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Phosphorus (P) efficiencies and mycorrhizal responsiveness of old and modern wheat cultivars

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Abstract

A pot experiment was carried out in a growth chamber to investigate P efficiencies and mycorrhizal responsiveness of modern (Krichauff and Excalibur) and old (Khapstein, Bobin, Comeback and Purple Straw) wheat cultivars (*Triticum aestivum*). The arbuscular mycorrhizal fungus (AMF) used in this study was *Glomus intraradices*. The growth medium was a soil/sand mixture with $NaHCO_3$-extractable P of 9.4 mg P kg^{-1} and no extra P was added. Plant P efficiencies (uptake, utilisation and agronomic) were found to differ significantly between cultivars, but no general trends of changes with the year of release of the cultivar were found. AMF colonisation was found to decrease plant growth under our experimental conditions with low light intensity. Mycorrhizal responsiveness (MR) was measured in terms of the improvement in plant P nutrition (shoot P concentrations). MR was found to be generally lower in modern cultivars than in old cultivars, indicating that modern breeding programs may have reduced the responsiveness of modern wheat cultivars to arbuscular mycorrhizal fungi. MR was also found to decrease in general with increased plant P utilisation efficiency.

Introduction

Phosphorus (P) is one of the most important nutrients (next only to nitrogen) limiting crop production in many regions of the world. To improve the P nutrition of plants, the traditional approach is to apply large amounts of P fertilisers to soils. However, the use-efficiency of applied P is generally very low, ranging from 10% to 30% in the year applied (McLaughlin et al., 1991). Continuous application of P fertilisers also increases the risk of P loss from soil to water, causing toxic algal blooms in water bodies (Sharpley et al., 2000). Improving plant uptake of P from soil is an important part of the management systems for low P soils and the enhancement of use efficiency of P fertilisers. Genetic variations in P uptake efficiencies have been widely reported in many crops, such as clover (Trolove et al., 1996) and maize (Silva and Gabelman, 1992). Plant traits responsible for P uptake efficiency include rhizosphere acidification, root exudation of organic anions, root morphology, uptake kinetics and symbiotic association with mycorrhizal fungi.

Symbiotic association between plants and arbuscular mycorrhizal fungi is ubiquitous and of particular importance in improving plant P uptake efficiency (Smith and Read, 1997). The effect is mainly due to the ability of mycorrhizal fungal hyphae to acquire P well beyond the limits of the rhizosphere depletion zone (Li et al., 1991). It has been widely reported that responsiveness to (or dependency on) mycorrhizal colonisation, in terms of improved growth and/or P nutrition, varies between crop cultivars (Baon et al., 1993; Bryla and Koide, 1990; Hetrick et al, 1993, 1995; Koide et al., 1988; Krishna et al., 1985; Kresava Rao et al., 1990; Mercy et al., 1990; Khalil et al., 1994). Hetrick et al. (1992) observed that modern bread wheat cultivars (released after 1950) have lower mycorrhizal responsiveness than many native grasses, suggesting that modern breeding practices

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have reduced responsiveness to mycorrhizal symbiosis. Genetic analysis showed that genes involved in determining mycorrhizal responsiveness may be derived from the D (Hetrick et al., 1993) or B genome in wheat (Hetrick et al., 1995). However, Koide and his colleagues have argued that modern cultivars of oats and tomato plants may be more responsive to mycorrhizal associations (Bryla and Koide, 1990; Koide et al., 1988). Therefore, there is no general conclusion on whether modern breeding practices under relatively fertile conditions have inadvertently contributed to changes in mycorrhizal responsiveness. Other factors such as inherent plant morphological and phenological traits, and adaptation to infertility based on mechanisms other than mycorrhizal symbiosis will also influence the mycorrhizal responsiveness in wild accessions.

In the present study, we selected four old and two modern wheat cultivars (all of Australian origin) to investigate the variation in P efficiencies (uptake, utilisation and agronomic) between cultivars, and to test if there is a general trend that modern breeding practices have affected their P uptake efficiencies and mycorrhizal responsiveness in terms of tissue P concentrations.

