Yield components drive phosphorus use efficiency in *Brassica oleracea* and correlate with root traits

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Submission date: 11 Dec 2008

Number of tables: 4

Number of figures: 8

Abstract

The environmental and financial costs of using inorganic Pi fertilisers to maintain crop yield and quality are high. Breeding crops that acquire and use phosphorus (P) more efficiently could reduce these costs. We quantified the variation in shoot P concentration (shoot-P) and various measures of P use efficiency (PUE) among 355 *Brassica oleracea* L. accessions, 74 current commercial cultivars and 90 doubled haploid (DH) mapping lines from a reference genetic mapping population. Accessions were grown at two or more external P concentrations ($[P]_{ext}$) in glasshouse experiments; commercial and DH accessions were also grown in replicated field experiments. Within the substantial species-wide diversity observed for shoot-P and various measures of PUE in *B. oleracea,* current commercial cultivars have greater PUE than would be expected by chance. This may be a consequence of breeding for increased yield, which is a significant component of most measures of PUE or early establishment. Root development and architecture correlate with PUE, in particular, lateral root number, length and growth rate. Significant QTL associated with shoot-P and PUE occur on chromosomes C3 and C7. These data provide information to initiate breeding programmes to improve PUE in *B. oleracea*.

Introduction

Phosphorus (P) is essential to plants. Their roots acquire P from the rhizosphere solution as phosphate (Pi), primarily in the form of $H_2PO_4^-$ (Vance *et al*., 2003; Hammond et al., 2004; White and Hammond, 2008). The concentration of Pi in the soil solution is often low (2 to 10 μM) and consequently, the supply of Pi to the root surface by diffusion is slow (Bieleski, 1973; Marschner, 1995). Hence, P is one of the least available mineral elements in the soil and frequently limits plant growth (Vance *et al*., 2003; Tiessen, 2008).

Crops are frequently supplied with inorganic Pi fertilisers to maintain crop yields and quality. However, the environmental and financial costs of using inorganic Pi fertilisers are high. With crop production relying on large inputs of Pi fertilisers, and most crops not recovering all of the Pi fertiliser applied, excess soluble inorganic Pi fertilisers added to crops can be leached or eroded from the soil into surface waters. The agriculture sector in Great Britain contributes over 12,000 tonnes of P to surface waters annually (White and Hammond, 2009), resulting in nutrient enrichment of adjacent environments, with a consequent loss of habitats and decline in biodiversity. The implementation of the EU Water Framework directive, which imposes strict requirements on water quality, will require large reductions in diffuse P losses to the environment. There are also financial costs involved in the use of P fertilisers, which will increase in the future as a result of i) unsustainable production of P fertilisers from commercially viable, but non-renewable, reserves of phosphate rock, which are predicted to last only 50-100 years (Runge-Metzger, 1995; Denison and Kiers, 2005; Cohen, 2007), ii) unstable

energy prices, which will have an impact on the mining, transport and spreading of phosphate rocks and fertilisers (Helsel, 1992), and iii) potential introduction of financial instruments associated with meeting climate change, the EU water framework directive and other soil management targets.

Breeding crops that acquire and/or use P more efficiently is one strategy to reduce the use of Pi fertilisers. Such crops would either produce comparable yields with lower inputs of inorganic Pi fertilisers or have reduced physiological P requirements and tissue P concentrations, thus reducing the amount of P removed by the crop and, thereby, the amount of P needed to maintain the availability of Pi in the soil. Several measures of P use efficiency (PUE) have been proposed (Table 1; White *et al.*, 2005; White and Hammond, 2008). A common measure of PUE is the increase in yield per unit of added P fertiliser (g DM g^{-1} P_f), often referred to as the agronomic P use efficiency (APE) in the literature. This is equivalent to the product of the increase in plant P content per unit of added P fertiliser (g P g^{-1} P_f), which has been referred to as plant P uptake efficiency (PUpE) in the literature, and the increase in yield per unit increase in plant P content (g DM g^{-1} P), which has been referred to as the P utilisation efficiency (PUtE) in the literature. The same relationship holds when yield and P content are determined at a specific P concentration in the rooting medium.

Other measures of PUE commonly encountered in the literature are: (i) yield divided by the amount of P in the plant (g DM g^{-1} P), which is also referred to as the P efficiency ratio (PER) and is equivalent to the reciprocal of tissue P concentration if the entire plant is harvested, (ii) yield divided by tissue P concentration at a given P concentration in the rooting medium (g^2

DM g^{-1} P) which may be referred to as physiological P use efficiency (PPUE), (iii) amount, or concentration, of P in the rooting medium required for a given percentage of maximum yield (g P), which may be expressed as either the 'Km' value required for half-maximal yield or the 'critical' value required for 90% yield, and (iv) tissue P concentration required for a given percentage of maximal yield, which is referred to as the 'critical' tissue P concentration if this is 90% of maximal yield (White *et al.*, 2005; White and Hammond, 2008). Crops and varieties that have low Km and critical soil P values will grow to their potential with minimal P fertilisation. Crops with lower critical tissue P concentrations are likely to tolerate soils with low Pi availability better and reduce P-fertiliser requirements since less 'maintenance' P-fertilisation is needed to maintain soil P concentration.

There is considerable within-species genetic variation in all these measures of PUE (see reviews by White *et al*. 2005, White and Hammond, 2008). However, differences in the response of yield to P fertilisation do not appear to be correlated with PUtE. Thus, selection for greater PUtE does not appear to be an effective strategy for developing crops that yield well on soils with low P availability. However, genotypes of crops that yield well and have lower tissue P concentrations can be used to reduce P-fertiliser inputs to soils that require only maintenance P fertilisation.

Natural genetic variation has been observed for various measures of PUE in common bean (*Phaseolus vulgaris* L.; Gabelman and Gerloff, 1983; Fageria and da Costa, 2000), wheat (*Triticum aestivum* L.; Fageria and Baligar, 1999; Osborne and Rengel, 2002; Wang *et al*., 2005), spring barley (*Hordeum vulgare* L.; Górny and Sodkiewicz, 2001), rice (*Oryza sativa* L.;

Fageria and Baligar, 1997*a*; Wissuwa *et al.*, 2002), maize (*Zea mays* L.; Fageria and Baligar, 1997*b*; Baligar *et al*., 1997), Arabidopsis (Krannitz *et al*., 1991; Narang *et al*., 2000; Hammond, 2004) and cowpea (*Vigna unguiculata* L.; Krasilnikoff *et al*., 2003). The continuous variation observed for these traits suggests that they are controlled by quantitative trait loci (QTL) (Duncan and Carrow, 1999; Ahmad *et al*., 2001; Baligar *et al*., 2001). In rice, a major QTL for P-deficiency tolerance, P uptake 1 (*Pup1),* has been mapped to a 150 kb region of Chromosome 12, containing 60 predicted genes, (Wissuwa *et al*., 2002; Ismail *et al*., 2007). Among Brassicaceae species, QTL have been associated with leaf and seed P and phytate concentration, and primary root growth responses to low P availability (Bentsink *et al*., 2003; Loudet *et al*., 2003; Hammond, 2004; Vreugdenhil *et al*., 2004; Reymond *et al*., 2006; Svistoonoff *et al*., 2007; Zhao *et al*., 2007, 2008). Notably, a multicopper oxidase gene involved in root cap sensing of P in *Arabidopsis* (Svistoonoff *et al*., 2007), has been cloned using such forward genetic approaches.

Here, we demonstrate large species-wide variation within *Brassica oleracea* L. for shoot P concentration (shoot-P), different measures of PUE, and their responsiveness to $[P]_{ext}$. Taking extreme phenotypes from within the species we demonstrate that the responsiveness of *B. oleracea* to $[P]_{ext}$ correlates with root development and architecture. Finally, we have used a forward genetic approach to identify QTL associated with different measures of PUE.

Materials and Methods

Plant material

The plant material used to study the species-wide variation in shoot-P and the responsiveness to available P in the domesticated genepool of *Brassica oleracea* L. has been described previously (Broadley *et al*., 2008). This consisted of a selected Diversity Foundation Set (DFS) of 376 sampled from the >4,300 C-genome *B. oleracea* accessions held in the Warwick HRI Genetic Resources Unit (HRI-GRU). Since theoretical studies of natural populations (Lawrence *et al*., 1995a, b) indicate that 400 accessions, collected from throughout the world, should contain 99% of the allelic polymorphism (i.e. for alleles with frequencies >2%) present in a species, this DFS is likely to represent most of the common allelic variation within the species. To assess existing genetic variation in current or recent cultivation in N. Europe, a further set of genotypes, primarily commercial cultivars, was also sampled, to represent the distinct major *B. oleracea* morphotypes.

Plant material for the QTL mapping experiments consisted of a sub-population of 90 DH lines selected from a larger segregating population of 206 lines representing the 'AGDH' mapping population (Broadley *et al*., 2008). The AGDH mapping population was generated through anther culture of the F_1 of a cross between a DH rapid-cycling accession *B. oleracea* var. *alboglabra* (‘A12DHd’) and a DH accession derived from an F_1 hybrid calabrese cultivar, ‘Green Duke’, *B. oleracea* var. *italica* (‘GDDH33’; Bohuon *et al*., 1996; Rae *et al*., 1999; Sebastian *et al*., 2000). A linkage map of 906 cM for the AGDH mapping population has been developed, with a mean distance between marker loci of 1.92 ± 3.49 cM, such that *c.* 90% of the genome was within 5 cM of a marker (Sebastian *et al*., 2000; Broadley *et al*.,

2008). To test the location of QTLs in the AGDH population, 20 substitution lines (the 'AGSL' population; Rae *et al*., 1999; Broadley *et al*., 2008) were grown.

Both A12DHd and GD33DH, and eight *B. oleracea* commercial cultivars, used previously to develop appropriate growth conditions (Greenwood *et al*., 2005, 2006), were used as common reference cultivars in all experiments (Broadley *et al*., 2008).

