of the mean. Blue color: significant at P<0.05 (t-test).

Metabolite	**Low P/adequate P**				**RAC875/Wyalkatchem**			
	RAC875		**Wyalkatchem**		**P10**		**P30**	
	P10/ P30	**sem**	**P10/ P30**	**sem**	**R/W**	**sem**	**R/W**	**sem**
Amino Acids and Amines								
4-amino-butyric acid	0.431	0.332	0.653	0.245	1.274	0.332	1.930	0.236
Adenine	0.791	0.071	0.856	0.118	1.097	0.071	1.188	0.074
Alanine	0.931	0.089	**0.700**	**0.112**	1.174	0.089	0.883	0.047
Aspartic acid	0.805	0.024	0.843	0.058	**1.180**	**0.024**	1.236	0.134
beta-alanine	1.061	0.113	0.867	0.159	**1.727**	**0.113**	**1.410**	**0.012**
Cytosine	0.922	0.102	1.270	0.074	0.883	0.102	1.217	0.096
Ethanolamine	0.987	0.218	0.795	0.077	1.010	0.218	0.813	0.058
Glutamic acid	0.878	0.085	0.981	0.083	1.066	0.085	1.190	0.150
Glutamine	0.724	0.185	0.489	0.170	**1.958**	**0.185**	1.322	0.145
Glycine	0.790	0.150	0.644	0.154	1.185	0.150	0.966	0.223
Guanine	1.211	0.082	1.133	0.036	1.027	0.082	0.961	0.078
Guanosine	1.102	0.057	0.768	0.067	**1.418**	**0.057**	0.988	0.061
Homoserine	0.945	0.218	0.702	0.212	1.145	0.218	0.850	0.303
Isoleucine	1.145	0.238	0.904	0.252	1.066	0.238	0.842	0.215
N-acetyl serine	**0.589**	**0.064**	0.912	0.143	0.695	0.064	1.075	0.124
Phenylalanine	1.026	0.066	0.977	0.116	0.848	0.066	0.807	0.110
Proline	0.812	0.527	0.795	0.354	1.772	0.527	1.735	0.327
Putrescine	0.856	0.137	**0.698**	**0.075**	0.780	0.137	**0.636**	**0.089**
Pyroglutamic acid	**0.529**	**0.093**	0.560	0.293	0.848	0.093	0.897	0.058
Serine	0.704	0.236	0.792	0.191	0.828	0.236	0.932	0.260
Threonine	0.775	0.083	0.715	0.118	1.172	0.083	1.081	0.096
Uracil	0.719	0.114	1.036	0.131	0.854	0.114	1.231	0.065
Valine	0.925	0.150	0.829	0.148	1.114	0.150	0.999	0.179
Organic Acids								
2- oxo-glutaric acid	1.259	0.086	0.778	0.107	1.288	0.086	0.796	0.090
2-methyl Maleic acid/Fumaric acid	0.854	0.043	1.240	0.049	0.909	0.043	1.319	0.079
4-hydroxy-benzoic acid	**0.607**	**0.095**	1.059	0.140	0.758	0.095	1.322	0.035

Table S 5.1. Fold-change in metabolite levels in leaves of efficient (RAC875) and inefficient (Wyalkatchem). Comparisons between low P supply and adequate P supply (low P/adequate P); and between RAC875 and Wyalkatchem (RAC875/Wyalkatchem) at each P supply. R: RAC875; W: Wyalkatchem; Low P: 10 mg P kg^{-1} soil (P10), adequate P: 30 mg P kg^{-1} soil (P30); sem: standard error of the mean. Blue color: significant at P<0.05 (t-test) (continue)