Materials and methods

Seeds

Seeds of four old and two modern wheat cultivars (*Triticum aestivum* L.) were obtained from the Australia Winter Cereal Collections (Tamworth, New South Wales) and Waite Institute (South Australia). Seeds of all six cultivars were grown to maturity under similar conditions in a glasshouse (25 $\pm$ 2 °C with ambient light intensity) in order to increase the seed supply and achieve similar mineral nutrient contents in the seeds used in the experiment, although inherent differences in seed size and nutrient composition between cultivars will remain, even after being grown in the same conditions. Plants were grown in UC mix (University of California formula, produced by the Plant Research Centre, The Waite Campus, Adelaide University, Australia; Barker et al., 1998) with about 2 g of compound fertiliser per pot (with N, P and K contents of 18%, 4.8% and 9.1%, respectively) spread on the soil surface. Seeds were harvested at maturity (around 3 months after sowing). Seed P content was determined by nitric-perchloric acid digestion in HNO_3 (70%)/$HClO_4$ (70%) mixture (6:1 v/v) followed by molybdenum-ascorbic acid colorimetry (Hanson, 1950). Seed P contents of the different cultivars are shown in Table 1.

Table 1. Cultivars of wheat (*Triticum aestivum* L.) used in this study, year of release and P contents of seeds used in the main experiment

Cultivars	Year of release	Seed P content (μg seed^{-1})
Krichauff	1996	150
Excalibur	1991	167
Khapstein	1955	161
Bobin	1925	147
Comeback	1897	198
Purple Straw	1860	145

Experimental growth medium

Soil/sand mixture (1:1 w/w) was used in this experiment. Laffer sand (sandy subsoil, almost pure sand) was washed in Reverse Osmosis (RO)-purified water at least five times in order to wash away the indigenous nutrients (salts) in the sand. Soil and sand samples were autoclaved to destroy indigenous mycorrhizal fungi. $NaHCO_3$ extractable P (Colwell, 1963) of this growth medium is 9.4 mg P kg^{-1}. Nutrients were added to the soil/sand mixture as the following chemical compounds (in solution form) per kg: 0.918 g of $Ca(NO_3)_2$, 0.174 g of K_2SO_4, 0.185 g of $MgSO_4$, 0.4 mg FeEDTA, 2 mg $CuSO_4.5H_2O$, 0.6 mg $MnSO_4.4H_2O$, 0.4 mg $CoSO_4.7H_2O$, 0.5 mg H_3BO_3 and $H_2MoO_4.H_2O$ and 2.2 mg $ZnSO_4.7H_2O$. No additional P was supplied.

To produce mycorrhizal plants, the soil/sand mixture was inoculated with 10% soil/sand inoculum (10/90 w/w) from a pot culture of *Glomus intraradices* Schenck & Smith (obtained from NPI, Utah, USA) grown on sorghum (*Sorghum bicolour* L.) for 4 months, thoroughly incorporated into the sterile mixture. The inoculum contained approximately 4000 spores plus root fragments and hyphae per pot, and no pathogenic microbes were observed in this inoculum. *G. intraradices* is an AM species widely used in experiments. It colonises roots of many plant species rapidly, normally producing a positive growth responses (Baon et al., 1993). Non-inoculated soil/sand mixture received 10% autoclaved soil/sand mixture (of the same composition as the inoculum material) without any plants being grown in it.

Growth conditions and plant analysis

Seeds of the six cultivars of *T. aestivum* were germinated on moist filter paper. Four pre-germinated seeds were sown in each pot (6.2 cm in diameter and 26 cm in depth) filled with 1 kg of soil/sand mixture (inoculated or non-inoculated). Each pot was thinned to two plants 3–4 days after emergence. Each treatment had 4 replicates. Pots were watered when necessary (by weighing) with reverse RO water to 12% water content (w/w). The experiment was carried out in a growth chamber set at 20 °C day/15 °C night with a 14 h light period (260–320 μE m^{-2} s^{-1}). The plants were harvested at 7 weeks after emergence. The reason to choose 7 weeks for harvest is that plant P uptake mainly occurs during the first several weeks of plant growth and mycorrhizal colonisation approaches plateau value at this stage according to our previous study under the same conditions (Zhu and Smith, 2001).

