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RESEARCH PAPER

Shoot yield drives phosphorus use efficiency in *Brassica oleracea* and correlates with root architecture traits

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Abstract

The environmental and financial costs of using inorganic phosphate fertilizers to maintain crop yield and quality are high. Breeding crops that acquire and use phosphorus (P) more efficiently could reduce these costs. The variation in shoot P concentration (shoot-P) and various measures of P use efficiency (PUE) were quantified 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 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 quantitative trait loci 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*.

Key words: *Brassica*, diversity, efficiency, *napus*, *oleracea*, phosphate, phosphorus, QTL, *rapa*, root.

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 (2–10 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 fertilizers to maintain yields and quality. However, the environmental and financial costs of using inorganic Pi fertilizers are high. With crop production relying on large inputs of Pi fertilizers, and most crops not recovering all of the Pi fertilizer applied, excess soluble inorganic Pi fertilizers added to crops can run off the soil into surface waters. The agriculture sector in Great Britain contributes over 12000 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

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costs involved in the use of P fertilizers, which will increase in the future as a result of (i) unsustainable production of P fertilizers from commercially viable, but non-renewable, finite reserves of phosphate rock, which at current rates of use could be exhausted in the next 100–400 years (Johnston, 2008), (ii) unstable energy prices, which will have an impact on the mining, transport, and spreading of phosphate rocks and fertilizers (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 fertilizers. Such crops could produce comparable yields with lower inputs of inorganic Pi fertilizers 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 fertilizer (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 fertilizer (g P g^{-1} P_f), often 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), or P utilization 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 are: (i) yield divided by the amount of P in the plant (g DM g^{-1} P), or P efficiency ratio (PER), which 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) or 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), expressed as the 'K_m' 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, 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 with low K_m and critical soil P values will grow to their potential with minimal P fertilization. Crops with lower critical tissue P concentrations are likely to tolerate soils with low Pi availability better and reduce P-fertilizer requirements since less 'maintenance' P fertilization 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 fertilization do not appear to correlate 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-fertilizer inputs to soils that require only maintenance P fertilization.

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 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, large species-wide variation within *Brassica oleracea* L. is demonstrated for shoot P concentration (shoot-P), different measures of PUE, and their responsiveness to

Table 1. Definitions of phosphorus use efficiency (PUE)

Y_{high}=yield on a high P/fertilized soil; Y_{low}=yield on a low P/unfertilized soil; P_{high}=tissue P concentration on a high P/fertilized soil; P_{low}=tissue P concentration on a low P/unfertilized soil; ΔP_{app}=difference in amount of P applied as fertilizer between high and low P treatments; DM=dry matter; P_f=fertilizer P.

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 utilization 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

external P concentration ($[P]_{ext}$). Taking extreme phenotypes from within the species it is demonstrated that the responsiveness of *B. oleracea* to $[P]_{ext}$ correlates with root development and architecture. Finally, a forward genetic approach has been used 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 gene pool of *Brassica oleracea* L. has been described previously (Broadley *et al.*, 2008). This consisted of a diversity foundation set (DFS) of 376 accessions selected from the >4300 C-genome *B. oleracea* accessions held in the Warwick HRI Genetic Resources Unit. Since theoretical studies of natural populations (Lawrence *et al.*, 1995*a*, *b*) indicate that 400 accessions, collected from throughout the world, should contain 99% of the allelic polymorphism 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 doubled haploid (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 ~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) as follows.

(i) A glasshouse experiment (GE1): 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 (7% P) per litre of compost (Greenwood *et al.*, 2005). Analysis of compost samples showed Olsen's extractable P to be 9.2 and 20.2 mg l^{-1} for low and high composts, respectively. P treatments were determined based on growth response curves for this crop under these conditions, providing a high $[P]_{ext}$ treatment where the plants had sufficient P, and a low $[P]_{ext}$ treatment, where the P availability was suboptimal, such that yield was negatively affected (Greenwood *et al.*, 2005, 2006; Broadley *et al.*, 2008). 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, and 37 d after sowing on occasions 1–6, respectively. Occasions represent independent experimental runs, containing a subset of the accessions being screened (Broadley *et al.*, 2008).