Metabolite	**Low P/adequate P**				**RAC875/Wyalkatchem**			
	RAC875		**Wyalkatchem**		**P10**		**P30**	
	P10/ P30	**sem**	**P10/ P30**	**sem**	**R/W**	**sem**	**R/W**	**sem**
Aconitic acid	**1.334**	**0.039**	**1.304**	**0.053**	0.985	0.039	0.963	0.088
Benzoic acid	**0.878**	**0.029**	0.990	0.046	0.975	0.029	1.099	0.028
Caffeic acid	0.941	0.067	1.147	0.152	0.823	0.067	1.003	0.017
Citric acid	0.714	0.133	0.920	0.155	0.897	0.133	1.156	0.262
Ferulic acid	1.094	0.143	1.232	0.151	1.129	0.143	1.271	0.070
Aconitic acid	**1.334**	**0.039**	**1.304**	**0.053**	0.985	0.039	0.963	0.088
Benzoic acid	**0.878**	**0.029**	0.990	0.046	0.975	0.029	1.099	0.028
Caffeic acid	0.941	0.067	1.147	0.152	0.823	0.067	1.003	0.017
Citric acid	0.714	0.133	0.920	0.155	0.897	0.133	1.156	0.262
Ferulic acid	1.094	0.143	1.232	0.151	1.129	0.143	1.271	0.070
Fumaric acid	1.205	0.074	1.231	0.056	0.976	0.074	0.997	0.053
Glyceric acid	0.997	0.062	1.100	0.079	1.025	0.062	1.131	0.111
Glyceric acid-3-phosphate	1.172	0.188	0.685	0.252	0.991	0.188	**0.579**	**0.190**
Glycolic acid	0.909	0.032	1.104	0.033	0.941	0.032	1.143	0.045
Iso Citric	**1.228**	**0.051**	1.055	0.069	1.125	0.051	0.966	0.051
Itaconate	0.953	0.082	**1.579**	**0.065**	**0.750**	**0.082**	1.243	0.115
Malic acid	1.150	0.046	1.144	0.039	0.940	0.046	0.936	0.110
Malonic acid	**1.647**	**0.172**	1.185	0.123	1.032	0.172	0.743	0.075
Oxalic acid	1.203	0.275	1.247	0.239	1.041	0.275	1.079	0.236
Qunic acid	**2.861**	**0.191**	1.226	0.230	0.899	0.191	**0.385**	**0.191**
Saccharic acid	**1.621**	**0.135**	1.166	0.174	1.064	0.135	0.765	0.107
Shikimic acid	**1.555**	**0.019**	**1.178**	**0.041**	0.999	0.019	**0.757**	**0.060**
Sinapic acid	0.886	0.060	1.119	0.082	**0.647**	**0.060**	0.818	0.145
Succinic acid	1.023	0.071	0.848	0.108	0.965	0.071	0.800	0.075
Threonic acid	1.199	0.093	0.939	0.160	1.101	0.093	0.862	0.055
Sugars, Sugar Alcohol and Sugar Phosphates								
Arabinose	1.015	0.043	1.101	0.029	0.945	0.043	1.025	0.009
Arabitol	0.746	0.187	1.197	0.143	0.969	0.187	**1.554**	**0.124**
Beta Gentibiose	1.032	0.178	1.099	0.088	1.111	0.178	1.183	0.097
Erythritol	0.894	0.078	1.126	0.122	1.127	0.078	**1.419**	**0.037**
Fructose	0.893	0.306	1.047	0.340	0.935	0.306	1.096	0.330

Table S 5.1. Fold-change in metabolite levels in leaves of efficient (RAC875) and inefficient (Wyalkatchem). Comparisons between low P supply and adequate P supply (low P/adequate P); and between RAC875 and Wyalkatchem (RAC875/Wyalkatchem) at each P supply. R: RAC875; W: Wyalkatchem; Low P: 10 mg P kg^{-1} soil (P10), adequate P: 30 mg P kg^{-1} soil (P30); sem: standard error of the mean. Blue color: significant at P<0.05 (t-test) (continue)