Field and glasshouse experiments

Plants were grown in a series of field and glasshouse experiments (Table 2; Broadley *et al*., 2008). These consisted of (1) a glasshouse experiment (GE1), in which the three replicates of the 376 DFS accessions and nine replicates of the 74 commercial cultivars were grown in peat based compost containing 5.25 mg L^{-1} (low $[P]_{ext}$) or 15.75 mg L^{-1} (high $[P]_{ext}$) of added P following the incorporation of 0.075 g and 0.225 g of sieved (500 µm) single superphosphate (SSP, 7% P) per litre of compost. Other nutrients were incorporated in the potting-mix in sufficient amounts to prevent deficiencies. Plant shoots were sampled at similar developmental stages, 39, 47, 49, 49, 42, 37 days after sowing on Occasions 1-6, respectively; (2) a field experiment (FE1) in which 72 commercial cultivars were grown on three occasions, with three replicates, at four $[P]_{ext}$ using an alpha design (Patterson and Williams, 1976). Each of the $[P]_{ext}$ treatments were imposed by addition of triple superphosphate (21% P, TSP) equivalent to 0, 298, 1125, or 2713 kg TSP ha^{-1}. TSP was incorporated to a depth of 0.10 m using a power harrow (Greenwood *et al*., 2005). Plant shoots were sampled after

101, 97, 93 days growth on Occasions 1-3, respectively. These timings were chosen to represent pre-commercial maturity; (3) a second glasshouse experiment (GE2), in which nine replicates of 90 AGDH lines plus the A12DHd and GDDH33 parents of the AGDH population, and eight reference commercial cultivars were grown at the same two $[P]_{ext}$ as GE1; (4) a second field experiment (FE2), in which three replicates of 72 cultivars (62 AGDH lines plus the two AGDH population parents and eight reference commercial cultivars) were grown at the same four $[P]_{ext}$ levels as FE1. Plant shoots were sampled after 105 days growth; (5) a third glasshouse experiment (GE3), in which three replicates of the 20 AGSLs were grown at the same two $[P]_{ext}$ as GE1 and GE2. Plant shoots were sampled 39 days after sowing; (6) a fourth glasshouse experiment (GE4) in which three replicates of 18 accessions (Table S1) from the DFS with extreme phenotypes were grown in compost under P replete conditions. In addition to shoot material being harvested, roots were also harvested, weighed, washed and imaged to calculate root length, area and volume (Fig. 1AB); (7) a growth room experiment (CE1) in which three replicates of the 18 accessions grown in GE4 were grown on Steel Blue Seed Germination Blotter paper (Fig. 1C; Bonser *et al*., 1996; Anchor Paper Company, MN, USA) supported on glass plates in a system similar to that described previously by Murphy and Taiz (1995). The glass plates/blotter papers were placed in a container containing MS salts solution (Murashige and Skoog, 1962; Hampton *et al*., 2004), modified to contain 0.625 (high) or 0.006 (low) μM P. Seedlings were transferred to the blotter paper 4 days after sowing and harvested 7 days after transfer. Images of the root system were taken at transfer and harvest (Fig. 1C). Seedlings were

placed in a growth room set to 24 °C, with 16 h light d^{-1}. Illumination was provided by a bank of 100 W 84 fluorescent tubes (Philips, Eindhoven, Netherlands) giving an intensity of 45 µmol photons m^{-2} s^{-1} at plant height.

In all experiments, shoot fresh weight (FW), comprising all above ground biomass, was recorded immediately, and shoot dry matter (DM) after oven-drying at 60 ºC for 72 h. For GE1, total shoot P concentration (shoot-P) was determined by a commercial foliar analysis laboratory (Yara Phosyn Ltd, Pocklington, York, UK). For all other experiments, shoot-P was determined using the micro Kjeldahl method, *ca* 0.1 g subsample of dried plant material was digested for 1 h, following the addition of 1 ml of H_2O_2 and 2 ml of a H_2SO_4/Se catalyst (Bradstreet, 1965). Inductively-coupled plasma emission spectrometry (JY Ultima 2, Jobin Yvon Ltd., Stanmore, Middlesex, UK) was used to determine mineral concentrations in digested shoot material.

Data analysis

Several measures of PUE were calculated from the data (Table 1). For PPUE and PER, values were calculated for plants grown at low and high $[P]_{ext}$. Data were analysed using REML procedures in GenStat (Release 9.1.0.147, VSN International, Oxford, UK) to allocate sources of variation and estimate accession means for individual experiments (Patterson and Thompson, 1971; Robinson, 1987). QTL mapping was performed with the QTL Café programme (Seaton, 2000) as described previously (Payne *et al*., 2004), and QTL Cartographer 2.0 (Wang *et al*., 2004), using the composite interval mapping (CIM) option as described previously (Broadley *et al*., 2008). In GE4, root system area was calculated using MatLab (Version 7.7;

The MathWorks, MA, USA). In CE1, total root length and lateral root length were calculated using ImageJ (Abramoff *et al*., 2004).

Results

Measures of PUE vary widely within B. oleracea *due to genetic and non-genetic factors*

We used a diversity foundation set (DFS) consisting of 376 founder lines, which included landrace, open-pollinated and more uniform F_1 or inbred lines that represent most of the common allelic variation within *B. oleracea*, in addition to 74 commercial varieties (Table S2). It was impractical to screen all accessions under a range of $[P]_{ext}$. Therefore, a method was developed for obtaining growth response parameters from two $[P]_{ext}$. (Greenwood *et al*., 2005, 2006). Subsequently, these accessions were screened in replicated trials under glasshouse conditions at two levels of $[P]_{ext}$.

Substantial species-wide variation was observed for shoot-P and various measures of PUE among the 355 diversity foundation set (DFS) accessions and 74 commercial cultivars successfully grown in experiment GE1 (Fig. 2). Shoot-P varied 4.9 fold at low $[P]_{ext}$ and 2.8 fold at high $[P]_{ext}$ between the 355 DFS accessions with mean shoot-P of 0.19 %P for plants grown at low $[P]_{ext}$ and 0.34 %P for accessions grown at high $[P]_{ext}$ (Fig. 2A; Table S2). Values for agronomic P use efficiency (APE), P uptake efficiency (PUpE) and P utilisation efficiency (PUtE) calculated for accessions in the DFS had a wider distribution than those calculated for current commercial cultivars (Fig. 2B, D, F). However, the mean values for APE, PUpE and PUtE

calculated for the commercial cultivars were all greater than the mean values calculated for accessions in the DFS. Physiological P use efficiency (PPUE) had the greatest range in values, varying between -294.7 and 1268.4 g^2 g^{-1} P for accessions grown at high $[P]_{ext}$, and varying between -62.9 and 1051.2 g^2 g^{-1} P for accessions grown at low $[P]_{ext}$ (Fig. 2E). Negative values arose due to lower yield at higher $[P]_{ext}$ for some cultivars and/or as a mathematical consequence of the REML procedure. The mean value for PPUE at high $[P]_{ext}$ was also greater than the population mean for PPUE at low $[P]_{ext}$ among both accessions in the DFS and commercial cultivars. Again, the variation in PPUE within commercial cultivars was less than that observed for accessions in the DFS (Fig. 2E). As expected the P efficiency ratio (PER) had a greater mean value when accessions were grown at low $[P]_{ext}$ than when grown at high $[P]_{ext}$. The mean PER for commercial cultivars was greater and the variation in PER was less, in commercial cultivars than in accessions of the DFS at both low and high $[P]_{ext}$ (Fig. 2C).

Since environment has a significant effect on shoot-P, we tested if shoot-P and measures of PUE correlated between glasshouse and field environments amongst the genetically uniform commercial cultivars. The distribution of values for shoot-P among commercial cultivars represented >60% of the species-wide distribution for shoot-P in the DFS. Among the 69 *B. oleracea* accessions grown in both GE1 and FE1, significant (P<0.01) positive correlations were obtained for shoot DM and shoot P. Thus, glasshouse conditions can be used to represent variation in measures of PUE, but environmental components significantly affect these traits and must be accounted for (Table S3). Treatment variation attributed to the accession

terms was 29.2 and 11.0% of the total variation for shoot-P at low and high $[P]_{ext}$ respectively (Table S3). Genetic variance components were highly significant (P<0.001) for APE, PUpE, PER and PPUE, but not for PUtE (P=0.998) and ranged between 2.3 and 15.1% of the total variation (Table S3).

Shoot-P differed significantly (P<0.001) between different subtaxa, with *botrytis* and *italica* subtaxa having the highest mean shoot-P and subtaxa with cabbage morphologies (*capitata*, *sabauda* and *tronchuda*) having the lowest mean shoot-P (Fig. 3A). APE, PUpE, PUtE, PPUE at high $[P]_{ext}$, PPUE at low $[P]_{ext}$, PER at high $[P]_{ext}$, and PER at low $[P]_{ext}$ differed significantly (P=0.024 to <0.001) between different subtaxa. Subtaxa representing cabbages and kales (*acephela*, *alboglabra* and *sabellica*), had higher mean APE, PUpE, PUtE, PPUE and PER compared to the *botrytis, gemmifera, gongylodes* and *italica* subtaxa (Fig. 3B-D).

The effect of shoot biomass accumulation on shoot-P was tested within subtaxa, to avoid confounding effects of shoot morphology. Shoot-P at high $[P]_{ext}$ was significantly (P<0.001) inversely correlated with shoot biomass for all subtaxa. For shoot-P at low $[P]_{ext}$, there was a significant (P<0.001) negative correlation for all subtaxa, except *sabauda* (P=0.157, n=15), *sabellica* (P=0.183, n=6), and *tronchuda* (P=0.606, n=17), possibly due to the small sample size for the latter subtaxa. These data suggest a growth dilution effect in the shoot material of *B. oleracea* for shoot-P.