(ii) A field experiment (FE1): Seventy-two 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 was

Table 2. Description of experiments

Experiment name	Location	Media	P treatment	Genotypes	Measurements
GE1	Glasshouse	Peat-based compost	5.25 mg l^{-1} (low), 15.75 mg l^{-1} (high)	DFS and commercial cultivars	Shoot FW, DM, P
GE2	Glasshouse	Peat-based compost	5.25 mg l^{-1} (low), 15.75 mg l^{-1} (high)	AGDH mapping population	Shoot FW, DM, P
GE3	Glasshouse	Peat-based compost	5.25 mg l^{-1} (low), 15.75 mg l^{-1} (high)	AG substitution lines	Shoot FW, DM, P
GE4	Glasshouse	Peat-based compost	15.75 mg l^{-1}	18 extreme phenotypes	Shoot FW, DM, P; root FW, DM, area
FE1	Field	Soil	0, 298, 1125, and 2713 kg TSP ha^{-1}	Commercial cultivars	Shoot FW, DM, P
FE2	Field	Soil	0, 298, 1125, and 2713 kg TSP ha^{-1}	AGDH mapping population	Shoot FW, DM, P
CE1	Controlled environment	Filter paper/nutrient solution	0.006 mM P (low), 0.625 mM P (high)	18 extreme phenotypes	Shoot FW, DM, P; root FW, DM, P, length; lateral root length, angle, number

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). Analysis of soil samples (to a depth of 30 cm) from these plots gave average Olsen's extractable P values of 40.7, 39.6, 81.7, and 152.1 mg P l^{-1} for the four fertilizer addition rates described above. Plant shoots were sampled after 101, 97, 93 d growth on occasions 1–3, respectively. These timings were chosen to represent pre-commercial maturity.

(iii) A second glasshouse experiment (GE2): 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.

(iv) A second field experiment (FE2): Three replicates of 72 cultivars (61 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 d growth.

(v) A third glasshouse experiment (GE3): Three replicates of the 20 AGSLs were grown at the same two $[P]_{ext}$ as GE1 and GE2. Plant shoots were sampled 39 d after sowing.

(vi) A fourth glasshouse experiment (GE4): Three replicates of 18 accessions (Table S1 in Supplementary data available at *JXB* online) 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. 1A, B).

(vii) A growth room experiment (CE1): Three replicates of the 18 accessions grown in GE4 were grown on Steel Blue Seed Germination Blotter paper (Anchor Paper Company, MN, USA) (Fig. 1C; Bonser *et al.*, 1996), 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) mM P. Seedlings were transferred to the blotter paper 4 d after sowing and harvested 7 d 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, The Netherlands) giving an intensity of 45 μmol photons m^{-2} s^{-1} at plant height.