Metabolite	**Low P/adequate P**				**RAC875/Wyalkatchem**			
	RAC875		**Wyalkatchem**		**P10**		**P30**	
	P10/ P30	**sem**	**P10/ P30**	**sem**	**R/W**	**sem**	**R/W**	**sem**
Fructose-6-phosphate	0.774	0.275	**0.581**	**0.201**	1.381	0.275	1.036	0.225
Galactinol	**1.306**	**0.039**	0.884	0.089	1.107	0.039	0.749	0.110
Galactitol	0.781	0.234	0.967	0.157	0.725	0.234	0.898	0.199
Galactonic acid	0.878	0.123	0.992	0.019	**0.720**	**0.123**	**0.813**	**0.071**
Galactose	0.990	0.191	1.177	0.144	0.788	0.191	0.936	0.100
Gluconate	0.956	0.149	1.041	0.149	1.114	0.149	1.212	0.094
Gluconate-6-phosphate	1.603	0.388	0.620	0.331	1.254	0.388	0.485	0.468
Glucose	0.980	0.191	1.012	0.256	1.134	0.191	1.171	0.224
Glucose-6-phosphate	0.875	0.295	0.560	0.243	1.353	0.295	0.865	0.168
Maltose	1.433	0.269	0.623	0.204	1.840	0.269	0.800	0.384
Manitol	0.643	0.106	0.858	0.319	0.397	0.106	0.530	0.177
Manose	0.894	0.144	0.861	0.159	1.185	0.144	1.141	0.076
myo-Inositol	0.983	0.038	1.018	0.101	1.062	0.038	1.099	0.062
Raffinose	1.101	0.097	0.972	0.310	1.031	0.097	0.910	0.176
Ribitol	0.981	0.035	1.006	0.060	0.941	0.035	0.965	0.070
Ribose	**0.765**	**0.065**	0.975	0.131	1.029	0.065	1.311	0.088
Sucrose	0.960	0.070	0.979	0.155	0.945	0.070	0.964	0.114
Threitol	0.755	0.191	1.086	0.152	1.232	0.191	**1.773**	**0.134**
Trehelose	1.254	0.034	0.884	0.093	1.248	0.034	0.880	0.098
Xylitol	0.756	0.148	0.961	0.092	1.374	0.148	**1.746**	**0.090**
Xylose	**1.336**	**0.061**	1.138	0.031	**1.248**	**0.061**	1.063	0.019
Others								
Glycerol	1.139	0.096	0.595	0.102	0.887	0.096	**0.464**	**0.144**
Hexadecanoic acid	1.185	0.070	1.047	0.054	1.082	0.070	0.956	0.022
Monomethylphosphate	0.851	0.098	0.867	0.165	0.996	0.098	1.015	0.056
Octadecanoic acid	1.056	0.033	1.043	0.027	1.050	0.033	1.036	0.035
Octadecatrienoic acid	**2.227**	**0.160**	1.312	0.201	1.024	0.160	0.603	0.142
Phosphoric acid	0.844	0.184	0.526	0.297	1.189	0.184	0.740	0.236
Threonic acid-1,4-lactone	0.918	0.052	0.856	0.051	0.996	0.052	0.929	0.118

Table S 5.2. Fold-change in metabolite levels in roots of efficient (RAC875) and inefficient (Wyalkatchem). Comparisons between low P supply and adequate P supply (low P/adequate P); and between RAC875 and Wyalkatchem (RAC875/Wyalkatchem) at each P supply. R: RAC875; W: Wyalkatchem; Low P: 10 mg P kg^{-1} soil (P10), adequate P: 30 mg P kg^{-1} soil (P30); sem: standard error of the mean. Blue color: significant at P<0.05 (t-test); green color: significant at P<0.05 with Bonferroni correction.

Metabolite	**Low P/adequate P**				**RAC875/Wyalkatchem**			
	RAC875		**Wyalkatchem**		**P10**		**P30**	
	P10/ P30	**sem**	**P10/ P30**	**sem**	**R/W**	**sem**	**R/W**	**sem**
Amino Acids and Amines								
Alanine	0.881	0.144	1.009	0.097	0.720	0.144	0.825	0.132
Alanine, beta-	1.269	0.051	1.122	0.040	0.996	0.051	0.881	0.133
Allantoin	1.204	0.182	**2.108**	**0.128**	0.874	0.182	1.531	0.219
Asparagine	0.772	0.274	1.057	0.236	0.901	0.274	1.233	0.190
Aspartic acid	0.795	0.108	0.889	0.061	0.804	0.108	0.899	0.140
Butyric acid, 4-amino-	0.891	0.092	0.958	0.067	1.223	0.092	1.314	0.171
Cytosine	0.871	0.031	0.945	0.064	0.915	0.031	0.993	0.124
Ethanolamine	0.822	0.152	0.853	0.074	1.088	0.152	1.129	0.199
Glutamine	0.818	0.552	0.913	0.188	0.829	0.552	0.925	0.357
Glycine	0.979	0.050	1.128	0.057	0.898	0.050	1.034	0.117
Guanine	0.867	0.118	1.180	0.094	0.912	0.118	1.241	0.145
Guanosine	1.756	0.173	1.261	0.173	1.098	0.173	0.788	0.218
Homoserine	0.976	0.105	0.946	0.070	1.052	0.105	1.021	0.162
Isoleucine	1.013	0.068	1.084	0.074	0.914	0.068	0.978	0.187
Leucine	1.008	0.105	1.171	0.054	0.914	0.105	1.062	0.188
Lysine	1.009	0.109	1.183	0.109	0.930	0.109	1.091	0.180
O-acetyl Serine	0.712	0.116	**0.794**	**0.073**	1.102	0.116	1.228	0.111
Phenylalanine	1.067	0.047	1.057	0.048	1.014	0.047	1.005	0.155
Proline	0.911	0.153	0.844	0.072	1.078	0.153	0.999	0.137
Putrescine	0.936	0.165	0.762	0.206	0.869	0.165	0.708	0.164
Pyroglutamic acid	0.892	0.114	0.747	0.060	1.348	0.114	1.130	0.152
Serine	**0.745**	**0.090**	0.815	0.055	1.117	0.090	1.221	0.090
Threonine	0.801	0.061	**0.835**	**0.046**	1.064	0.061	1.110	0.115
Tyrosine	0.926	0.105	0.924	0.080	1.085	0.105	1.083	0.184
Uracil	**0.478**	**0.167**	1.055	0.222	0.565	0.167	1.247	0.167
Valine	0.914	0.090	0.938	0.056	1.071	0.090	1.099	0.136
Organic Acids								
4-Hydroxycinnamic acid	0.800	0.088	1.091	0.074	0.888	0.088	1.211	0.145
Citric acid	1.199	0.046	1.082	0.064	1.076	0.046	0.972	0.067
Ferulic acid, trans-	0.652	0.097	1.122	0.093	**0.667**	**0.097**	1.148	0.139
Fumaric acid	1.075	0.110	1.079	0.057	**0.704**	**0.110**	0.706	0.164
Galactonic acid	1.070	0.053	**1.342**	**0.015**	***0.600***	***0.053***	0.752	0.090
Gluconate	1.042	0.101	**1.368**	**0.056**	0.848	0.101	1.113	0.206

