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ORIGINAL ARTICLE

Variation in root system architecture and morphology of two wheat genotypes is a predictor of their tolerance to phosphorus deficiency

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Abstract

Variation in root system architecture (RSA) and morphology are important for improving phosphorus use efficiency (PUE) in wheat. This work aims to analyze the root system architecture and morphology of wheat genotypes to explain their variation in PUE. Two genotypes differing in PUE, RAC875 (P efficient) and Wyalkatchem (P inefficient) were grown in a sandy soil amended with adequate basal nutrients and two P rates (10 and 30 mg P kg^{-1}). RSA traits were measured by freely available software, GiA roots and DIRT, and root hair features were measured using a microscope with an attached camera and LAS v3.6 software. Under low P supply, RAC875 produced greater shoot dry matter (DM) at 24, 27 and 48 days after sowing (DAS), and at maturity, RAC875 also had a higher grain yield at maturity. Enhanced P efficiency (biomass and seed yield at inadequate P supply relative to adequate P supply) was observed more so in RAC875 at all harvest times. P supply affected most RSA traits, with low P leading to reductions in convex hull area (CHA), root surface area, root volume, total root length and root tip number. RAC875 produced significantly greater CHA than Wyalkatchem at low P supply while Wyalkatchem had significantly larger CHA than RAC875 when P was non-limiting. RAC875 also had greater root hair density (RHD) than Wyalkatchem at low P level, but no difference was observed at adequate P. When grown under low P supply, a greater CHA and RHD in RAC875 were likely to contribute to improved P uptake, resulting in its higher shoot biomass and grain yield.

Keywords Convex hull area (CHA) · Phosphorus use efficiency (PUE) · Rhizoboxes · Root hair density (RHD) · Root system architecture (RSA)

Abbreviations

CHA	Convex hull area
DAS	Day after sowing
DM	Dry matter
ICP-MS	Inductively coupled plasma mass spectrometry
P	Phosphorus
PAE	Phosphorus acquisition efficiency
PUE	Phosphorus use efficiency
PUE_{GY}	Phosphorus use efficiency calculated as the ratio of grain yield under low P to grain yield under adequate P
PUE_{SM}	Phosphorus use efficiency calculated as the ratio of shoot matter under low P to shoot matter under adequate P
PUtE	Phosphorus utilization efficiency
RSA	Root system architecture
RHD	Root hair density

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Introduction

As roots are the conduit for mineral and water acquisition from soils, they are logically a target for manipulation to improve crop productivity on soils with poor nutrition (Meister et al. 2014). Root system architecture (RSA) and morphology are factors used to define properties of root systems; therefore, it is vital to identify their favorable traits for

the improvement of crop yield. Root morphology refers to the features of a single root axis as an organ, including root hairs, root diameter and cortical senescence. RSA relates to the whole root system or a large subset of the root system and may be described as topological or geometric measures of the root shape (Lynch 1995; Bucksch et al. 2014). Wheat has the monocot root system that consists of several seminal roots and adventitious roots (Sinha et al. 2018). Traits often used for wheat roots are total root length, root surface area, root volume, root angle, number of roots and root diameter (Ahmadi et al. 2018; York et al. 2018). Although a number of platforms have been developed for root phenotyping, genetic studies on root traits are hindered due to their complexity, underground location and interactions with the environment (Kuijken et al. 2015; Paez-Garcia et al. 2015; Topp et al. 2016). Therefore, suitable methods need to be developed to explore root traits.

Current efforts to study the structure of crop root systems have resulted in a number of root phenotyping platforms that are able to elucidate RSA under various conditions, including laboratory, greenhouse 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 has been developed for RSA characterization including manual, semi-automatic and automatic programs (Lobet et al. 2015). Root images can be obtained with or without the destruction of root systems, depending on the cultivation method and imaging techniques. Each method has its own advantages and disadvantages. Using a gel-based growth platform, roots are visible and can be easily imaged in situ (Iyer-Pascuzzi et al. 2010), but root behavior in gel may not be comparable to root behavior in soils. In contrast, intact root systems cannot be imaged in soil growth systems as roots need to be extracted before being imaged (da Silva et al. 2016); therefore, some root traits such as root angle and CHA are not able to be accurately measured. Although advanced techniques such as X-ray (Mooney et al. 2012) or magnetic resonance imaging (MRI) (van Dusschoten et al. 2016) can be applied for non-destructive imaging in soils, these are expensive approaches. Glass bead-based cultivation in specialized rhizoboxes is a good approach to retain two-dimensional (2D) RSA in rice (Courtois et al. 2013), but this method does not use soil-based cultivation. Thus, the development of specialized soil-based growth rhizoboxes to obtain root systems without destruction is ideally needed for RSA analysis.

Phosphorus (P) deficiency occurs in the majority of terrestrial ecosystems and reduces crop productivity (Shenoy and Kalagudi 2005; Lynch 2011). This sub-optimal supply 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 (Jones et al. 1989; Liu 2015; Roberts and Johnston 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; Lambers et al. 2006; Lynch 2007; Wang et al. 2010).

In response to P deficiency, plants may display a variety of adaptation mechanisms, one of which is modification of RSA/morphology (Zhu et al. 2006; Niu et al. 2013). Indeed, plants can adjust their root systems to P stress via stimulation of root and lateral root growth (Gaume et al. 2001; Zhu et al. 2005a), enhancement of root hair development (Foehse and Jungk 1983; Bates and Lynch 1996) and formation of cluster root (Wasaki et al. 2003; Abdolzadeh et al. 2010). The increase in root hair length and root hair density under low P was observed in wheat (Wang et al. 2016b). Genotypic variation in RSA and morphology under P starvation has been observed in food crops. For example, studies in wheat have shown that root hair length and density correlate with P acquisition (Gahoonia et al. 1997; James et al. 2016). Wheat genotypes with a shallow root system exhibit greater P uptake efficiency (da Silva et al. 2016). Also, 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 more P-efficient crops through plant breading or genetic modification.