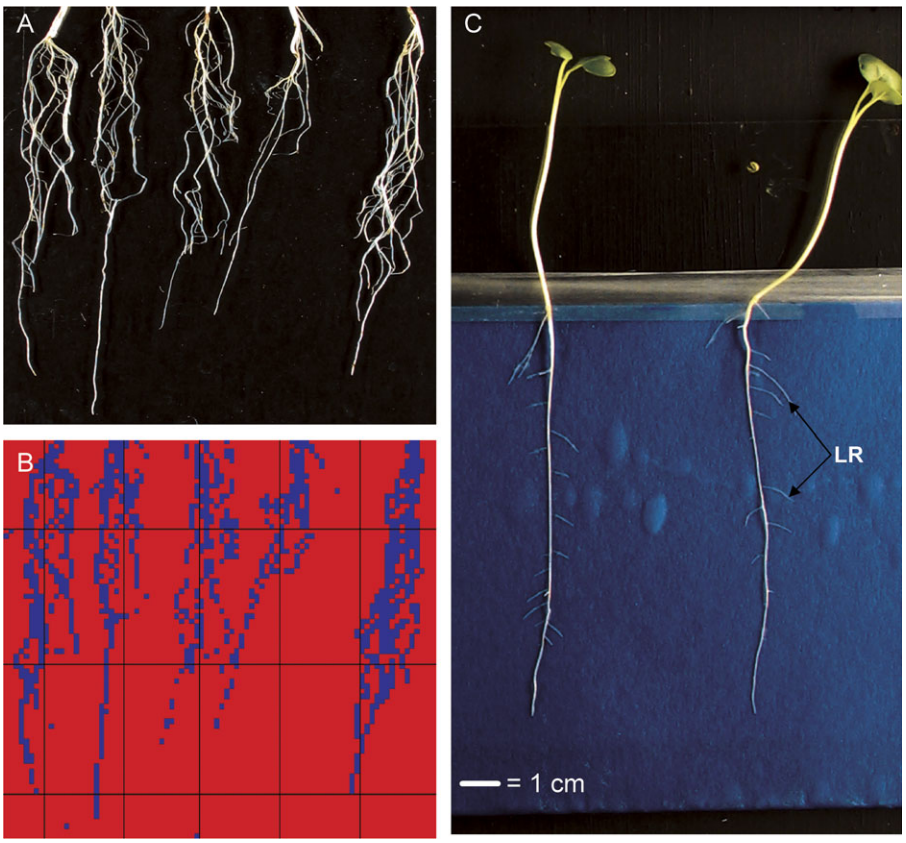

Fig. 1. (A, B) Image of roots grown in compost (GE4) and washed clean (A), before being analysed using an image analysis program to determine root area (B). (C) Roots of plants grown for 10 d on vertical glass plates supported on blue blotter paper (CE1) to determine root architectural traits. LR=lateral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

Determination of tissue P concentration

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 °C for 72 h. For GE1, total shoot-P was determined by a commercial foliar analysis laboratory (Yara Analytical Ltd, Pocklington, York, UK). Dried samples were ashed at 500 °C. The ashed samples were then digested in concentrated hydrochloric acid, and P determined using inductively coupled plasma emission spectrometry. For all other experiments, shoot-P was determined using the micro-Kjeldahl method: ~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.

Determination of root architecture traits

In GE4, root system area was calculated using MatLab (Version 7.7; The MathWorks, Natick, MA, USA). In CE1, total root length and lateral root length were calculated using ImageJ (Abramoff *et al.*, 2004). In *B. oleracea*, the lateral roots are secondary roots emerging from the primary root (Fig. 1). Differences in root and lateral root lengths between images of the root system taken at transfer and harvest were used to calculate root growth rates. Lateral root angles were measured using a protractor. The primary root was aligned to 0° and lateral root angle measured relative to this, with lateral roots growing perpendicular to the primary root having a growth angle of 90°.

Data analysis

Several measures of PUE were calculated (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 program (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).

Results

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

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*, was used in addition to 74 commercial varieties (Table S2 in Supplementary data available at *JXB* online). 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 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 in Supplementary data available at *JXB* online). Values for APE, PUpE, and 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. PPUE had the greatest range in values, varying between –294.7 and 1268.4 g^2 DM g^{-1} P for accessions grown at high $[P]_{ext}$, and varying between –62.9 and 1051.2 g^2 DM g^{-1} P for accessions grown at low $[P]_{ext}$ (Fig. 2E). Negative values arose 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 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, shoot-P and measures of PUE were tested to find out if there is a correlation 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 in Supplementary data available at *JXB* online). 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