Table S 5.2. Fold-change in metabolite levels in roots of efficient (RAC875) and inefficient (Wyalkatchem). Comparisons between low P supply and adequate P supply (low P/adequate P); and between RAC875 and Wyalkatchem (RAC875/Wyalkatchem) at each P supply. R: RAC875; W: Wyalkatchem; Low P: 10 mg P kg^{-1} soil (P10), adequate P: 30 mg P kg^{-1} soil (P30); sem: standard error of the mean. Blue color: significant at P<0.05 (t-test); green color: significant at P<0.05 with Bonferroni correction (continue).

Metabolite	**Low P/adequate P**				**RAC875/Wyalkatchem**			
	RAC875		**Wyalkatchem**		**P10**		**P30**	
	P10/ P30	**sem**	**P10/ P30**	**sem**	**R/W**	**sem**	**R/W**	**sem**
Glutamic acid	0.860	0.140	0.840	0.080	0.901	0.140	0.879	0.127
2- oxo-glutaric acid	0.765	0.248	0.656	0.138	0.790	0.248	0.677	0.198
Glyceric acid	1.156	0.042	**1.363**	**0.055**	**0.772**	**0.042**	0.910	0.140
Glycolic acid	**0.629**	**0.099**	1.108	0.161	0.654	0.099	1.152	0.117
Iso Citric	1.016	0.021	1.070	0.061	0.893	0.021	0.941	0.079
Itaconate	**0.378**	**0.127**	1.053	0.232	**0.468**	**0.127**	1.305	0.166
Maleate	0.552	0.095	1.181	0.121	**0.519**	**0.095**	1.111	0.170
Malic acid	1.209	0.055	**1.287**	**0.081**	**0.700**	**0.055**	0.745	0.149
Malonic acid	1.123	0.089	1.095	0.126	0.705	0.089	0.687	0.200
Nonaoic acid	1.083	0.024	1.179	0.057	1.066	0.024	1.161	0.097
Pipecolate	0.568	0.235	**0.684**	**0.122**	0.803	0.235	0.968	0.234
Pyruvic acid	1.084	0.109	0.921	0.114	1.052	0.109	0.894	0.107
Quinic acid	1.375	0.117	1.181	0.130	0.760	0.117	0.652	0.260
Saccharic acid	1.058	0.073	0.988	0.084	**1.392**	**0.073**	1.300	0.155
Shikimic acid	1.436	0.090	1.151	0.114	1.313	0.090	1.053	0.133
Succinic acid	0.944	0.093	0.878	0.046	0.829	0.093	0.771	0.150
Threonic acid	1.014	0.048	1.018	0.060	**0.683**	**0.048**	**0.686**	**0.134**
Sugars, Sugar Alcohol and Sugar Phosphates								
1-kestose	**2.952**	**0.175**	**2.049**	**0.142**	**2.026**	**0.175**	1.406	0.269
Arabinose	0.966	0.136	**1.178**	**0.017**	0.919	0.136	1.121	0.108
Arabitol	0.871	0.037	1.272	0.296	0.811	0.037	1.184	0.389
Beta Gentibiose	1.445	0.182	1.431	0.321	0.727	0.182	0.720	0.113
Fructose	0.933	0.117	0.993	0.052	0.848	0.117	0.903	0.186
Fructose-6-phosphate	1.173	0.161	**0.530**	**0.147**	1.334	0.161	0.603	0.176
Fucose	1.041	0.136	1.058	0.084	1.054	0.136	1.071	0.212
Galactinol	0.996	0.053	0.890	0.110	1.063	0.053	0.949	0.281
Galactose	0.900	0.140	1.003	0.062	0.835	0.140	0.930	0.204
Galactosylglycerol	1.531	0.102	**2.017**	**0.087**	0.772	0.102	1.017	0.174
Glucose	0.889	0.141	1.014	0.060	0.826	0.141	0.942	0.207
Glucose-6-phosphate	1.003	0.126	**0.492**	**0.149**	1.306	0.126	0.641	0.174
Inositol myo	1.015	0.082	1.083	0.063	0.915	0.082	0.976	0.136
Maltose	1.300	0.097	1.468	0.033	1.016	0.097	1.148	0.230
Mannitol	1.330	0.164	2.374	0.398	0.695	0.164	1.240	0.392
Mannose	1.307	0.150	**1.283**	**0.039**	1.108	0.150	1.087	0.189
Raffinose	**2.805**	**0.159**	**2.002**	**0.133**	**1.897**	**0.159**	1.354	0.252