Genotypic variations that are linked with yield improvements and mechanisms for coping with limited P supply are an important area of study. Modification of root architecture is one of the mechanisms for plants to cope with P stress; however, previous wheat RSA studies have had technical limitations (as highlighted above). The aim of this study was to develop a simple, non-destructive method for studying the RSA of wheat grown in soil to characterize RSA traits of two genotypes differing in PUE.

Materials and methods

Wheat varieties

Two wheat genotypes, RAC875 and Wyalkatchem, were used for the experiments. RAC875 is a breeding line and Wyalkatchem is a cultivar grown in Australia. From field experiments (McDonald et al. 2015) and our own

preliminary results, RAC875 was P efficient and Wyalkatchem was P inefficient.

Experiment 1. Genotypic evaluation for P use efficiency

Experimental design and plant growth conditions

Plants were grown in round pots (dimensions: 18.5 cm deep × 17.5 cm top diameter × 16.0 cm base diameter) filled with 4.2 kg of soil at two P rates: 10 and 30 mg P kg^{-1}. Basal nutrients (expressed in mg kg^{-1}) consisting of $Ca(NO_3)_2{\cdot}4H_2O$ (918), K_2SO_4 (113.6), $MgSO_4{\cdot}7H_2O$ (140), $FeSO_4{\cdot}7H_2O$ (1.4), $Na_2MoO_4{\cdot}2H_2O$ (0.61), $CuSO_4{\cdot}5H_2O$ (2.25), $MnSO_4{\cdot}4H_2O$ (3.68), $ZnSO_4{\cdot}7H_2O$ (6.6), and H_3BO_3 (0.28) were added and mixed thoroughly into the soil. P (in the form of KH_2PO_4) was then added at two different rates; 10 and 30 mg P kg^{-1} (low and adequate P, respectively) and mixed thoroughly into the soil.

Wheat grains were sterilized in 2% hypochlorite for 10 min and then rinsed with Milli-Q water. The sterilized grains were then placed in Petri dishes lined with moistened filter papers and kept in a dark place at room temperature for 3 days. Five germinated grains were sown into each pot and the experiment was conducted with four replicates in a controlled environment growth room with the following conditions: 20 °C/10 °C, 13-h/11-h day/night cycle, and light intensity of 700 µmol m^{-2} s^{-1} at the leaf level. Light source was a combination of fluorescent and incandescent. Pots were arranged in a completely randomised design and were rotated every 3–7 days to minimize the effect of light gradient within the growth room. The plants were watered to 8–10% of the soil weight every 2–3 days.

Harvest and measurements

Two plants per pot were harvested at 27 days after sowing (DAS), one plant was harvested at 48 DAS and the remaining two plants were grown to maturity. At maturity, wheat heads were separated from shoots and stems were detached from roots at the crown. Shoots and stems were rinsed with Milli-Q water and dried for 48 h at 85 °C for dry matter measurements. Heads were dried at 37 °C for 5 days and grains collected using a Haldrup thresher for measurements of grain yield. The dry mass of roots was also measured after drying at 85 °C for 48 h.

Experiment 2. RSA characterization

The rhizoboxes used in this study were based on the design of those in the Kono et al. (1987) and Courtois et al. (2013) study with modifications. The rhizoboxes have three main parts: a plastic box (L × W × D: 29.5 × 20 × 4 cm) with holes for watering, a foam base with a grid of toothpicks to maintain the root system architecture and a transparent plastic sheet to sit under the root system (Fig. 1).

Plants were grown in rhizoboxes filled with 1.2 kg of double-washed sandy soil with added basal nutrients as described in the previous experiment with two P rates: 10 and 30 mg P kg^{-1}. Wheat grains were also sterilized and germinated as described in the previous experiment, and one germinated seed was sown into each rhizobox. The experiment was carried out in triplicates in a growth room with the conditions as described in the previous experiment, except light intensity was 500 µmol m^{-2} s^{-1}. The rhizoboxes were kept at a 60° angle for the duration of the experiment and plants were watered every 2–3 days to 8% of the soil weight.

Plants were harvested at 24 DAS and shoots were detached from roots at the crown level. Soil was gently washed away by slow agitation in a tank of water; the toothpicks within the rhizobox minimize the root movement