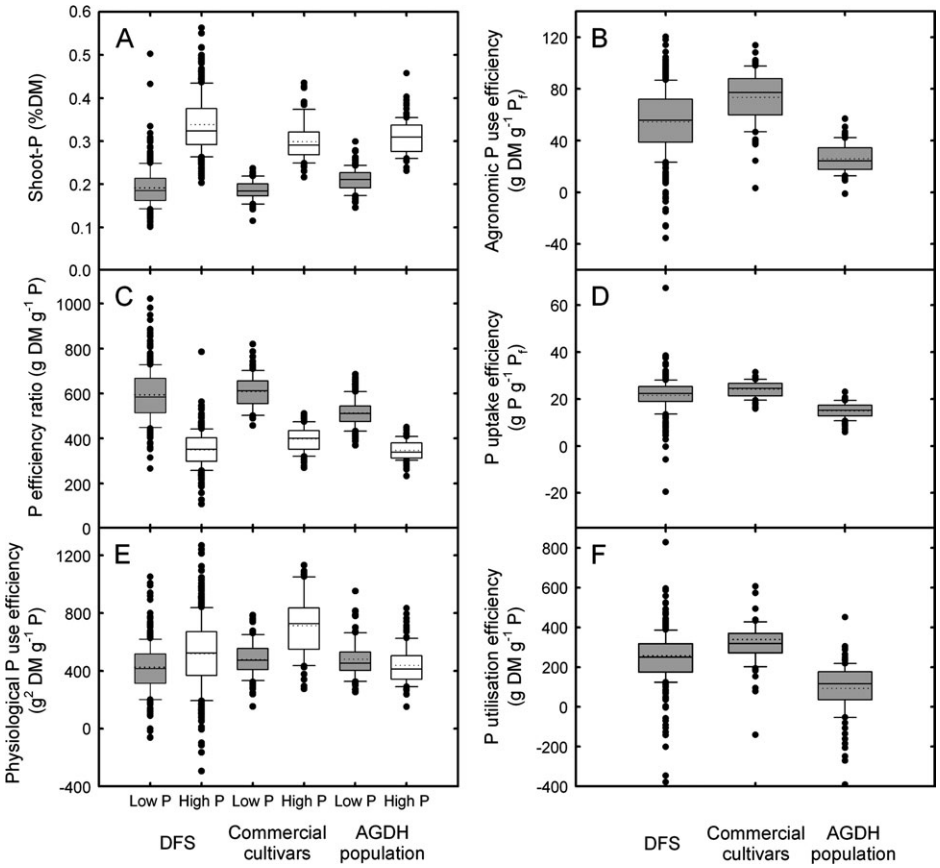

Fig. 2. Shoot P concentration (A), agronomic P use efficiency (B), P efficiency ratio (C), P uptake efficiency (D), physiological P use efficiency (E), and P utilization efficiency (F) for *Brassica oleracea* diversity foundation set (DFS) accessions, current commercial cultivars in GE1, and AGDH mapping 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 to and farthest from zero indicate the 25th and 75th percentiles, respectively. The continuous and dotted lines within the box indicate the median and mean, respectively. Error bars indicate the 10th and 90th percentiles. Circles indicate outliers.

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 with 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 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 in Supplementary data available at *JXB* online). Five groups of accessions were selected representing accessions that have low (Group 1), average (Group 2), and high (Group 3)