Table S 5.2. Fold-change in metabolite levels in roots of efficient (RAC875) and inefficient (Wyalkatchem). Comparisons between low P supply and adequate P supply (low P/adequate P); and between RAC875 and Wyalkatchem (RAC875/Wyalkatchem) at each P supply. R: RAC875; W: Wyalkatchem; Low P: 10 mg P kg^{-1} soil (P10), adequate P: 30 mg P kg^{-1} soil (P30); sem: standard error of the mean. Blue color: significant at P<0.05 (t-test); green color: significant at P<0.05 with Bonferroni correction (continue).

Metabolite	**Low P/adequate P**				**RAC875/Wyalkatchem**			
	RAC875		**Wyalkatchem**		**P10**		**P30**	
	P10/ P30	**sem**	**P10/ P30**	**sem**	**R/W**	**sem**	**R/W**	**sem**
Rhamnose	1.087	0.103	1.015	0.088	1.012	0.103	0.945	0.145
Ribitol	1.017	0.110	**0.828**	**0.073**	1.035	0.110	0.843	0.176
Ribose	1.080	0.095	0.989	0.094	1.027	0.095	0.941	0.159
Sucrose	1.343	0.075	1.046	0.048	1.180	0.075	0.919	0.101
Trehelose	0.946	0.072	1.001	0.083	1.090	0.072	1.153	0.167
Xylitol	0.972	0.085	1.013	0.080	1.031	0.085	1.074	0.128
Xylose	1.182	0.042	**1.268**	**0.047**	0.927	0.042	0.995	0.144
Others								
Benzoic acid, 4-hydroxy-	**0.589**	**0.123**	0.938	0.149	0.679	0.123	1.079	0.135
Glycerol	0.886	0.082	0.952	0.091	0.882	0.082	0.947	0.183
Glycerol-3-P	**0.547**	**0.137**	**0.523**	**0.099**	0.946	0.137	0.905	0.163
Hexadecanoic acid	1.185	0.047	1.208	0.064	1.128	0.047	1.150	0.118
Monomethylphosphate	1.321	0.267	0.791	0.212	1.094	0.267	0.655	0.178
Octadecadienoic acid,9,12-Z,Z	1.443	0.151	1.016	0.230	1.201	0.151	0.846	0.141
Octadecanoic acid, n-	1.540	0.129	1.151	0.120	1.474	0.129	1.101	0.124
Octadecatrienoic acid, 9,12,15-Z,Z	1.489	0.133	0.980	0.303	1.014	0.133	0.668	0.093
Phosphoric acid	***0.360***	***0.056***	***0.358***	***0.072***	**0.727**	**0.056**	**0.724**	**0.098**
Threonic acid-1,4-lactone	0.774	0.050	0.834	0.082	**0.583**	**0.050**	**0.629**	**0.156**
Urea	1.460	0.134	**2.613**	**0.117**	0.825	0.134	1.476	0.213