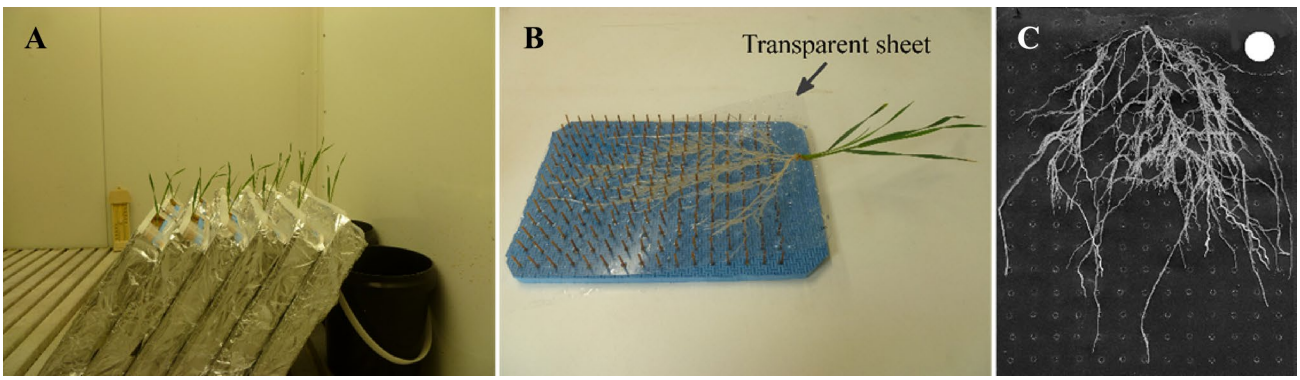

Fig. 1 Rhizoboxes with plants (**a**), a foam base with a grid of toothpicks and a transparent sheet (**b**) and a root image at 24 DAS (**c**). The root image scale of **c** is 2-cm circle

during washing. The transparent sheet with the root system was removed from the rhizobox and the root system was gently blotted with absorbent paper and scanned (8-bit gray scale, 400 dpi) on an Epson Perfection V700 Photo scanner for RSA analysis. 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). GiA roots were used to measure convex hull area (CHA, the smallest area that encloses the whole root system), root surface area, total root length and root volume. DIRT was used to measure root tip number, medium root width, root density, root angle, 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; the RTP skeleton is a loop-free sampling of the medial axis derived from the root shape visible in the image) and accumulated width over the depth (D).

Shoot dry matter (Shoot DM) was measured after 3 days of freeze-drying and root dry matter (root DM) was measured after 48 h at 85°C to analyze the correlation between root traits and shoot DM.

Experiment 3. Root hair characterization

Wheat grains were grown in transparent plastic rootboxes filled with 600 g of sandy soil, adding basal nutrients as described in the previous experiment, and at two P rates of 10 and 30 mg P kg^{-1} soil. The rootbox size was L × W × D: 24 × 24 × 2.5 cm. The experiment was carried out in four replicates in a growth room as described in the previous experiment, except light intensity was 650 µmol m^{-2} s^{-1} at the leaf level. Rootboxes were diagonally placed at a 45° angle, to ensure roots attached to the surface of the rootbox. Soil was moistened to 10% of the soil weight. Seminal root diameter, root hair length (RHL) and root hair density (RHD) on the longest seminal roots were measured at 5 DAS using a microscope (LEICA MZ16, × 12.5 and × 20 magnifications) with an attached camera (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. Eight measurements from each replicate were taken for seminal root diameter and RHL. The root hair density (RHD) was estimated by the equation described by Vandamme et al. (2013): $RHD = (\pi r_s^2)^{-1}$, in which r_s is the half-mean distance between the root hairs, measured by counting the number of root hairs per 0.5 mm of root length. Four measurements were taken from each replicate.

Analysis and calculations

The P concentration in shoot, grain and straw was determined by inductively coupled plasma mass spectrometry (ICP-MS) (Agilent Technologies, Model 7500cx) at Flinders University using the closed-tube digestion method developed by Wheal et al. (2011). PUE of wheat genotypes was evaluated by five criteria as described below (Osborne and Rengel 2002).

(1) Shoot biomass and grain yield at low P.

(2) $PUE_{SM} = \frac{\text{Shoot DM at low P}}{\text{Shoot DM at adequate P}} * 100.$

(3) $PUE_{GY} = \frac{\text{Grain yield at low P}}{\text{Grain yield at adequate P}} * 100.$

(4) P acquisition efficiency (PAE): $PAE = \frac{\text{Shoot P uptake}}{\text{Amount of P supplied}} * 100.$

(5) P utilization efficiency (PUtE): $PUtE = \frac{\text{Shoot DM}}{\text{Shoot P uptake}}.$

Statistical analysis

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

Results

Genotypic evaluation for P use efficiency

Shoot DM at 27 DAS slightly increased with the addition of P, but significant ($P < 0.01$) responses to P occurred at 48 DAS and at maturity (Table 1). When compared to Wyalkatchem, RAC875 produced significantly ($P < 0.05$) greater shoot DM at 27 DAS, 48 DAS and at maturity. No significant G × P interaction was observed. Under low P availability, RAC875 had significantly ($P < 0.05$) greater shoot DM at all three growth stages and higher grain yield than Wyalkatchem. No significant genotypic variation for these traits was found under adequate P supply (Table 1). PUE_{SM} of RAC875 was 11, 3 and 15% higher than those in Wyalkatchem at 27 DAS, 48 DAS, and at maturity, respectively. RAC875 also showed 17% greater PUE_{GY} than Wyalkatchem (Table 2).

At maturity, RAC875 had a significantly ($P < 0.01$) smaller root to shoot ratio and a smaller ($P < 0.001$) root DM than Wyalkatchem (Table 3). At this growth stage,

Table 1 The effect of P supply on shoot DM at 27 DAS, 48 DAS and at maturity and grain yield at maturity of two wheat genotypes, RAC875 and Wyalkatchem

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 $plant^{-1}$)
10	RAC875	$0.44 \pm 0.03^{a,*}$	$2.89 \pm 0.04^{a,**}$	$11.4 \pm 0.5^{a,**}$	$4.8 \pm 0.3^{a,**}$
	Wyalkatchem	0.33 ± 0.02^{b}	2.42 ± 0.08^{b}	8.3 ± 0.5^{b}	3.5 ± 0.2^{b}
30	RAC875	0.47 ± 0.03	3.77 ± 0.28	16.4 ± 1.3	7.3 ± 0.7
	Wyalkatchem	0.40 ± 0.03	3.29 ± 0.15	15.5 ± 0.4	7.2 ± 0.2
F ratio					
Genotype (G)		11.034**	9.136*	7.971*	3.054 ns
P supply (P)		2.762 ns	30.733**	79.443***	59.203***
G×P		0.581 ns	0.085 ns	2.613 ns	0.171 ns