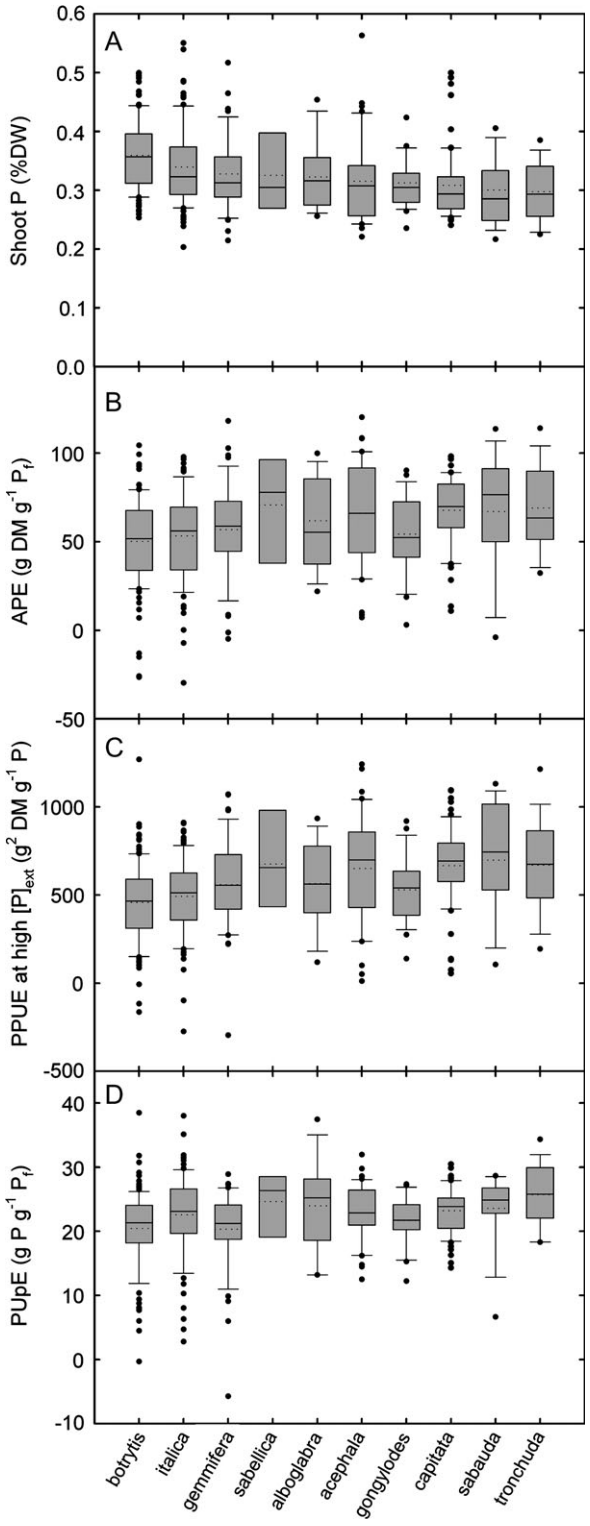

Fig. 3. Subtaxa (varietas) rankings of mean shoot P concentration for plants at low and high $[P]_{ext}$ (A), agronomic phosphorus use efficiency (APE) (B), physiological P use efficiency (PPUE (C), and P uptake efficiency (PUpE) (D) in GE1. The boundaries of the box closest to and farthest from zero indicate the 25th and 75th percentiles, respectively. The continuous and dotted lines within the box indicate the median and mean, respectively. Error bars indicate the 10th and 90th percentiles. Circles indicate outliers.

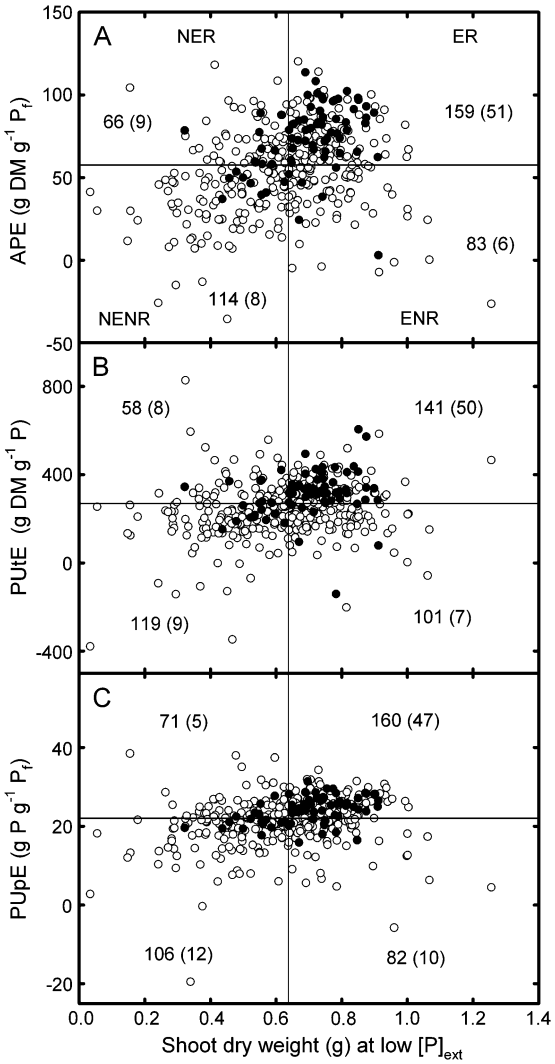

Fig. 4. Relationship between shoot dry matter (DM) and responsiveness to $[P]_{ext}$ measured as agronomic P use efficiency (APE) (A), P utilization efficiency (PUtE) (B), and P uptake efficiency (PUpE) (C) for diversity foundation set (DFS) accessions (open circles) and commercial cultivars (filled circles) grown in GE1. Continuous 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 the commercial cultivar given in parenthesis.

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. 1A, B). Root DM, root area, and specific root area 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 specific root area compared with Group 1, which contains accessions with low yields at low and high $[P]_{ext}$ (Fig. 6).