Commercial cultivars are more efficient and responsive to P

Accessions from the DFS and commercial cultivars were divided into four groups based on their responsiveness to $[P]_{ext}$, measured as APE, PUtE or PUpE, and their yield at low $[P]_{ext}$ (Fig. 4; *sensu* Fageria and Baligar, 1993). The first group contained efficient and responsive (ER) accessions, with above average yield at low $[P]_{ext}$ and responsiveness to $[P]_{ext}$, measured as APE, PUtE or PUpE (Fig. 4). Commercial cultivars were significantly (P<0.001) over-represented in this category for all measures of responsiveness to $[P]_{ext}$. Of the 74 commercial cultivars screened, 45 were in the ER group for all measures of responsiveness to $[P]_{ext}$. Eight commercial cultivars were consistently grouped as non-efficient and non-responsive (NENR) for all measures of responsiveness to $[P]_{ext}$.

Root traits correlate with measures of PUE

Root biomass and architectural traits were measured in a subset of extreme accessions from GE1, to investigate the underlying traits associated with improved PUE. Extreme accessions were selected and grouped together based on their yield at low and high $[P]_{ext}$ (Fig. 5; Table S1). Five groups of accessions were selected representing accessions that have low (Group 1), average (Group 2) and high (Group 3) yields when grown at high or low $[P]_{ext}$, accessions that have high yields when grown at high $[P]_{ext}$ and average yields when grown at low $[P]_{ext}$ (Group 4) and accessions that have low yields when grown at high $[P]_{ext}$ but average yields when grown at low $[P]_{ext}$ (Group 5).

Root DM and root areas were measured for extreme accessions grown in compost under glasshouse conditions with high $[P]_{ext}$ (GE4; Fig.

1AB). Root DM, root area and specific root area (SRA) differed significantly (P<0.05) between Groups (Fig. 6). Groups that have average to high yields at low $[P]_{ext}$ had greater root areas and lower SRA compared to Group 1, which contains accessions with low yields at low and high $[P]_{ext}$ (Fig. 6).

Since differences in root architecture can affect a plant's ability to intercept $[P]_{ext,}$ the root architectures of these accessions were studied in more detail. Lateral root number was higher for Groups with average to high yields at low $[P]_{ext}$, with lateral root number increasing with yield potential of the Group (Fig. 7A). Total lateral root length and lateral root growth rate were higher for Groups 3 and 4, which have the greatest yields at low and high $[P]_{ext}$ (Fig. 7BC). Interestingly, there was no significant effect of Group or $[P]_{ext}$ on lateral root angle (Table S1). There were significant effects of Group and $[P]_{ext}$ on total root length, primary root length and total root growth rate and root DM (Table S1). All accessions had greater total root length and greater growth rates when grown at low $[P]_{ext}$ compared to when they were grown at high $[P]_{ext}$, but most accessions had lower root DMs when grown at low $[P]_{ext}$. Various measure of PUE correlated significantly with root architectural traits (Table 3). Lateral root growth rate, lateral root length and lateral root number had significant (P<0.05) positive correlations with APE, PPUE at high $[P]_{ext}$ and PUtE. There was also a significant (P<0.05) positive correlation between lateral root angle and PER and PPUE at low $[P]_{ext}$.

Characterisation of genetic material for detection of QTLs associated with measures of PUE

Variation in measures of PUE among the species-wide genepool was compared to variation in measures of PUE associated with allelic combinations within a population derived from two homozygous DH parental accessions, again using plants grown at low and high $[P]_{ext}$ in the glasshouse (GE2) and field (FE2). Genetic loci associated with the responsiveness to $[P]_{ext}$ were mapped using these DH accessions (GE2, FE2), and these loci were confirmed and resolved using substitution lines in a further glasshouse experiment (GE3).

Shoot-P varied 2.0 fold at low $[P]_{ext}$ and 1.9 fold at high $[P]_{ext}$ between the 90 DH accessions with mean shoot-P of 0.21 %P for plants grown at low $[P]_{ext}$ and 0.31 %P for accessions grown at high $[P]_{ext}$ (Table S4). Genetic variance components for DH accessions approximate the population-wide additive genetic variation (V_A), or narrow-sense heritability. The treatment variance component attributed to accession (genetic variance) accounted for 17.5% and 15.1% of the total variation in shoot-P at low and high $[P]_{ext}$, respectively (Table S3). Genetic variance components were highly significant for shoot-P at low and high $[P]_{ext}$ (P<0.001). The proportion of the spread of values observed in the species-wide data set (GE1 and FE1; Table S2), captured by the forced recombination of alleles in the DH accessions was 38 and 63% for shoot-P at low and high $[P]_{ext}$. Similar data values and genetic variance components were observed for the 62 accessions successfully grown under field conditions (FE2; Table S4).

Measures of PUE also varied among 90 DH accessions grown in the glasshouse (GE2; Table S4). Trait data ranges were: APE -1.2 to 56.8 g DM g^{-1} P_f, PUpE 6.0 to 23.0 g P g^{-1} P_f, PUtE -2716.6 to 450.7 g DM g^{-1} P, PPUE

at low $[P]_{ext}$ 252.0 to 951.9 g^2 DM g^{-1} P, PPUE at high $[P]_{ext}$ 150.9 to 833.3 g^2 DM g^{-1} P, PER at low $[P]_{ext}$ 368.7 to 684.8, and PER at high $[P]_{ext}$ 232.7 to 449.7 (Table S4).

The treatment variance component attributed to accession was highest for PPUE at low and high $[P]_{ext}$, accounting for 31.5 and 28.1% of the total variation, respectively (Table S3). The treatment variance component attributed to accession for PER at low and high $[P]_{ext}$ was 14.7 and 15.2% respectively. Only 4.2 and 3.5% of the treatment variation was attributed to accession for APE and PUpE respectively. Genetic variance components were highly significant for all traits (P<0.001), except PUtE (P=0.496). The proportion of the spread of values observed in the species-wide data set (GE1 and FE1; Table S2), captured by the forced recombination of alleles in the DH accessions was substantial for all traits. The spread of data values for PPUE at low and high $[P]_{ext}$ captured most of the spread of data observed in GE1, representing 44 and 63% of the species-wide spread respectively.

Significant and positive correlation coefficients were obtained among the nine reference *B. oleracea* accessions grown in both GE1 and GE2 for shoot-P at low and high $[P]_{ext}$ and for all measures of PUE, except for PUtE (data not shown). Similarly, there were positive correlations between the measures of PUE under glasshouse and field conditions among the 61 AGDH accessions, 2 parent lines, and 8 reference cultivars grown in both FE2 and in GE2. Therefore, in general, measures of PUE for *B. oleracea* accessions responded consistently between replicate experiments and environments, under field and glasshouse conditions. Thus, the choice of genetic material, and the glasshouse experimental conditions were

considered sufficiently robust for mapping QTL associated with measures of PUE.

Quantitative trait loci (QTL) associated with measures of PUE are located on chromosomes C3 and C7

Marker means for shoot-P at low and high $[P]_{ext}$ were calculated for the 90 DH accessions grown in GE1 and the 62 DH accessions grown in FE2. For shoot-P at both low and high $[P]_{ext}$ in GE1, there was a significant negative effect of the A12DHd parental allele on the top of chromosome C3 and a significant positive effect of the A12DHd parental allele on the bottom of C3 (Fig. 8; Table 4). There was also a significant negative effect of the A12DHd parental allele in the middle of C7. The significant negative effect on the top of C3 coincides with significant positive effects of the A12DHd parental allele for FW and DM at low and high $[P]_{ext}$. Marker regression was used to identify the presence of significant (P<0.05) QTL on individual chromosomes associated with shoot-P at low and high $[P]_{ext}$. Significant (P<0.05) QTL associated with shoot-P at low and high $[P]_{ext}$ were identified between 30 and 32 cM and between 106 and 108 cM on C3 (Fig. 8; Table 4). A significant (P<0.05) QTL associated with shoot-P at high $[P]_{ext}$ was identified at 32 cM on C7. Composite interval mapping confirmed the presence of both significant QTL on C3, but not those C7. Analysis of marker means and marker regression data from FE2 confirmed the presence of a significant QTL associated with shoot-P on C7, with a negative effect of the A12DHd parental allele. No QTL associated with shoot-P were identified on C3 in FE2.

Marker means for different measures of PUE were calculated for the 90 DH accessions grown in GE1. There was a significant positive and a significant negative effect of the A12DHd allele on C3 for APE, PUpE, PPUE at high $[P]_{ext}$, PPUE at low $[P]_{ext}$, PER at high $[P]_{ext}$, and PER at low $[P]_{ext}$, and a significant positive effect of the A12DHd allele on C7 for APE, PPUE at low $[P]_{ext}$. No significant effects were observed for PUtE. Significant QTL (P<0.05) associated with APE, PUpE, PPUE at high $[P]_{ext}$, PPUE at low $[P]_{ext}$, PER at high $[P]_{ext}$, and PER at low $[P]_{ext}$, were identified between 22 and 28 cM on C3 (Fig. 8; Table 4). Significant QTL (P<0.05) associated with APE, PPUE at high $[P]_{ext}$, PER at high $[P]_{ext}$, and PER at low $[P]_{ext}$, were identified between 24 and 38 cM on C7. Composite interval mapping also identified a significant QTL at 23 cM on C3 for PPUE at high $[P]_{ext}$.