At harvest, plant roots were thoroughly washed, and plants were divided into shoots and roots. A weighed subsample of root material was removed for determination of mycorrhizal colonisation. The remaining material was oven dried at 70 °C for 24 h. Dry weights of shoots and roots were recorded. Tissue P concentration was determined by nitric-perchloric acid digestion in HNO_3 (70%)/$HClO_4$ (70%) mixture (6:1 v/v) followed by molybdenum-ascorbic acid colorimetry (Hanson, 1950). For examination of mycorrhizal colonisation, the subsamples of root material were cleared in 10% KOH solution (w/v) and stained using trypan blue (a modification of the method of Phillips and Hayman, 1970, omitting phenol from the solutions). Mycorrhizal colonisation was determined by the grid-line intersection method (Giovannetti and Mosse, 1980).

Data analysis

We define mycorrhizal responsiveness here as improvement of P nutrition, and is expressed as the percentage increase in plant shoot P concentrations in response to mycorrhizal colonisation, and is calculated using Equation (1):

$$\text{Mycorrhizal responsiveness} = \frac{\text{Shoot[P]}_{\text{M}} - \text{Shoot[P]}_{\text{NM}}}{\text{shoot[P]}_{\text{NM}}} \times 100 \quad (1)$$

where Shoot $[P]_M$ and Shoot $[P]_{NM}$ are P concentrations (mg g^{-1} DW) in shoots of mycorrhizal plants and non-mycorrhizal plants, respectively.

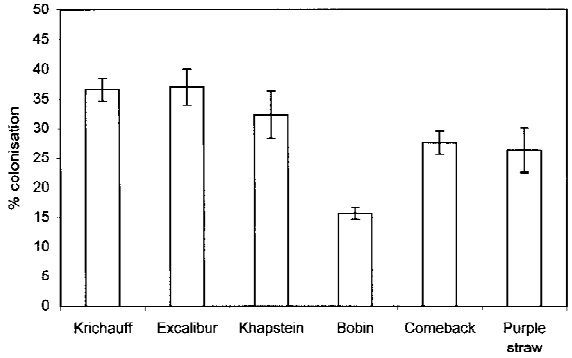

Figure 1. Percentage of root length of spring wheat plants colonised by arbuscular mycorrhizal fungi. Error bars indicate standard errors.

Specific P uptake (SPU) is expressed as total P uptake (mg P) per gram of dry root mass, and was calculated using Equation (2):

$$\text{SPU} = \frac{\text{Total P in plant (mg)}}{\text{root dry weight (g)}} \quad (2)$$

P uptake efficiency is defined as the total P uptake (mg P pot^{-1} or $plant^{-1}$); P utilisation efficiency is expressed as shoot biomass per unit total P uptake (g shoot DW mg^{-1} P).

All data were subjected to analysis of variance (ANOVA) using commercially available Genstat.

Results

Mycorrhizal colonisation

The percentage root length colonised by arbuscular mycorrhizal fungi was significantly different between different wheat cultivars, varying from 16% for Bobin to 37% for Excalibur ($P < 0.001$, Figure 1). The two modern cultivars had slightly higher colonisation than the four old cultivars, but there is no obvious trend of change with regards to the year of release of the cultivar.