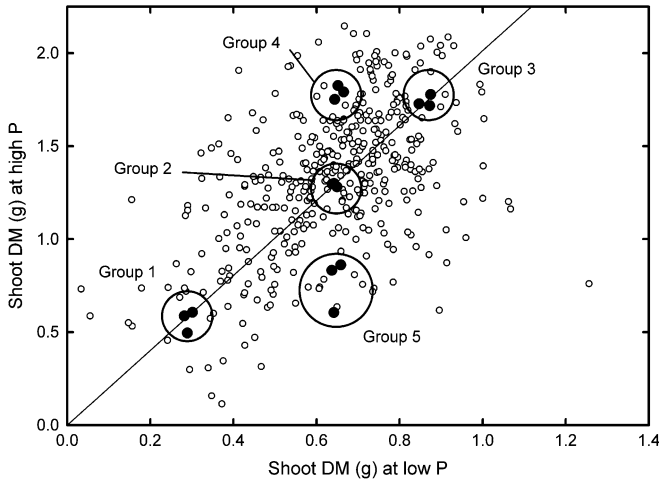

Fig. 5. Relationship between shoot dry matter (DM) at low and high $[P]_{ext}$. The continuous line represents the line of best fit through the data $y=2.01x$, $r=0.36$. Groups represent extreme phenotypes (see text for full description).

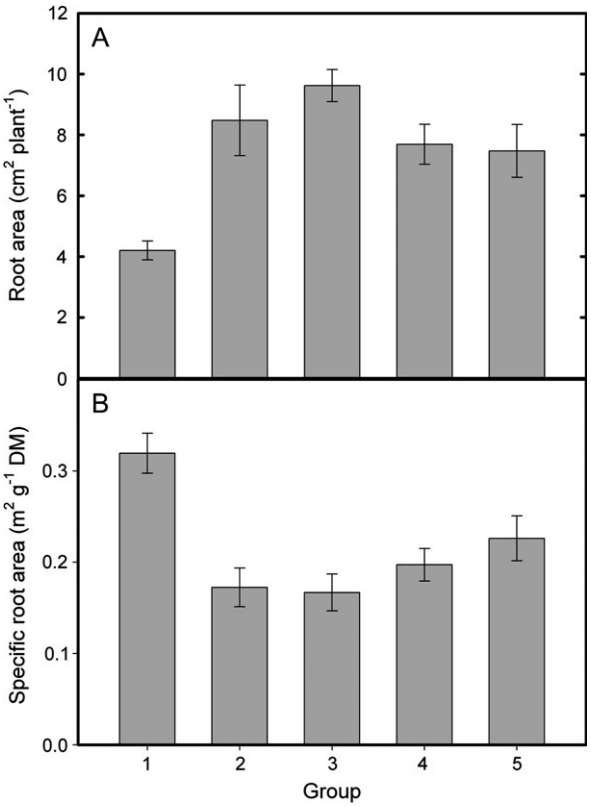

Fig. 6. Root area (A) and specific root area (B) for extreme phenotypes (see text for full description) grown in compost under glasshouse conditions (GE4). Bars represent means ±SEM ($n=3$).

Since differences in root architecture can affect a plant's ability to intercept P, the root architectures of these accessions were studied in more detail (CE1). 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. 7B, C). Interestingly, there was no significant effect of group or $[P]_{ext}$ on lateral root angle (Table S1 in Supplementary data available at *JXB* online). 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 (0.006 mM) $[P]_{ext}$ compared with when they were grown at high (0.625 mM) $[P]_{ext}$, but most accessions had lower root DMs when grown at low $[P]_{ext}$. Various measures 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. Interestingly, with the exception of lateral root angle, there were no significant (P <0.05) correlations between root traits and PER or PPUE at low $[P]_{ext}$ (Table 3).