Data represent the mean ± standard error ($n = 4$). *F* ratios are from two-way ANOVA analysis

ns not significant

*, ** and *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively

Different letters show 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 2 PUE of two wheat genotypes, RAC875 and Wyalkatchem measured at 27 DAS, 48 DAS and at maturity

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

PUE_{SM} ratio between shoot DM at low P and adequate P supply, PUE_{GY} ratio between grain yield at low P and adequate P supply

under low P supply, P uptake of RAC875 was significantly ($P < 0.01$) higher than that of Wyalkatchem, while no difference in this variable between the two genotypes was observed at adequate P. RAC875 exhibited higher root efficiency than Wyalkatchem under low P supply with root efficiency of RAC875 almost double that of Wyalkatchem (Table 3).

Table 3 The effect of P supply on root DM, root to shoot ratio, P uptake and root efficiency at maturity of two wheat genotypes, RAC875 and Wyalkatchem

P supply (mg P kg^{-1} soil)	Genotype	Root DM at maturity (g $plant^{-1}$)	Root to shoot ratio (g g^{-1})	P uptake (mg $plant^{-1}$)	Root efficiency (mg shoot P uptake/g root DM)
10	RAC875	$1.7 \pm 0.13^{a,*}$	$0.15 \pm 0.01^{a,**}$	$6.8 \pm 0.4^{a,**}$	$4.0 \pm 0.3^{a,**}$
	Wyalkatchem	2.6 ± 0.26^{b}	0.32 ± 0.04^{b}	5.2 ± 0.2^{b}	2.1 ± 0.3^{b}
30	RAC875	$3.0 \pm 0.26^{a,**}$	$0.19 \pm 0.02^{a,**}$	21.5 ± 2.3	$7.7 \pm 0.1^{a,*}$
	Wyalkatchem	4.2 ± 0.17^{b}	0.27 ± 0.01^{b}	21.0 ± 0.4	5.0 ± 0.2^{b}
F ratio					
Genotype (G)		26.262***	29.919**	10.324*	24.537***
P supply (P)		52.558***	0.031 ns	815.297***	49.666***
G×P		0.760 ns	2.723 ns	2.242 ns	0.692 ns

Values represent the mean ± standard error ($n = 4$). Analysis of variance performed on original data except for P uptake transformed using log10. *F* ratios are from two-way ANOVA analysis

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

ns not significant

*, ** and *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively

RSA characterization and responses to P of two wheat genotypes grown in rhizoboxes

RSA traits

Results indicate that P supply did not significantly affect root angle, average root diameter, spatial root distribution (*X*) ($RDISTR_x$) and average density (Table 4). However, low P resulted in significant reductions in CHA, total root length, root surface area, root volume, root tip number, medium root with, and the absolute value of spatial root distribution (*Y*) ($RDISTR_y$) (Table 4).

On low-P soils, RAC875 showed a moderate 11.2% decrease in CHA, a 14.5% decrease in total root length, a 16% decrease in root surface area, a 17.2% decrease in root volume, a 22.3% decrease in root tip number and a 14.8% decrease in medium root width, and showed no decline in the absolute value of spatial root distribution (*Y*). Meanwhile, Wyalkatchem had a marked 43.5% reduction in CHA, 47% reduction in total root length, 47.2% reduction in root surface area, 48.5% reduction in root volume, 41.5% reduction in root tip number and 44.8% reduction in medium root width, and also showed no significant decrease in the absolute value of spatial root distribution (*Y*) (Table 4, Fig. 2). 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, 19 and 30%, respectively, greater in RAC875 than in Wyalkatchem (Table S 1).

A significant G × P interaction for CHA was observed. At low P supply, CHA in RAC875 was 16.1% larger than that in Wyalkatchem ($P < 0.05$), but 19.4% smaller at adequate P supply ($P < 0.01$) (Fig. 2)

No genotypic variation in specific accumulated width (D) over depth was observed under low P supply, except at D90 where RAC875 showed a significantly ($P < 0.05$) greater accumulated width over depth when compared to Wyalkatchem. Under adequate P supply, D values in Wyalkatchem were higher than those in RAC875 (Fig. 3).

Responses to P and PUE of two wheat genotypes

Shoot DM was significantly affected by P supply at 24 DAS. Shoot DM at adequate P was about 74% greater than that at low P supply (10 mg P kg^{-1}) ($P < 0.01$) (Table S2). Under low P condition, shoot DM in RAC875 was 56% higher than Wyalkatchem ($P = 0.07$), while there was no difference between these two wheat genotypes at adequate P supply (Fig. 4). Consequently, RAC875 showed 55% higher PUE_{SM} than Wyalkatchem.

A significant G × P interaction in shoot P concentration was observed ($P < 0.05$) (Table S2), indicating 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 supply, while at adequate P supply, Wyalkatchem showed significantly ($P < 0.05$) higher shoot P concentration (Table S2).

PUtE significantly ($P < 0.001$) declined with an increase in P supply and RAC875 showed significantly greater PUtE than Wyalkatchem (Table S2). No significant differences in P uptake, PAE and root efficiency were observed between the two wheat genotypes, but RAC875 was generally higher in these parameters under low P supply (Table S2).