CHAPTER 6

General discussion

6.1 Responses to P of wheat genotypes and environmental effects

Responses to P differed among the six selected wheat genotypes (RAC875, Scout, Gladius, Mace, Correll and Wyalkatchem) but were not all consistent between growth conditions (in a greenhouse and in a growth room) (Chapter 2). Scout and Mace highly responsive to high P supply under both controlled growth conditions, while RAC875 and Wyalkachem were not responsive genotypes. Responsiveness to high P supply also occurred in Mace, whereas RAC875 was not responsive to high P under field conditions (McDonald et al., 2015). Although Mace and Scout produced high yield at high P, they had low P use efficiency (PUE) (defined as relative grain yield ratio between low P and adequate P) under both growth conditions. RAC875 and Wyalkatchem differed in PUE between the two growth environments of greenhouse and growth room. Indeed, PUE was high in RAC875 but low in Wyalkatchem under growth room conditions, while this value was the opposite under greenhouse conditions. The effect of growth conditions affected P responses to wheat was observed, in which most wheat genotypes behave differently in their response to low P under greenhouse and field conditions (Gunes et al., 2006). It is, therefore, important to select growth conditions where plants show similar responses to P when compared to field evaluation and this particularly important for mechanistic studies of PUE. This study showed that under growth room conditions, RAC875 had greater PUE than Wyalkatchem and this was consistent with published field responses (McDonald et al.,

2015).

Reasons for the differences in response witnessed between greenhouse and growth room were of a complex nature. One would expect similar expression of yield potential and P efficiency between the two growth environments as the same soil type was use and extraneous environmental effects were kept to a minimum, but the responses between the two environments were dissimilar. Subtle environmental factors (i.e. light intensity, temperature) may have resulted in differences in PUE of RAC875 and Wyalkatchem under different growth conditions and light intensity was one obvious discrepancy between the two environments. An experiment was conducted in the growth room at two different light intensities to identify whether responses to P in these two wheat genotypes were affected by this parameter (Chapter 3). The results showed that light intensity impacted responses to P of the two wheat genotypes. Compared to RAC875, Wyalkatchem highly responsive to adequate P under low light intensity, while their responses were similar under high light intensity. Yin et al. (2012) also indicated that responses to P of two weed species behaved differently to light intensity. Although light intensity had some effect on P responsiveness in the two wheat genotypes, RAC875 showed greater PUE than Wyalkatchem under both light intensities, so it is unclear why differences in efficiency exist between the two environments. It is possible that there could be differences in temperature or the selective penetration of wavelengths with the thick walled polycarbonate greenhouse selectively inhibiting the penetration of red light but this was not investigated and could be explored in follow-up studies.

The results showed that PUE of two wheat genotypes RAC875 and Wyalkatchem were

consistent between experiments under growth room conditions. Indeed, RAC875 showed greater PUE than Wyalkatchem at maturity in three different experiments (Table 2.7, Figure 3.3, Table S 4.3). At vegetable stages (27 DAS, 28 DAS and 48 DAS), RAC875 also presented higher shoot dry matter than Wyalkatchem under low P supply in two different experiments (Table S 4.1, Figure 5.2 A). Furthermore, RAC875 also had greater PUE than Wyalkatchem in published results from field experiments (McDonald et al., 2015). Since there is the consistence in PUE between greenhouse and field condition, the growth room was used to evaluate the mechanisms of PUE in RAC875.

6.2 Root traits and PUE

Roots are important for plants to uptake nutrients and water (Paez-Garcia et al., 2015) and it is crucial to investigate root traits associated with PUE in plants. Results from Chapter 2 showed that root dry matter (DM) and root volume varied among the six wheat genotypes. RAC875 and Scout possess small root systems, while Wyalkatchem harbors a large root system (Chapter 2). In contrast, RAC875 and Scout produced greater grain yield, whereas Wyalkatchem had low grain yield under low P. It appears that root DM and grain yield are negatively correlated. Indeed, root dry matter of the six wheat genotypes had a strongly negative correlation to grain yield under low P (Chapter 2). This result agrees with a recent report in which wheat genotypes with smaller root systems had greater P acquisition efficiency than wheat genotypes with larger root systems (da Silva et al., 2016). Although a number of studies indicated that plants enhanced their root growth in response to P starvation (Gaume et al., 2001; Horst et al., 1996; Steingrobe et al., 2001), root efficiency seems to be more important

than the root size. Root efficiency is calculated as shoot P uptake per unit of root dry matter and appears to be a beneficial trait when screening for P efficiency in wheat (Jones et al., 1989). In fact, this research revealed that root efficiency was positively correlated with PUE at both low and adequate P supply under growth room conditions (Chapter 2). In other words, the wheat genotypes having greater root efficiency showed higher PUE. According to Jones et al. (1989) root efficiency is an indication as to structure of a root system and its soil explorative capacity; therefore, an exploration of further root architectural and morphological traits could be useful for improved P efficiency in wheat.