Plant growth

Table 2 shows that dry weights of plant shoots and roots differed significantly between cultivars. Mycorrhizal colonisation had negative effect on biomass production of all cultivars, and the extent of the growth depression varied between cultivars. Differences in root/shoot ratio were highly significant between cultivars. Mycorrhizal colonisation slightly

Table 2. Dry weights of plant tissues of wheat plants grown in pot culture with (M) or without (NM) arbuscular mycorrhizal fungi

Cultivars	Shoot		Root	
	NM	M	NM	M
Krichauff	6.97	4.53	2.14	1.20
Excalibur	6.43	4.50	3.07	1.87
Khapstein	6.00	4.01	3.99	2.41
Bobin	6.07	3.57	2.60	1.50
Comeback	7.53	5.04	2.09	1.43
Purple Straw	5.47	2.99	3.29	1.81
	Analysis of variance			
Cultivar (C)	P <0.001		P <0.001	
Mycorrhiza (M)	P <0.001		P <0.001	
C × M	P =0.263		P <0.010	

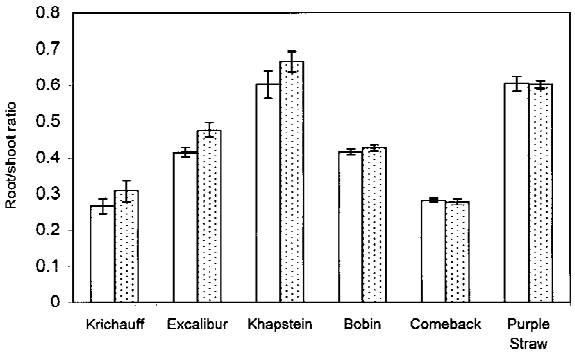

Figure 2. Root/shoot ratio of wheat plants grown in pot culture with (open columns) and without (dotted columns) arbuscular mycorrhizal colonisation. Error bars indicate standard errors.

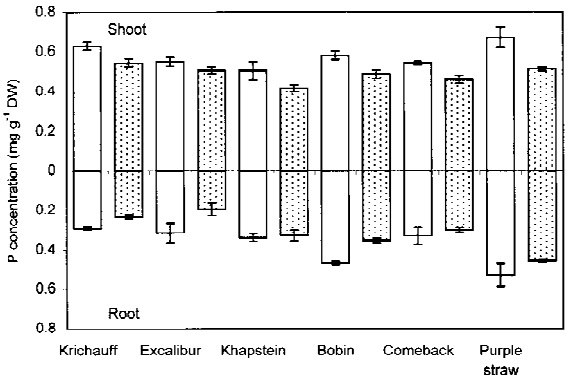

Figure 3. Tissue P concentrations of wheat plants grown in pot culture with (open columns) and without (dotted columns) arbuscular mycorrhizal colonisation. Error bars indicate the standard errors.

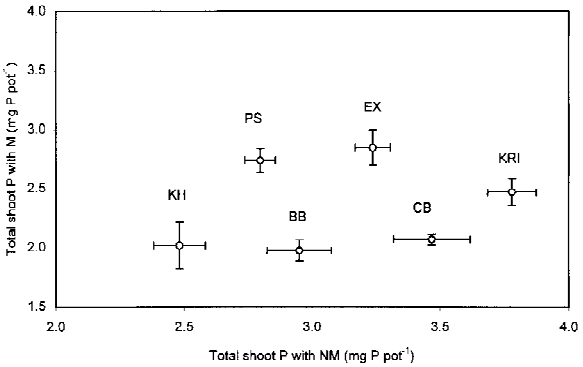

Figure 4. Total P accummulation in aboveground parts of wheat plants grown in pot culture with (M) or without (NM) mycorrhizal colonisation. Error bars indicate standard errors. Letters on each point indicate names of cultivars.

reduced root/shoot ratios in the more recently released cultivars Krichauff, Excalibur and Khapstein, but not in the older cultivars Bobin, Comeback and Purple Straw (Figure 2).

Plant P uptake

Shoot and root P concentrations are shown in Figure 3, and statistical analysis (ANOVA) showed significant differences between cultivars, irrespective of mycorrhizal colonisation (P<0.001). Mycorrhizal colonisation significantly increased P concentrations in both roots and shoots (P<0.001). No significant interactions between AMF colonisation and cultivars were found. In the absence of mycorrhizal colonisation, root P concentrations in modern cultivars were generally lower than those in old cultivars, but no such trend was found in shoot P concentrations. There were considerable variations in P accumulation in shoots, both with and without mycorrhizal colonisation, but no obvious trend between modern and old cultivars was found (Figure 4). Figure 5 shows that mycorrhizal responsiveness (MR) differed considerably between cultivars, and it appeared that old cultivars were generally more responsive to AMF than modern cultivars using this criterion.