Shoot P uptake per unit of RSA traits was calculated to evaluate how the RSA traits affect shoot P uptake (Table 5). Under low P supply, a significant reduction occurred in shoot P uptake per unit of root trait (including CHA, root surface area, root volume, total root length and root tip number); however, significant differences in these parameters were not found between genotypes. On low-P soils, shoot P uptake per unit of average root density in RAC875 was significantly ($P < 0.05$) higher than in Wyalkatchem but no variation in this parameter was observed between the two wheat genotypes in adequate-P soils (Table 5).

Root hair length and density, and seminal root diameter

For the estimation of root hair features, a preliminary trial showed that root hairs of wheat were sparse and delicate, and they were mostly lost or were aggregated when harvested from the pot experiment. Thus, growing wheat in transparent rootboxes for root hair measurements is a viable approach. The results illustrated that root hairs were well observed and captured through the transparent rootboxes under a microscope with a camera attached (Fig. 5).

Results in Table 6 and Fig. 5a show that RAC875 had significantly ($P < 0.001$) greater ($P < 0.001$) seminal root diameter than Wyalkatchem, while P supply did not affect the seminal root diameter. Both genotype and P supply did not have a significant impact on RHL (Table 6, Fig. 5b). At low P supply, RAC875 showed significantly ($P < 0.05$) higher RHD than Wyalkatchem, while no difference in RHD was observed at adequate P supply (Fig. 5c).

Discussion

Responses to P and PUE of two wheat genotypes

The consistency in response to P was observed between plants grown in the rhizoboxes and in pots as well as in the field, therefore, the RSA traits were measured from the roots grown in the rhizoboxes could be used in practice. Field trial experiments showed that RAC875 produced high grain yield under low P (McDonald et al. 2015), and RAC875 exhibited

Table 4 The effect of P supply on root system architecture of two wheat genotypes, RAC875 and Wyalkatchem

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)	Average root diameter (mm)	Spatial root distribution[1] (X component)	Spatial root distribution (Y component)	Average root density[2]
10	RAC875	18.8 ± 1.9	171.6 ± 16.5	1.64 ± 0.16	490 ± 68	66.8 ± 1.0	9.8 ± 1.8	0.29 ± 0.00	− 0.39 ± 0.42	− 19.0 ± 1.3	0.42 ± 0.03
	Wyalkatchem	15.1 ± 1.9	140.1 ± 17.5	1.34 ± 0.17	445 ± 46	66.8 ± 1.1	9.0 ± 0.5	0.29 ± 0.00	− 0.32 ± 0.49	− 15.9 ± 0.7	0.53 ± 0.03
30	RAC875	22.0 ± 4.1	204.4 ± 34.9	1.98 ± 0.31	631 ± 104	68.0 ± 1.5	11.5 ± 2.3	0.30 ± 0.00	− 0.41 ± 0.36	− 18.7 ± 0.2[a,*]	0.51 ± 0.02
	Wyalkatchem	28.5 ± 3.6	265.3 ± 33.4	2.60 ± 0.33	759 ± 66	67.5 ± 1.2	16.3 ± 2.0	0.30 ± 0.00	− 0.97 ± 0.30	− 20.2 ± 0.5[b]	0.34 ± 0.06
F ratio											
Genotype (G)		0.209 ns	0.297 ns	0.409 ns	0.319 ns	0.072 ns	1.195 ns	0.182 ns	0.374 ns	1.008 ns	0.051 ns
P supply (P)		7.430*	8.577*	9.967*	9.401*	0.632 ns	6.156*	2.212 ns	0.699 ns	6.240*	0.042 ns
G × P		2.765 ns	2.942 ns	3.320 ns	1.353 ns	0.045 ns	2.431 ns	0.393 ns	0.619 ns	8.122*	6.448*

Plants were harvested 24 DAS. Values represent the mean ± standard error ($n = 3$). *F* ratio is from two-way ANOVA analysis. *, *** significant at $P<0.05$ and $P<0.001$, respectively

ns not significant

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

[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 in the root shape (Bucksch et al. 2014)

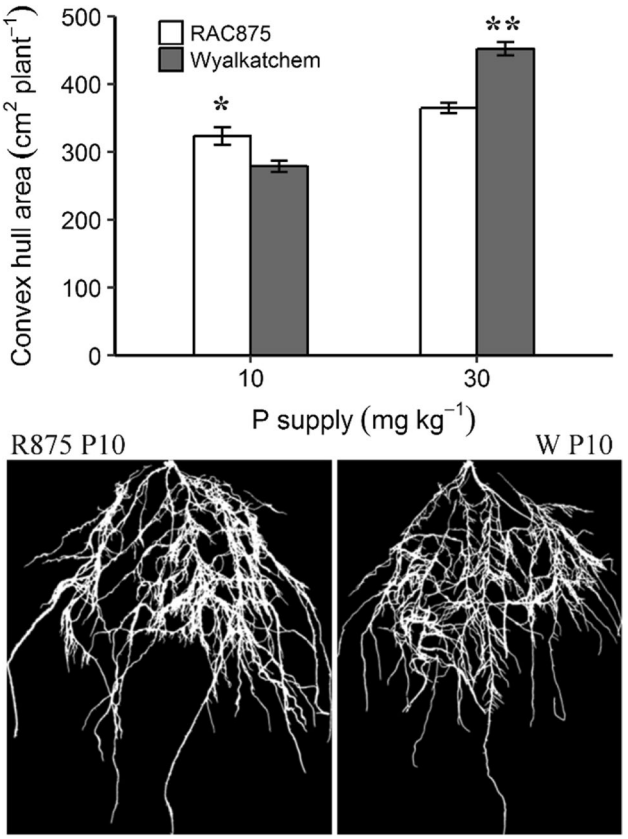

Fig. 2 Convex hull area of two wheat genotypes RAC875 and Wyalkatchem grown in rhizoboxes at 24 DAS. Data were the means ± standard errors ($n = 3$). *, ** significant within the same P supply at $P < 0.05$, $P < 0.01$, respectively. Mean comparisons between genotypes at each P treatment were performed by independent t-test. Root system architecture of RAC875 and Wyalkatchem grown in the rhizoboxes at two P rates (P10 and P30: 10 and 30 mg P kgle 5); representative photos show RAC875 (left) and Wyalkatchem (right) at 24 DAS grown under low P levels (10 mg P kg^{-1})