A new simple soil-based cultivation was developed for root architectural phenotyping to investigate whether RAC875 possesses beneficial root traits leading to a greater root efficiency when compared to Wyalkatchem. This method used rhizoboxes which were modified from a similar design reported by Courtois et al. (2013), where instead of a hydroponic system and the use of glass beads, soil based culture was used. This cultivation method is simple and has some advantages when compared to previous methods. For instance, original root architectures were altered when roots were harvested from a pot experiment (da Silva et al., 2016), while roots were obtained without destruction of root systems in the method reported here. Roots can be visualized and able to be imaged directly, without destruction as when using gel-based growth in glass tubes (Iyer-Pascuzzi et al., 2010). Roots may also behave differently when grown in gels and soil is the preferred growth medium. Advanced techniques such as X-ray (Mooney et al., 2012) or magnetic resonance imaging (MRI) (van Dusschoten et al., 2016) can be used for root imaging in soils without root destruction,

but they are very costly. Thus, the rhizobox design presented in this study can be beneficial to the scientific community for analyzing 2-D architectural root traits (Chapter 4).

A variety of root architectural traits were measured including root top angle, convex hull area (CHA), root surface area, total root length, root volume and root tip number. Root surface area, root volume, root tip number and total root length reduced under low P compared to adequate P. A reduction in root surface area, root tip number and total root length was also observed on barley under low P (Wang et al., 2015), but low P enhanced root length in wheat grown under hydroponic conditions (Horst et al., 1996) and in barley in a field experiment (Steingrobe et al., 2001). These differences could be due to different growth methods or harvest stages. Under low P supply, no significant variation was witnessed for root surface area, root volume, root tip number and total root length between the two wheat genotypes, however relative ratios of these parameters between low P and adequate P in RAC875 were greater than those in Wyalkatchem. Although topsoil foraging is beneficial for P acquisition in common bean and maize under low P supply (Zhu et al., 2005b; Lynch and Brown, 2001), root top angle was not different between the two wheat genotypes, suggesting that wheat may have different adaptive mechanisms in response to P deficiency. RAC875 had greater CHA than Wyalkatchem under low P, while Wyalkatchem showed larger CHA than RAC875 under adequate P. Besides, CHA was positively correlated to shoot DM under low P, therefore CHA may be associated with great production of shoot biomass and grain yield in RAC875 under low P (Chapter 4).

Root hairs are important for P acquisition and therefore the correlation of root hair

features and PUE was investigated. In contrast to previous studies showing that root hair length (RHL) increased in *Arabidopsis thaliana* (Bates and Lynch, 2000a), in maize (Zhu et al., 2010) and in rice (Vejchasarn et al., 2016) under low P, this study found that low P had no significant effect on RHL (Chapter 4). Genetic differences between plants may lead to the differences in their responses to P. Yuan et al. (2016) indicated that RHL increased when P supply increased. Apparently, results varied among studies, thus a large number of genotypes should be evaluated to understand whether plants enhance RHL in response to low P. On contrary to RHL, low P significantly increased root hair density (RHD) in RAC875, but had no impact on Wyalkatchem. In regard to variation between genotypes, Wyalkatchem had greater RHL than RAC875 under both low and adequate P supply, while RAC875 showed greater RHD than Wyalkatchem under low P (Chapter 4). RAC875 also harboured larger rhizosheath size than Wyalkatchem under low P. This suggests that denser root hairs seem to correlate with larger rhizosheath size in RAC875 under low P although it is reported that rhizosheath size is correlated with root hair length (Delhaize et al., 2015). The presence of root hairs is important for nutrient uptake and plant growth (Bates and Lynch, 2000b) and rhizosheath size is associated with shoot biomass (James et al., 2016). Thus, denser root hairs and larger rhizosheath size may contribute to greater shoot biomass and grain yield production in RAC875 under low P supply.