Testing QTL associated with measures of PUE

The presence of QTL associated with shoot-P and measures of PUE were tested using recurrent backcross substitution lines (AGSLs), in which segments of the GDDH33 line are introgressed into the A12DHd background (Rae *et al*., 1999; Broadley *et al*., 2008). Of the AGSLs screened AGSL118, 119, 134, 169 and 173 were informative for QTL regions associated with shoot-P. AGSLs 118 and 169 had higher shoot-P at low and high $[P]_{ext}$ than the A12DHd parent, consistent with a negative effect of the A12DHd parental allele on C7 (Table 4; Table S5). AGSL173 had a lower shoot-P at low and high $[P]_{ext}$ than the A12DHd parent, consistent with a positive effect of the A12DHd parental allele on C3 and AGSL134 had higher shoot-P at high $[P]_{ext}$ than the A12DHd parent, but not at low $[P]_{ext}$, partly consistent with a

negative effect of the A12DHd parental allele on C3. The trait values for AGSL134 for APE, PPUE at high $[P]_{ext}$, and PER at high $[P]_{ext}$, were lower than the value for the A12DHd parent, consistent with the negative effect of the GDDH33 allele on C3. The trait values for AGSL173 for APE, PPUE at low and high $[P]_{ext}$, and PER at high $[P]_{ext}$, were higher than the value for the A12DHd parent, consistent with the positive effect of the GDDH33 allele on C3. The trait values for AGSL118 and 169 for PER at low and high $[P]_{ext}$, were lower than the value for the A12DHd parent, consistent with the negative effect of the GDDH33 allele on C7 (Table S5). Trait values for AGSL118 and AGSL169 for APE and PPUE at high $[P]_{ext}$ were not consistent with the negative effect of the GDDH33 allele. Further backcrosses will be required to verify and resolve these loci.

Discussion

There is large species-wide variation within *B. oleracea* for shoot-P and various measures of PUE (Fig. 2). Using extreme phenotypes from within the species we have demonstrated that the responsiveness of *B. oleracea* to $[P]_{ext}$ and various measures of PUE correlate with root development and architecture (Table 3). In particular, there were significant correlations between lateral root number, length and growth rate and many measures of PUE. Using a forward genetic approach we have identified QTL associated with shoot-P and measures of PUE (Fig. 8; Table 4) and tested several QTL using substitution lines (Table 4).

Variation in shoot-P and measures of PUE between genotypes is consistent with other studies. Previous studies of commercial *Brassica*

cultivars have shown limited variation in shoot-P between a restricted number of commercial and advanced breeding lines (Shi *et al*., 2004; Solaiman *et al*., 2007; Akhtar *et al*., 2008). However, this study has shown large species-wide variation in shoot-P for *B. oleracea* (Fig. 2A), which is consistent with the large variation in leaf-P among five mapping populations of *B. rapa* (Wu *et al*., 2008; Zhao *et al*., 2008). Few studies have assessed the PUE of *Brassica* plants. Akhtar *et al*. (2008) demonstrated a 10-fold range of values for PPUE in their analysis of 14 *B. napus* cultivars, which is similar to the 4 to 5-fold range of values observed for PPUE in commercial cultivars screened in this study (Fig. 2E). The natural genetic variation observed between genotypes of *B. oleracea* demonstrates the potential for breeding cultivars with improved PUE, which will ultimately utilise applied inorganic Pi fertilisers more efficiently. Interestingly, when shoot-P and measures of PUE are separated into subtaxa (Fig. 3), those representing *B. oleracea* inflorescence mutants (e.g. cauliflower [*botrytis*], broccoli [*italica*]) had higher shoot-P and lower measures of PUE compared to leafy *B. oleracea* subtaxa, when harvested prior to floral initiation (Fig. 3). This may represent previous selection for quality and early vigour traits in cauliflower and broccoli.

Commercial cultivars were more likely to be classed as efficient and responsive to $[P]_{ext}$ than would be expected by chance (Fig. 4). This implies that responsiveness to $[P]_{ext}$ has been inadvertently selected for as part of current commercial breeding programmes. A major component of this is likely to be breeding for increased yield, which is a significant component of all measures of PUE, or early establishment in the field. Increasing yield,

whilst maintaining or decreasing shoot-P (as an effect of dilution), will lead to increased PUE. The assessment of yield has previously been suggested as a potential criterion for evaluating genotypes for PUE in young plants (Römer and Schenk, 1998; Akhtar *et al*., 2008). However, this does not provide information on the underlying processes driving PUE or responsiveness to $[P]_{ext}$.

Root system architecture, morphology and biochemistry can greatly affect the ability of a plant to acquire nutrients from the soil, in particular P, and thus their PUE and responsiveness to $[P]_{ext}$ (White *et al*., 2005, 2007; Lynch, 2007; Hammond and White, 2008). To investigate the relationship between root traits and measures of PUE, we selected a subset of extreme phenotypes (Fig. 5) and scored their root systems for root growth and architectural traits (Fig. 6 and 7). In Arabidopsis, an increase in the initiation and elongation of lateral roots has been observed under low $[P]_{ext}$ conditions (Williamson *et al*., 2001; Linkohr *et al*., 2002; López-Bucio *et al*., 2002, 2003, 2005; Al-Ghazi *et al*., 2003; Nacry *et al*., 2005). Accessions that had greater yields under high $[P]_{ext}$ (Group 3 and 4) had a greater number of lateral roots, which were longer and grew faster compared to accessions that had average or low yields at high $[P]_{ext}$ (Fig. 7). Accessions that had higher yields at low $[P]_{ext}$ (Groups 2, 3 4 and 5) also had a greater number of lateral roots, which were longer compared to accessions that had low yields at low $[P]_{ext}$ (Group 1). Most accessions also had a greater number of lateral roots that were also longer at low $[P]_{ext}$ compared to high $[P]_{ext}$, suggesting they are able to explore a greater volume of soil and thus access more P. In a comparison between two *B. napus* cultivars with either high or low PPUE, Akhtar *et al*.

(2008) showed a significant difference in lateral root length, with the high PPUE cultivar having a greater lateral root length compared to the low PPUE cultivar. Similarly, Solaiman *et al*. (2007) demonstrated that a P efficient canola cultivar had a greater total root length compared to a P inefficient cultivar. Lateral root traits also had the greatest correlations with various measures of PUE (Table 3). In *Phaseolus vulgaris*, P starvation results in a change in the growth angle of basal roots, generating a shallower root phenotype, allowing it to forage for available Pi in the top soil (Bonser *et al*., 1996; Lynch and Brown, 2001; Rubio *et al*., 2003; Lynch and Brown, 2008). Analysis of root angle between $[P]_{ext}$ treatments and groups of phenotypes, revealed no significant differences between groups or treatments, suggesting it might not be a major strategy for acquiring P in *B. oleracea* under the conditions used in this study. In Arabidopsis, P starvation has been shown to induce a cessation of primary root growth, through a decrease in meristematic activity (Ticconi *et al*., 2004; Sánchez-Calderón *et al*., 2005). However, there appeared to be no effect of $[P]_{ext}$ on the growth rate of the *B. oleracea* primary roots studied here (data not shown).

We detected several regions of the *B. oleracea* genome associated with shoot-P and responsiveness to $[P]_{ext}$ (Fig. 8; Table 4). The loci associated with several of these traits co-localise, including a significant QTL for shoot DM, shoot-P and measures of PUE at approximately 30 cM on C3 (Fig. 8), with a positive additive effect of the A12DHd parental allele for shoot DM and all measures of PUE, in contrast to the negative additive effect for shoot-P. This suggests a greater influence of shoot DM accumulation on measures of PUE, in contrast to more efficient accumulation or internal use

of P within the plant. Several traits also have loci that co-localise at 108 cM on C3, including a positive additive effect for shoot DM and shoot-P at low and high $[P]_{ext}$, and a negative additive effect for APE, PER at low and high $[P]_{ext}$ and PPUE at low and high $[P]_{ext,}$ and at 28 cM on C7 with a positive additive effect for APE, PPUE at high [P] and PER at low and high $[P]_{ext}$ and a negative additive effect for shoot-P at high $[P]_{ext}$ (Table 4).

Previously, QTL for leaf-P have been identified in Arabidopsis and *B. rapa* mapping populations (Bentsink *et al*., 2003; Loudet *et al*., 2003; Wu *et al*., 2008; Lisec *et al*., 2008; Zhao *et al*., 2008). The high co-linearity and synteny between the Arabidopsis, *B. rapa*, and *B. oleracea* genomes enables the identification of conserved loci between these species (Parkin *et al*., 2005). QTL for leaf-P have been located in *B. rapa* on chromosomes A1, A3 and A8, at 27, 40 and 47 cM respectively (Zhao *et al*., 2008). QTL for leaf-P on A1 co-localise with QTL identified in this study for PPUE and shoot DM on C1. However, QTL for shoot-P, shoot DM and various measures of PUE on C3 do not co-localise with QTL for leaf-P identified on A3 (Zhao *et al*., 2008). Alignment with QTL identified in Arabidopsis for shoot-P reveals some co-localisation between loci. QTL mapped to the top of C1 and C3 in this study co-localise with QTL for shoot-P identified previously (Hammond 2004; Lisec *et al*., 2008) on the bottom of chromosome 4 and the top of chromosome 5 in Arabidopsis respectively. This suggests loci for shoot-P may be conserved in the Brassicaceae, but further work, including identification of the genes responsible for these QTL, is required to confirm this.

Conclusion

We have successfully characterised the species-wide diversity in *B. oleracea* for shoot-P and various measures of PUE. Significant QTL associated with shoot-P and measures of PUE were identified on C3 and C7, and confirmed using substitution lines. Further fine mapping of these loci is required to improve their resolution and identify the genes underlying them. These data will provide sufficient information to initiate breeding programmes to develop *B. oleracea,* and potentially broad-acre oil seed rape *B. napus*, varieties with improved PUE. These crops will ultimately require less fertiliser, providing environmental and financial benefits by reducing the use of inorganic Pi fertilisers.

Supplementary Material

Table S1. Accessions with extreme phenotypes used to study root traits in GE4 and CE1.

Table S2. Trait means for diversity foundation set (DFS) accessions and commercial cultivars of *Brassica oleracea* in glasshouse (*GE1*) and field (*FE1*) experiments.

Table S3. Variance components analyses of shoot dry matter (DM), shoot P concentration, and measures of PUE for *Brassica oleracea* grown in the glasshouse (GE1, GE2).

Table S4. Trait means for the AGDH mapping population and reference accessions of *Brassica oleracea* in glasshouse (*GE2*) and field (*FE2*) experiments.