greater PUE than Wyalkatchem (PUE was calculated from supplemental data provided by McDonald). In this study, under low P, RAC875 also showed greater shoot DM at different growth stages (27 DAS, 48 DAS and at maturity) and higher grain yield than Wyalkatchem in the pot experiment under growth room conditions (Table 1). PUE of RAC875 was also higher than that of Wyalkatchem at all three growth stages (Table 2). Similar results were also observed in the rhizobox experiment (Fig. 4). The consistency of the results indicates that the rhizoboxes are a suitable soil-based method to elucidate the root RSA traits of wheat.

Lower P requirement for its normal growth would allow RAC875 to have a higher PUtE. At maturity and under low P supply, P uptake in RAC875 was 30.7% greater than that in Wyalkatchem (Table 3) and this value was also higher in RAC875 than in Wyalkatchem at 24 DAS (Table S2). Wyalkatchem had a significantly higher shoot P concentration when compared to RAC875, although it produced less biomass at 24 DAS (Table S2). This would indicate that Wyalkatchem requires more P for its normal growth in comparison with RAC875. Furthermore, RAC875 utilized P more efficiently than Wyalkatchem since PUtE in RAC875 was significantly higher than that in Wyalkatchem (Table S2).

Plants with a smaller root system would provide more energy for shoot growth. 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, the root to shoot ratio in RAC875 was significantly smaller than that in Wyalkatchem. RAC875 also has a smaller root biomass than Wyalkatchem at maturity (Table 3). In agreement with these findings, da Silva et al. (2016) also reported that P-efficient wheat genotypes possessed smaller root systems than P-inefficient genotypes. It

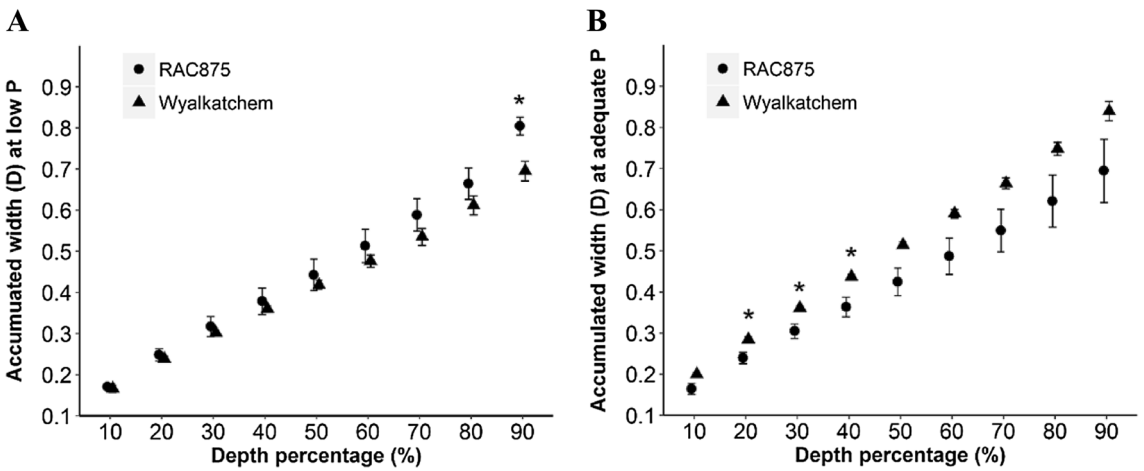

Fig. 3 Accumulated root width over root depth (%) for two wheat genotypes, RAC875 and Wyalkatchem at **a** low P supply (10 mg P kg^{-1}) and **b** adequate P supply (30 mg P kg^{-1}). Data were the means ± standard errors ($n = 3$). *Significantly different between genotypes at specific depth ($P < 0.05$). Mean comparisons between genotypes at each P treatment were performed by independent t-test

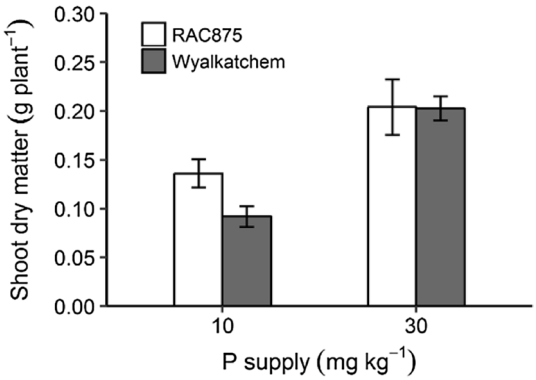

Fig. 4 Shoot DM of two wheat genotypes, RAC875 and Wyalkatchem, grown in rhizoboxes at 24 DAS. Data were the means ± standard errors (n = 3). Mean comparisons between genotypes at each P treatment were performed by independent t-test

appears that plants with smaller root systems may require less energy for root growth; therefore, they can sustain biomass under limited P nutrition. However, a question arises as to how small a root system can be to maintain shoot biomass under P deficiency, and a critical level study would be beneficial to determine this. A study on a large set of germplasm can provide an answer to this question.