6.3 Accumulation of raffinose and amino acids and remaining levels of glucose-6-P and fructose-6-P contribute to greater P efficiency

Although previous studies have shown that plants adjust their metabolic responses to adapt to P deficiency (Huang et al., 2008; Ganie et al., 2015; Muller et al., 2015), to

my knowledge, this information on wheat has not been reported. Metabolite profiling in two wheat genotypes with contrasting PUE can contribute to the exploration of metabolic mechanisms of PUE in wheat. This research led to some very interesting results. In this study, low P was found to increase the raffinose level in roots, which is consistent with a previous observation (Huang et al., 2008). Interestingly, the P efficient RAC875 accumulated greater raffinose in roots (Chapter 5). In this context, a greater accumulation of raffinose appears to be associated with higher P efficiency in RAC875 and this seems to agree with a previous report that raffinose functions as a stored carbohydrate and a stress tolerant factor (Van den Ende, 2013). A wider evaluation of germplasm for raffinose is a worthwhile outcome of this thesis as it indicates a potential trait for P efficiency in wheat.

Glycolysis is important for plants since it not only generates energy through respiration for cellular activities, but it also converts sugars from photosynthesis into intermediates for biosynthesis of other essential molecules for plant growth and development (Plaxton, 1996). Indeed, P deficiency decreased phosphorylated sugars (glucose-6-P and fructose-6-P) which are important for glycolysis (Huang et al., 2008; Muller et al., 2015) and this could be a reason resulting in a reduction in plant yield. Although alternative bypass reactions of glycolysis can be activated to maintain cellular functions under low P (Plaxton and Podesta, 2006; Plaxton and Tran, 2011), maintaining normal glycolysis under low P would be associated with P efficiency in plants. Indeed, the phosphorylated sugars, glucose-6-P and fructose-6-P remained unchanged at low P supply in the P efficient wheat, while they were reduced in the P inefficient wheat (Chapter 5).

Several leaf amino acids including aspartate, glutamine and β-alanine accumulated greater in the P efficient wheat under low P supply (Chapter 5). Aspartate is important for biosynthesis of amino acids within aspartic acid family and other essential cellular compounds (pyrimidine and NAD) (Jander and Joshi, 2009; Reitzer, 2004), and glutamine plays a key role in nitrogen metabolism (Sheppard, 2015). The increased accumulation of aspartate and glutamine may consequently assist RAC875 maintain its amino acid and nitrogen metabolism under low P. Higher β-alanine may also contribute to greater P efficiency in RAC875 since β-alanine is a precursor for the biosynthesis of β-alanine betaine (Duhazé et al., 2003) which has a protective role for plants from abiotic stress (Singh et al., 2015).

6.4 Conclusions and significance of research

Variation in PUE was observed in six wheat genotypes and responses to P were shown to be affected by differing environment. It was possible to find a controlled environment where one could get similar responses to low P supply, when compared to field conditions. The soil type used in the study is most likely one of the major reasons for this success and allows for a larger screening of germplasm, cultivars and elite lines, in follow-up experiments.

A simple soil-based cultivation was developed to obtain roots for 2-D root architectural analysis. This method is advantageous since it is soil-based and roots can be obtained without destruction. Therefore, this method can be used to study root architectural traits in plants, particularly in crops with sparse root systems which are difficult to obtain without affecting their original architecture from soil-based growth.

The P efficient wheat, RAC875 possessed a greater convex hull area, denser root hair and larger rhizosheath size than the P inefficient wheat (Wyalkatchem) under low P. Thus, convex hull area, root hair density and rhizosheath size can be beneficial for screening P efficient wheat. A wider use of these potential indicators of P efficiency is warranted.

RAC875 has an ability to maintain adequate levels of phosphorylated sugars (glucose-6-P and fructose-6-P), which are important for glycolysis, in leaves and roots under low P, suggesting a mechanism of efficiency in RAC875.

Under low P, RAC875 was found to accumulate greater raffinose, a carbohydrate resource and an abiotic tolerant factor, in roots and higher levels of amino acids in leaves including glutamine, which is important for nitrogen metabolism, than Wyalkatchem. Again, it would seem that this is a part of the mechanism of efficiency in RAC875, but further evaluation is required in a larger pool of germplasm, cultivars or elite lines.

This work is to my knowledge, the first investigation of variation in metabolites between P efficient and inefficient wheat genotypes grown under P deficiency. It provides for follow-up research to explore the mechanism of efficiency even further and provides potential targets for further genetic manipulation so that plant breeders have traits for breeding P efficient wheat.

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