Table S5. Trait means for the AGSL substitution population and reference accessions of *Brassica oleracea* used in glasshouse experiment *GE3*.

Acknowledgements

The authors acknowledge the financial support from Department of Environment, Food and Rural affairs (All) and The Scottish Government Rural and Environment Research and Analysis Directorate (PJW). The authors also acknowledge Nick Parsons for the development of the MatLab software for root area determination.

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Table 1. Definitions of phosphorus use efficiency (PUE)

Name	Abbreviation	Calculation	Units
Agronomic P use efficiency	APE	$(Y_{high}-Y_{low})/\Delta P_{app}$	g DM g^{-1} P_f
P uptake efficiency	PUpE	$((P_{high} \times Y_{high})-(P_{low} \times Y_{low}))/\Delta P_{app}$	g P g^{-1} P_f
P utilisation efficiency	PUtE	$(Y_{high}-Y_{low})/((P_{high} \times Y_{high})-(P_{low} \times Y_{low}))$	g DM g^{-1} P
Physiological P use efficiency	PPUE	Y_{high} / P_{high} or Y_{low} / P_{low}	g^2 DM g^{-1} P
P efficiency ratio	PER	$Y_{high} / (P_{high} \times Y_{high})$ or $Y_{low} / (P_{low} \times Y_{low})$	g DM g^{-1} P

Y_{high} = Yield on a high P/fertilised soil
Y_{low} = Yield on a low P/unfertilised soil
P_{high} = Tissue P concentration on a high P/fertilised soil
P_{low} = Tissue P concentration on a low P/unfertilised soil
ΔP_{app} = Difference in amount of P applied as fertiliser between high and low P treatments
DM = Dry matter
P_f = Fertiliser P

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Table 2. Description of experiments

Experiment Name	Location	Media	External [P]	Genotypes
GE1	Glasshouse	Peat based compost	5.25 mg L^{-1} (low) 15.75 mg L^{-1} (high)	DFS and commercial cultivars
GE2	Glasshouse	Peat based compost	5.25 mg L^{-1} (low) 15.75 mg L^{-1} (high)	AGDH mapping population
GE3	Glasshouse	Peat based compost	5.25 mg L^{-1} (low) 15.75 mg L^{-1} (high)	AG substitution lines
GE4	Glasshouse	Peat based compost	15.75 mg L^{-1}	18 extreme phenotypes
FE1	Field	soil	0, 298, 1,125, and 2,713 kg TSP ha^{-1}	Commercial cultivars
FE2	Field	soil	0, 298, 1,125, and 2,713 kg TSP ha^{-1}	AGDH mapping population
CE1	Controlled environment	Filter paper /nutrient solution	0.006 µM P (low) 0.625 µM P (high)	18 extreme phenotypes

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Table 3. Correlation coefficients between root traits and measures of phosphorus use efficiency (PUE)

	APE	PER at high $[P]_{ext}$	PER at low $[P]_{ext}$	PPUE at high $[P]_{ext}$	PPUE at low $[P]_{ext}$	PUpE	PUtE
Lateral root angle at high $[P]_{ext}$	**-0.528**	-0.335	-0.175	-0.387	0.171	-0.294	**-0.571**
Lateral root angle at low $[P]_{ext}$	-0.258	-0.130	**0.566**	-0.068	**0.640**	0.124	-0.350
Lateral root growth rate at high $[P]_{ext}$	**0.718**	0.499	0.046	**0.549**	0.350	**0.631**	**0.652**
Lateral root growth rate at low $[P]_{ext}$	**0.659**	0.454	-0.111	**0.518**	0.039	0.417	**0.616**
Lateral root length at high $[P]_{ext}$	**0.665**	**0.576**	-0.082	**0.565**	0.234	**0.530**	**0.601**
Lateral root length at low $[P]_{ext}$	**0.670**	**0.655**	-0.080	**0.634**	0.214	**0.510**	**0.616**
Lateral root number at high $[P]_{ext}$	0.500	**0.520**	-0.226	0.478	0.180	0.363	0.486
Lateral root number at low $[P]_{ext}$	**0.721**	**0.750**	-0.124	**0.716**	0.200	0.457	**0.675**
Primary root length at high $[P]_{ext}$	0.193	0.308	-0.150	0.201	0.046	0.129	0.299
Primary root length at low $[P]_{ext}$	**0.582**	**0.728**	0.099	**0.655**	0.227	0.361	**0.623**
Total root length at high $[P]_{ext}$	**0.535**	**0.520**	-0.125	0.462	0.174	0.437	**0.543**
Total root length at low $[P]_{ext}$	**0.673**	**0.725**	-0.011	**0.681**	0.234	0.484	**0.651**
Total root growth rate at high $[P]_{ext}$	**0.537**	0.478	-0.214	0.426	0.084	0.366	**0.557**
Total root growth rate at low $[P]_{ext}$	**0.656**	**0.689**	-0.082	**0.656**	0.212	0.428	**0.657**
Root DM at high $[P]_{ext}$	**0.680**	**0.721**	-0.268	**0.642**	-0.013	0.269	**0.698**
Root DM at low $[P]_{ext}$	0.491	**0.573**	-0.118	0.470	0.021	0.207	**0.604**
Shoot DM at high $[P]_{ext}$	**0.793**	**0.687**	-0.069	**0.682**	0.177	**0.549**	**0.789**
Shoot DM at low $[P]_{ext}$	**0.840**	**0.790**	0.058	**0.782**	0.193	**0.557**	**0.818**

Correlation coefficients in bold type are significant at the 5% level (P<0.05).

Table 4. Significant (P<0.05) QTL associated with shoot-P and measures of phosphorus use efficiency (PUE) in *Brassica oleracea*

Shoot DM, shoot-P and measures of PUE were determined in 90 DH accessions of the AG mapping population. Plants were grown under glasshouse conditions in compost containing 5.25 or 15.75 mg P L^{-1}. Trait means for each accession were used to identify QTLs associated with these traits by marker regression and interval mapping in the QTL Café programme (Seaton, 2000).

Trait	Chromosome	Location (cM)	Additive effect[a]	Genetic variance explained by QTL[b]	Confirmed by AGSL[c]
APE	C1	43.3	3.42	27.00%	nc
PPUE at high $[P]_{ext}$	C1	4.0	-81.71	46.26%	nc
PPUE at high $[P]_{ext}$	C1	24.0	87.14	52.61%	nc
PPUE at low $[P]_{ext}$	C1	0.0	-57.33	22.65%	nc
PPUE at low $[P]_{ext}$	C1	34.0	56.63	22.10%	nc
PUpE	C1	48.7	1.01	28.58%	nc
Shoot DM at high $[P]_{ext}$	C1	42.7	0.07	11.49%	nc
Shoot DM at low $[P]_{ext}$	C1	0.0	-0.06	22.09%	nc
Shoot DM at low $[P]_{ext}$	C1	32.0	0.06	22.09%	nc
Shoot DM at low $[P]_{ext}$	C2	69.8	-0.04	7.87%	123, 127
APE	C3	30.0	4.40	44.61%	134
APE	C3	106.0	-2.97	20.32%	173
PER at high $[P]_{ext}$	C3	30.0	13.46	12.68%	134
PER at high $[P]_{ext}$	C3	112.0	-13.15	12.10%	173
PER at low $[P]_{ext}$	C3	40.0	17.54	9.19%	-
PER at low $[P]_{ext}$	C3	106.0	-19.64	11.52%	-
PPUE at high $[P]_{ext}$	C3	32.0	49.20	16.77%	134
PPUE at high $[P]_{ext}$	C3	112.0	-37.56	9.77%	173
PPUE at low $[P]_{ext}$	C3	38.0	37.84	9.87%	-
PPUE at low $[P]_{ext}$	C3	112.0	-27.80	5.32%	-
PUpE	C3	36.7	0.99	27.47%	-
Shoot DM at high $[P]_{ext}$	C3	32.0	0.10	21.01%	134
Shoot DM at high $[P]_{ext}$	C3	112.0	-0.06	9.05%	173
Shoot DM at low $[P]_{ext}$	C3	34.0	0.05	11.64%	-
Shoot DM at low $[P]_{ext}$	C3	116.0	-0.02	2.78%	173
Shoot-P at high $[P]_{ext}$	C3	30.0	-0.01	8.03%	134
Shoot-P at high $[P]_{ext}$	C3	108.0	0.01	8.03%	173
Shoot-P at low $[P]_{ext}$	C3	32.0	-0.01	18.08%	-
Shoot-P at low $[P]_{ext}$	C3	106.0	0.01	18.08%	173
APE	C7	26.7	3.58	29.51%	-
PER at high $[P]_{ext}$	C7	30.6	11.95	9.99%	118, 169
PER at low $[P]_{ext}$	C7	38.5	22.71	15.41%	118, 169
PPUE at high $[P]_{ext}$	C7	29.6	35.90	8.93%	118
Shoot-P at high $[P]_{ext}$	C7	33.6	-0.01	11.57%	118, 169
PER at high $[P]_{ext}$	C9	23.2	15.94	17.77%	-
PPUE at low $[P]_{ext}$	C9	24.3	30.77	6.52%	-
PUpE	C9	43.0	-1.24	42.90%	122, 129

[a]Additive effect equals half the difference between homozygous allele at the QTL, positive number indicates an additive allelic effect of A12DHd parental allele. [b]The additive effect squared as a proportion of the line variance. [c]QTL location confirmed as a consistent effect in AG substitution line, see Table S5 for line values, nc = region not covered by the AGSL lines tested.

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Figure Legends

Figure 1. Image of roots (A) grown in compost (GE4) and washed clean, before being analysed using image analysis programme to determine root area (B). Roots of plants (C) grown for 10 days on vertical glass plates supported on blue blotter paper (CE1) to determine root architectural traits.