RSA responds to P supply

Growth methods and conditions as well as harvest stage could affect RSA trait measurements. In our study, at 24 DAS, a sub-optimal level of P supply led to a significant reduction in CHA, 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). Similarly, low P resulted in a decrease in total root length, root surface area and root tip number in barley at 28 DAS, under hydroponics (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). The variations 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. (1993) examined root traits at 14 DAS in a hydroponic experiment; Steingrobe et al. harvested only the 0–30 layers in the field but not the whole root system]. It appears that at a very early stage of growth, plants under low P stimulate greater root growth to obtain more P and their root length is higher than under adequate P. However, when plants grow longer, plants under adequate P grow larger and result in a larger root system, as our results indicate.

Larger CHA could help to explain the greater persistence under abiotic stress. Although previous studies show that topsoil foraging is advantageous for phosphorus acquisition in common bean and maize under low P supply (Lynch and Brown 2001; Zhu et al. 2005b), in this study root top angle did not vary between the two wheat genotypes (Table 4). Thus, RAC875 could have different mechanisms of PUE. Indeed, under P deficiency, RAC875 had significantly larger CHA than Wyalkatchem, while the result was reversed under adequate P supply (Fig. 4). Therefore, the mechanism of PUE seems to be interesting in this case. Under limited P supply, the root system of RAC875 expanded and occupied a larger area than that of Wyalkatchem. RAC875 produced

Table 5 The effect of P supply on 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, RAC875 and Wyalkatchem

P supply (mg P kg^{-1})	Genotype	Shoot P uptake/CHA (μg cm^{-2})	Shoot P uptake/RSurA (μg cm^{-2})	Shoot P uptake/root volume (mg cm^{-3})	Shoot P uptake/total root length(μg m^{-1})	Shoot P uptake/root tip number (μg tip^{-1})	Shoot P uptake/average root density[(1)]
10	RAC875	1.16 ± 0.17	2.17 ± 0.14	0.23 ± 0.02	19.9 ± 1.5	0.77 ± 0.06	1.00 ± 0.11[a*]
	Wyalkatchem	1.03 ± 0.14	1.89 ± 0.22	0.20 ± 0.02	17.4 ± 2.2	0.60 ± 0.12	0.57 ± 0.04[b]
30	RAC875	3.05 ± 0.52	5.46 ± 0.38	0.56 ± 0.04	51.0 ± 3.4	1.76 ± 0.05	2.70 ± 0.04
	Wyalkatchem	3.00 ± 0.19	5.19 ± 0.33	0.53 ± 0.03	48.5 ± 3.1	1.80 ± 0.12	3.26 ± 0.24
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**

Plants were harvested 24 DAS. Values represent the mean ± standard error (n = 3). F ratios are from two-way ANOVA analysis

ns not significant

*, *** significant at $P < 0.05$ and $P < 0.001$, respectively. Different letters show 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