Figure 6 shows that mycorrhizal colonisation significantly increased specific P uptake in all cultivars, and there were also considerable variations between cultivars. The differences in specific P uptake between mycorrhizal and non-mycorrhizal plants did not correlate with mycorrhizal colonisation (see Figure 1). There was no consistent trend in specific P uptake with the year of release of cultivars.

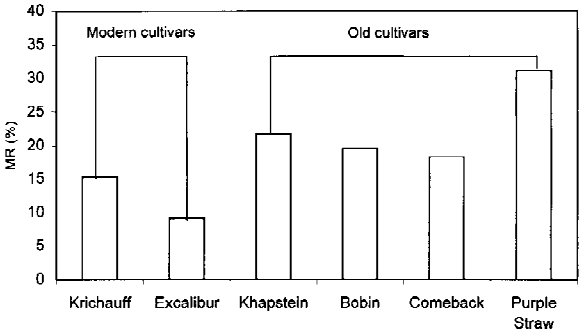

Figure 5. Mycorrhizal responsiveness of modern and old wheat cultivars. Each value represents the means of 4 replicates.

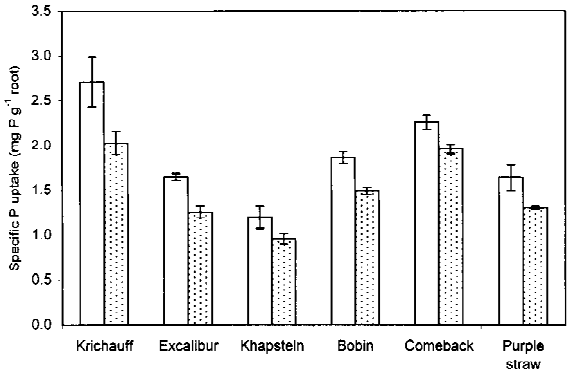

Figure 6. Specific P uptake of different wheat cultivars with (open columns) and without (dotted columns) arbuscular mycorrhizal colonisation. Error bars indicate standard errors.

Discussion

Considerable variations in P uptake and utilisation efficiencies were observed in modern and old wheat cultivars (Figure 3). The growth medium we used in this study is relatively low in bioavailable P ($NaHCO_3$-extractable P 9.4 mg kg^{-1} soil/sand mixture), therefore plants were under severe P stress. P concentration for optimal wheat growth is usually in the range of 3–5 mg g^{-1} dry weight (Marschner, 1995), while tissue P concentrations observed in our experiment were all below 1 mg g^{-1} dry weight (Figure 3). Under P stress, no obvious trends with the year of release of the cultivar were found in specific P uptake (SPU) or shoot P accumulation. This finding is in general agreement with results of Chapin et al. (1989), showing that P absorption potential did not differ consistently among wild and cultivated *Hordeum* species. We calculated SPU on the basis of root dry weight, and we expect that the ranking of SPU may change if it was calculated on the basis of root length. The ideal measurement of P uptake capacity of the root system would be the root surface area, which is related to root length and diameter. Without knowing root diameter, we believe that root weight- and root length-based SPU are equally effective, because root surface area could be variable either per unit root dry weight or per unit root length. Further studies on root morphology of old and modern cultivars are warranted for better understanding of the changes in plant P nutrition and mycorrhizal responsiveness in old and modern cultivars. P contents in seeds used in this study were relatively uniform (Table 1), therefore it is unlikely that seed P reserves would have confounded the results obtained here.