Figure 2. Shoot phosphorus (P) concentration (A), agronomic phosphorus use efficiency (PUE, B), P efficiency ratio (C), P uptake efficiency (D), physiological PUE (E) and P utilisation efficiency (F) for *Brassica oleracea* Diversity Foundation Set (DFS) accessions, current commercial cultivars in GE1 and AGDH mapping population in GE2. Data are residual maximum likelihood (REML)-estimated means, for plants grown in compost under glasshouse conditions at low and high $[P]_{ext}$. The boundaries of the box closest and farthest to zero indicate the 25th and 75th percentiles, respectively. The solid and dotted lines within the box indicate the median and mean, respectively. Error bars indicate the 10th and 90th percentiles. Circles indicate outliers.

Figure 3. Subtaxa (varietas) rankings of mean shoot phosphorus (P) concentration for plants at low and high $[P]_{ext}$ (A), agronomic phosphorus use efficiency (APE, B), physiological P use efficiency (PPUE; C), and P uptake efficiency (PUpE; D) in GE1. The boundaries of the box closest and farthest to zero indicate the 25th and 75th percentiles, respectively. The solid and

dotted lines within the box indicate the median and mean, respectively. Error bars indicate the 10th and 90th percentiles. Circles indicate outliers.

Figure 4. Relationship between shoot dry matter (DM) and responsiveness to $[P]_{ext}$ measured as agronomic phosphorus (P) use efficiency (APE; A), P utilisation efficiency (PUtE; B) and P uptake efficiency (PUpE; C) for Diversity Foundation Set (DFS) accessions (open circles) and commercial cultivars (filled circles) grown in GE1. Solid lines represent the mean value for the axis. NER = non-efficient and responsive, ER = efficient and responsive, ENR = efficient and non-responsive, NENR = non-efficient and non-responsive. Values represent the total number of accessions in each quadrant, with the number of commercial cultivar given in the parentheses.

Figure 5. Relationship between shoot dry matter (DM) at low and high $[P]_{ext}$. Solid line represents the line of best fit through the data y = 2.01x, r=0.36. Groups represent extreme phenotypes (see text for full description).

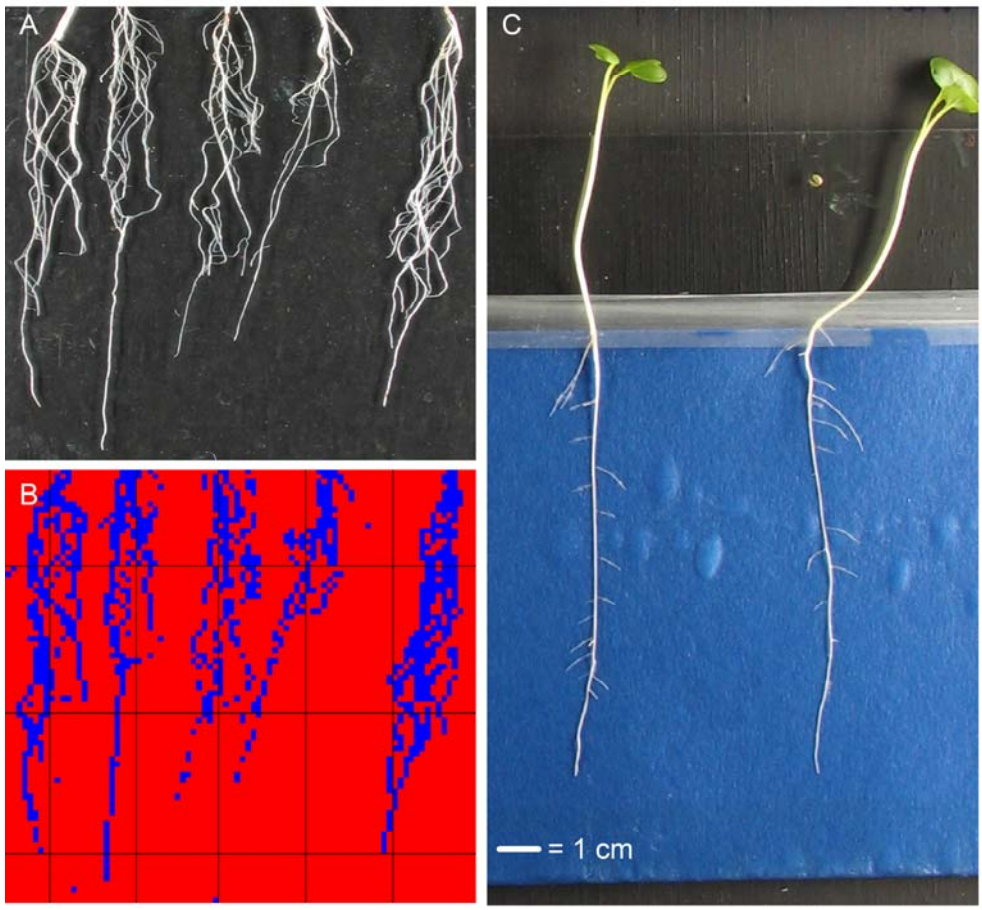
Figure 6. Root area (A) and specific root area (B) for extreme phenotypes (see text for full description) grown in compost under glasshouse conditions (GE4). Bars represent means ± SEM (n=3).

Figure 7. Number of lateral roots (A), total lateral root length (B), and lateral root growth rate (C) for extreme phenotypes (see text for full description) grown in CE1 on filter paper soaked in nutrient solution containing 0.006 µM

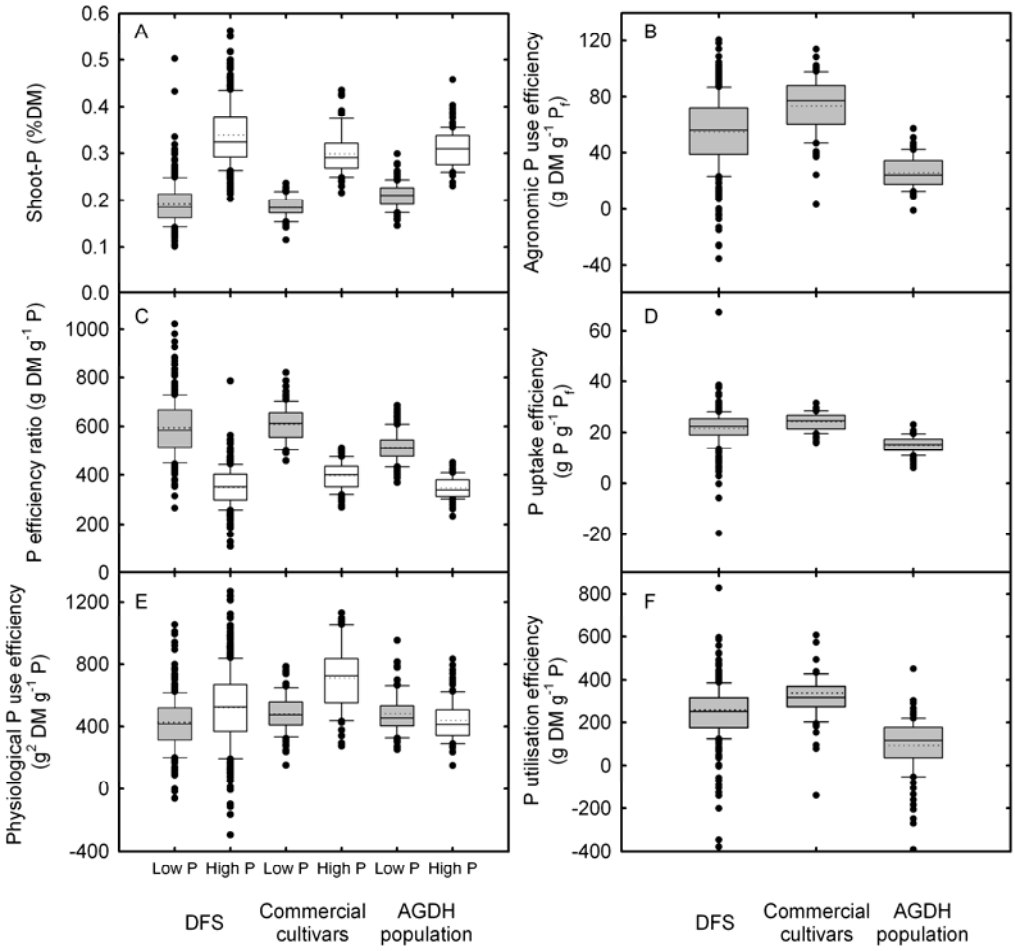
P (grey bars) or 0.625 μM P (open bars). Bars represent means ± SEM (n=3).

Figure 8. QTL associated with shoot-P and measures of phosphorus use efficiency (PUE) on chromosomes C1, C3 and C7 in *Brassica oleracea.* Shoot DM, shoot-P and measures of PUE were determined in 90 DH accessions of the AG mapping population (GE2). QTL associated with these traits were identified by multiple marker regression in the QTL Café programme (solid lines [one QTL model] or shaded arrows [two QTL model]; Seaton, 2000) and CIM in QTL Cartographer 2.0 (box and whiskers; Wang *et al*., 2004). For CIM, the box indicates the 1-LOD interval, and the whisker line the extent of the 2-LOD interval; for multiple marker regression with a one QTL model, the midpoint of the simulated QTL is shown by a horizontal mark, with the 95% confidence interval shown by the vertical whisker line; for multiple marker regression with a two QTL model the shaded arrow indicates the presence of two QTL on the chromosome, but error estimates are not given in QTL Café for a two QTL model. Chromosomes are colour coded for regions with homology to *Arabidopsis thaliana* chromosomes (Parkin *et al*., 2005).