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

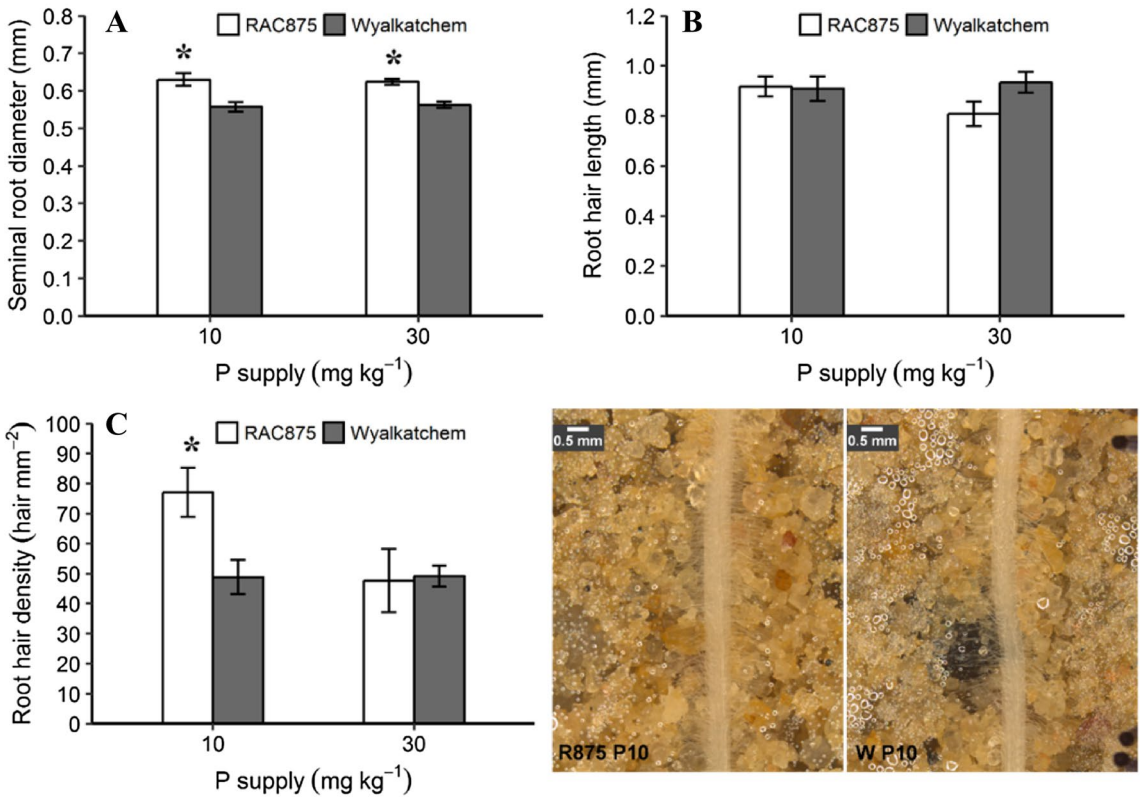

Fig. 5 Variations in seminal root diameter (**a**), RHL (**b**) and RHD (**c**) of two wheat genotypes, RAC875 and Wyalkatchem, under different P supply. Results were means ± standard errors ($n=4$). *Significant differences $P<0.05$ between genotypes grown under the same P supply at each growth stage. Mean comparisons between genotypes at each P treatment were performed by independent t-test. The root picture shows examples of two representative root systems of RAC875 (left) and Wyalkatchem (right) at low P supply (10 mg P kg^{-1})

higher shoot DM than Wyalkatchem under low P supply. This may help explain a mechanism for better growth and P uptake of RAC875 under low P and thus CHA appears to be a potential indicator for screening P-efficient wheat under low P. Large CHA was a characteristic of drought tolerance in wheat (Belachew et al. 2018). A study by Kenobi et al. (2017) also indicated that CHA can be used as a discriminant to identify high-nitrogen uptake efficiency in wheat genotypes.

Table 6 The effect of genotype and P supply on seminal root diameter and root hair features of two wheat genotypes, RAC875 and Wyalkatchem

Effect	Seminal root diameter (mm)	Root hair length (mm)	Root hair density (root hair mm^{-2})
	P value	*P* value	*P* value
Genotype (G)	<0.001	00.226	00.083
P supply (P)	10.000	00.376	00.063
G×P	00.637	00.168	00.058

Plants were grown at two P treatments (10 and 30 mg P kg^{-1}) and root features were measured at 5 days after sowing

Root hairs respond to P and relations between root hair features and PUE

Root hairs are important traits for improvement of PUE since their characteristics affect P acquisition (Gahoonia et al. 1997; Gahoonia and Nielsen 2003; Haling et al. 2013). Although previous studies have demonstrated that root hairs became longer under low P, such as in *Arabidopsis thaliana* (Bates and Lynch 2000), maize (Zhu et al. 2010) and rice (Vejchasarn et al. 2016), this study showed that P supply did not affect RHL (Table 6). This difference might simply be attributed to genetic differences between these plants. A recent study also 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 the evaluation of plant adaptation to low P, by promoting RHL.

The role of RHL in PUE is still not obvious. In the study presented here, RHL did not show any contribution to higher PUE in RAC875 because RAC875 and Wyalkatchem were not different in RHL (Table 6, Fig. 5b). Brown et al. (2012) also pointed out that RHL is not important for grain yield but

for shoot P accumulation. However, Gahoonia and Nielsen (2004) have shown that barley genotypes with long root hairs improved grain yield in comparison with short root hair genotypes. Recently, quantitative trait loci (QTLs) for RHL have been identified in wheat and they co-located with loci for yield components (Horn et al. 2016). Thus, a population study should be implemented to identify if RHL is important for grain yield under low P.

Dissimilar to RHL, RHD is not only responding to low P supply but also related to PUE. In this study, RHD increased ($P = 0.063$) when P supply was low (Table 6, Fig. 5c) and this result agrees with a number of studies (Bates and Lynch 2000; Ma et al. 2001; Hill et al. 2010; Hu et al. 2010). A previous study was able to show that higher RHD enhanced P uptake under low P in wheat (Wang et al. 2016a) and this could be important for later plant growth. Similarly, in this study, 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 in low P conditions.

What root features could be associated with PUE in RAC875?

Root efficiency can be considered to be involved in greater PUE. da Silva et al. (2016) found that root volume was negatively correlated with PUE in wheat. It appears that the efficiency of a root system to acquire P is more important than the actual root size. 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 (Wissuwa 2005; Mori et al. 2016). In this study, RAC875 was shown to have greater root efficiency and to produce higher yield under low P than Wyalkatchem. Thus, root efficiency would be important for screening P-efficient genotypes.

The increased root efficiency of RAC875 could be related to greater CHA and more dense root hairs. Indeed, under low P supply, CHA in RAC875 was larger than that in Wyalkatchem (Fig. 2). Also, under low P, RAC875 had greater root hair density than Wyalkatchem (Fig. 5). Keyes et al. (2013) reported that roots and root hairs equally contribute to P uptake, in which root hairs are more important for localized P acquisition. Therefore, it appears that the enlargement of a root system and the development of more root hairs under low P ensure that the P-efficient genotype with a small root system (in terms of root dry matter) can support higher yield production.

Conclusions

In summary, the specially designed rhizoboxes can be used to grow wheat in soils and obtain root systems without destruction for RSA analysis. Larger CHA and more dense root hairs appear to lead to enhanced shoot DM and grain yield in RAC875 under low P conditions. These root characteristics would ensure that a small root system as in RAC875 can support relatively greater biomass and yield production. Thus, it seems that small but efficient root systems would be a beneficial indicator for screening P-efficient crops. Root surface area, total root length and root volume would contribute to high productivity of RAC875 on low-P soils since these parameters were positively correlated with shoot DM and shoot P uptake. A larger screening of genotypes is also recommended to further validate this screening technique and its application in marker development could also be realized with a dedicated marker discovery study in a suitable mapping population.

Author contribution statement VLN and JS designed the research. VLN implemented the experiments and performed the data analyses. VLN wrote the manuscript. JS made the revision of the manuscript. All the authors approved the final revision to be published.

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