The old cultivars had somewhat lower percentage colonisation than modern cultivars and also had lower root dry weight. There was, therefore, no evidence that lower percentage colonisation was the outcome of greater or more rapid root growth. Mycorrhizal colonisation was found to depress plant growth in all cultivars. The growth depression was probably due to low irradiance (260–320 μE m^{-2} s^{-1}), which is well known to result in changes in symbiotic interactions, such as low colonisation, low growth responses or growth depressions (Graham et al., 1982; Son and Smith, 1988). However, even with the low irradiance used, the magnitude of growth depression in this study (around 30%) was comparable to that observed by Graham and Abbott (2000) for wheat, grown with relatively high light intensities. We suggest that pot size (i.e. soil volume) used in experiments may also partly affect the expression of mycorrhizal responsiveness, particularly for plants with large root systems such as spring wheat. Mycorrhizal responsiveness is likely to be greater when root length density is lower (i.e. in larger pots), because in this situation the exploration of the soil volume by mycorrhizal hyphae would become more critical for plant acquisition of P and other immobile nutrients. Pot size used in previous researches varied from just over 0.5 kg (Hetrick et al., 1993) to around 6 kg (Khalil et al., 1994), and the large variations in pot size will certainly reduce the comparability of different data sets.

Our results indicated that mycorrhizal colonisation increased tissue P concentrations in all cultivars (Figure 3). As tissue P concentrations observed in this study were well below the optimal level, increase in tissue P concentrations can be considered as an improvement in plant P nutrition. The general decrease in MR with the year of release of the cultivar observed in this study supported the assumption that modern cultural practices (including plant breeding) may have inadvertently limited or inhibited the symbi-

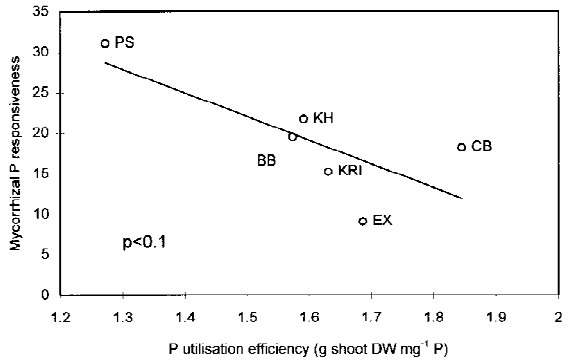

Figure 7. The relationship between mycorrhizal P responsiveness and P utilisation efficiency of wheat cultivars grown with no mycorrhizal colonisation.

otic contribution to crop plants (Hetrick et al., 1995). This highlights the importance of including mycorrhizas in breeding programs for maximising the efficiency of nutrient (particularly P) uptake and use by crops (Smith et al., 1992). However, in some species (e.g. *Lycopersicon esculentum* and *Avena sativa/fatua*) wild plants may benefit to a lesser extent than cultivated plants from mycorrhizal colonisation (Bryla and Koide, 1990; Koide et al., 1988). This inconsistency may be related to inherent morphological, physiological and phenological traits in different plant species (Bryla and Koide, 1990) and their different adaptations to soil infertility (Koide et al., 1988). Mycorrhizal responsiveness (either dry weight- or P concentration-based) is also likely to be altered by P efficiencies (uptake, utilisation or agronomic efficiencies). For example, Baon et al. (1993) found that mycorrhizal responsiveness (dry weight-based) was negatively correlated with agronomic P efficiency (g dry matter pot^{-1} at no P addition) in barley cultivars. Our results (Figure 7) show that increase in P utilisation efficiency with no mycorrhizal colonisation may reduce mycorrhizal responsiveness, but agronomic P efficiency and P uptake efficiency did not correlate with mycorrhizal responsiveness (data not shown).

In conclusion, this study has demonstrated that there is considerable variation in P efficiencies (uptake, utilisation and agronomic) between modern and old wheat cultivars, but no obvious trend of change with the year of release of the cultivar was found. Mycorrhizal P responsiveness may decrease with the year of release of the cultivar, which indicates that modern breeding programs may have inadvertently select cultivars with low mycorrhizal responsiveness. Mycorrhizal P responsiveness was also found to decrease generally with the P utilisation efficiency of the cultivar in the absence of mycorrhizal colonisation.

Acknowledgements

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