Figure 1



1 Figure 2



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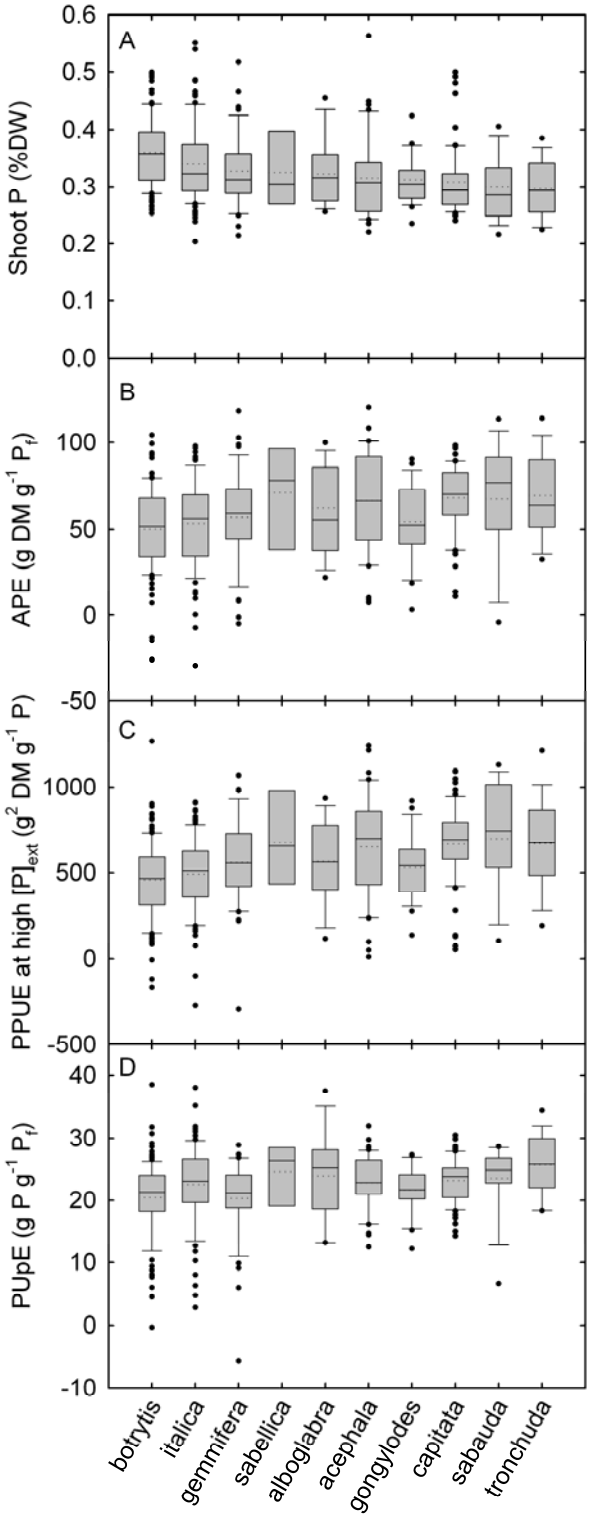
1 Figure 3


A
B
C
D
Shoot P (%DW)
APE (g DM g-1 Pf)
PPUE at high [P]ext (g2 DM g-1 P)
PUpE (g P g-1 Pf)
botrytis
italica
gemmifera
sabellica
alboglabra
acephala
gongylodes
capitata
sabauda
tronchuda

2

Figure 4

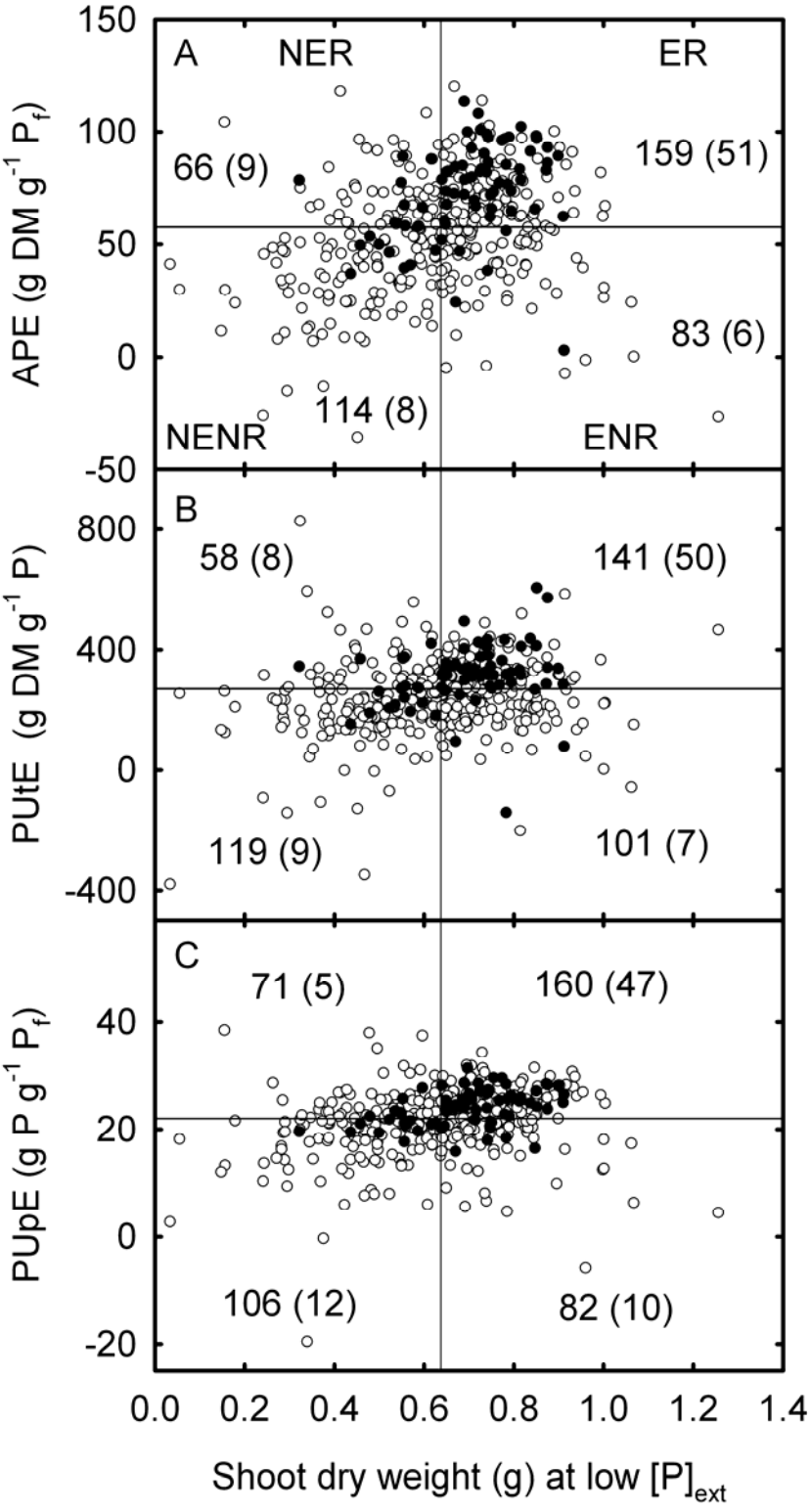

A
NER
ER
66 (9)
159 (51)
83 (6)
114 (8)
NENR
ENR
B
58 (8)
141 (50)
119 (9)
101 (7)
C
71 (5)
160 (47)
106 (12)
82 (10)
APE (g DM g-1 Pf)
PUtE (g DM g-1 P)
PUpE (g P g-1 Pf)
Shoot dry weight (g) at low [P]ext

Figure 5

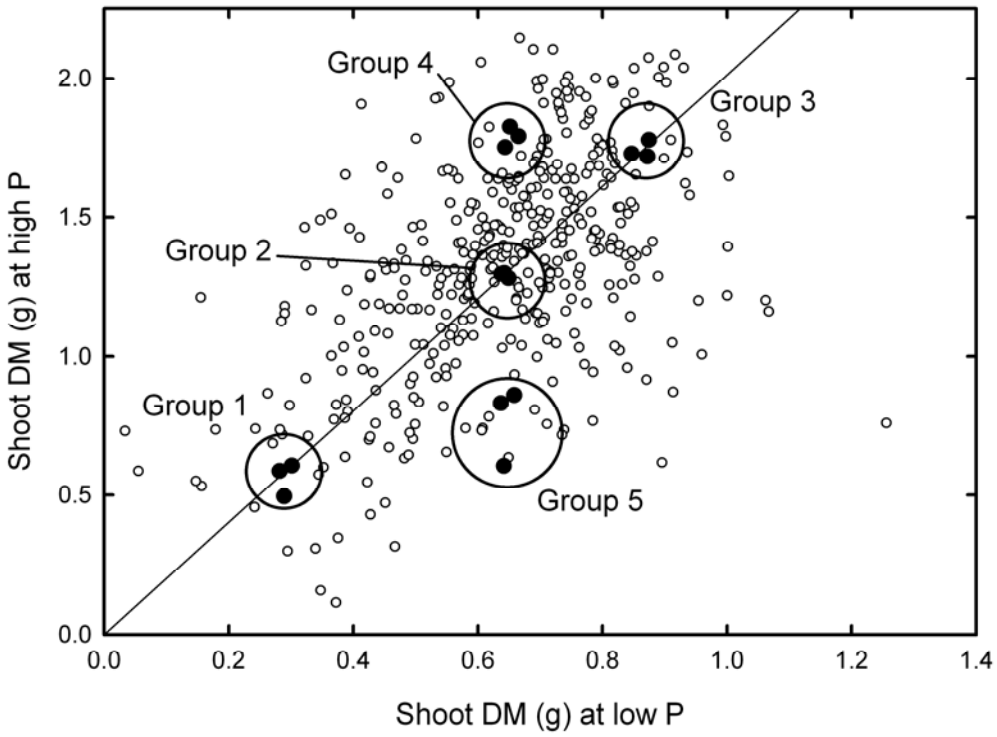

Group 4
Group 3
Group 2
Group 1
Group 5
Shoot DM (g) at high P
Shoot DM (g) at low P
0.0
0.5
1.0
1.5
2.0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4

Figure 6

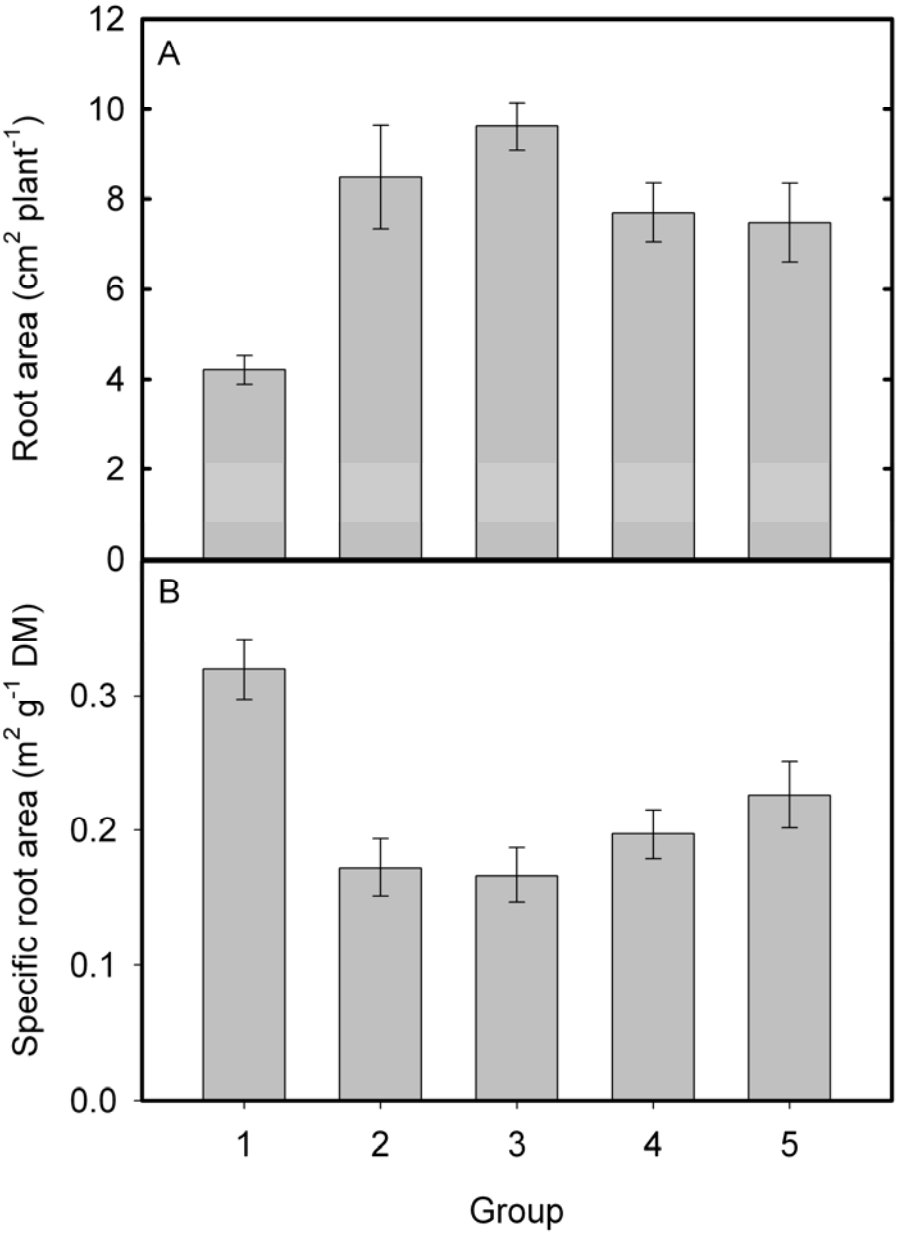
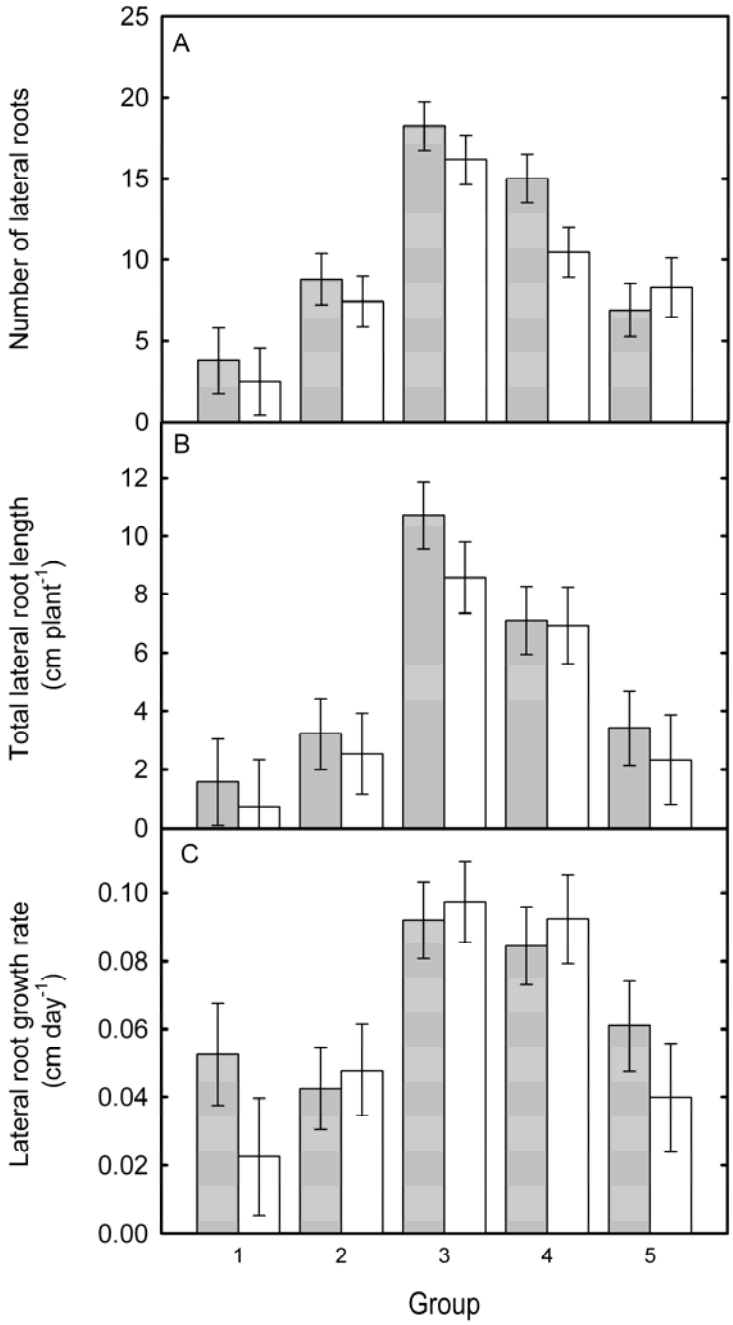
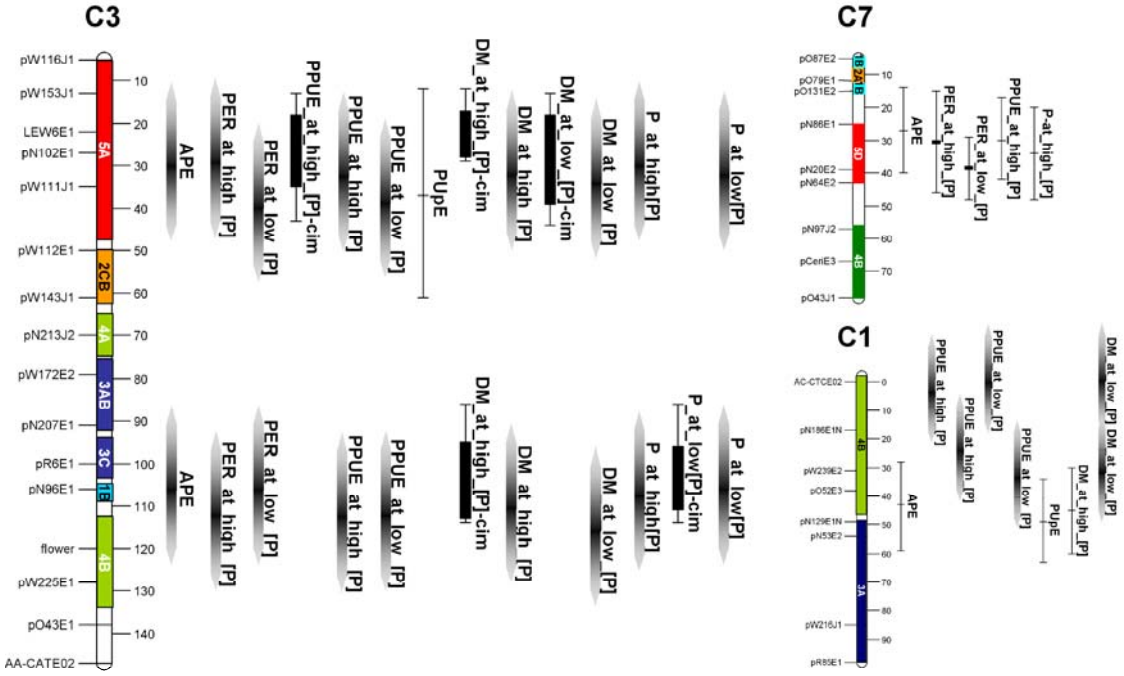

A
B
Root area (cm^2 plant^{-1})
Specific root area (m^2 g^{-1} DM)
12
10
8
6
4
2
0
0.3
0.2
0.1
0.0
1
2
3
4
5
Group

Figure 7


A
B
C
Number of lateral roots
Total lateral root length (cm plant-1)
Lateral root growth rate (cm day-1)
Group
0
5
10
15
20
25
2
4
6
8
12
0.00
0.02
0.04
0.06
0.08
0.10
1
2
3
4
5

1 Figure 8



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3

4