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

Variation in measures of PUE among the species-wide gene pool was compared with 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 in Supplementary data available at *JXB* online). Genetic variance components for DH accessions approximate the population-wide additive genetic variation 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 61 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 in Supplementary data available at *JXB* online). Trait data ranges were: APE, –1.2–56.8 g DM g^{-1} P_f; PUpE, 6.0–23.0 g P g^{-1} P_f; PUtE, –2716.6–450.7 g DM g^{-1} P; PPUE at low $[P]_{ext}$, 252.0–951.9 g^2 DM g^{-1} P; PPUE at high $[P]_{ext}$, 150.9–833.3 g^2 DM g^{-1}

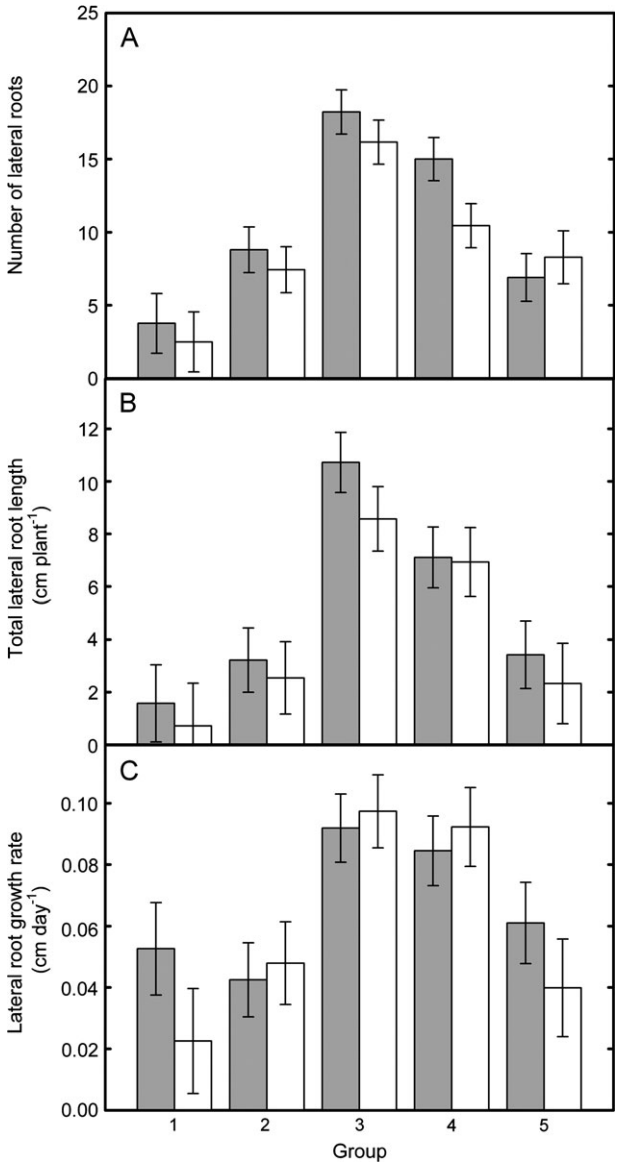

Fig. 7. Number of lateral roots (A), total lateral root length (B), and lateral root growth rate (C) for extreme phenotypes (see text for full description) grown in CE1 on filter paper soaked in nutrient solution containing 0.006 mM P shaded columns) or 0.625 mM P (open columns). Bars represent means ±SEM (n=3).

P; PER at low $[P]_{ext}$, 368.7–684.8 g DM g^{-1} P, and PER at high $[P]_{ext}$, 232.7–449.7 g DM g^{-1} P (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 in Supplementary data available at *JXB* online). 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, two parent lines, and eight 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.

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 61 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 on 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, and 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 and PPUE at low $[P]_{ext}$. No significant effects were observed for PUtE. Significant QTL (P <0.05) associated with APE, PUpE, and PPUE at high $[P]_{ext}$,

Table 3. Correlation coefficients between root traits and measures of phosphorus use efficiency (PUE)
Correlation coefficients in bold type are significant at the 5% level (P <0.05).

	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**

PPUE at low $[P]_{ext}$, PER at high $[P]_{ext}$, and PER at low $[P]_{ext}$, were identified between 22 cM and 28 cM on C3 (Fig. 8; Table 4). Significant QTL (P <0.05) associated with APE and PPUE at high $[P]_{ext}$, PER at high $[P]_{ext}$, and PER at low $[P]_{ext}$, were identified between 24 cM 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 was 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 in Supplementary data available at *JXB* online). 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 and 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 it has been 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, QTL associated with shoot-P and measures of PUE have been identified (Fig. 8; Table 4) and several QTL tested 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

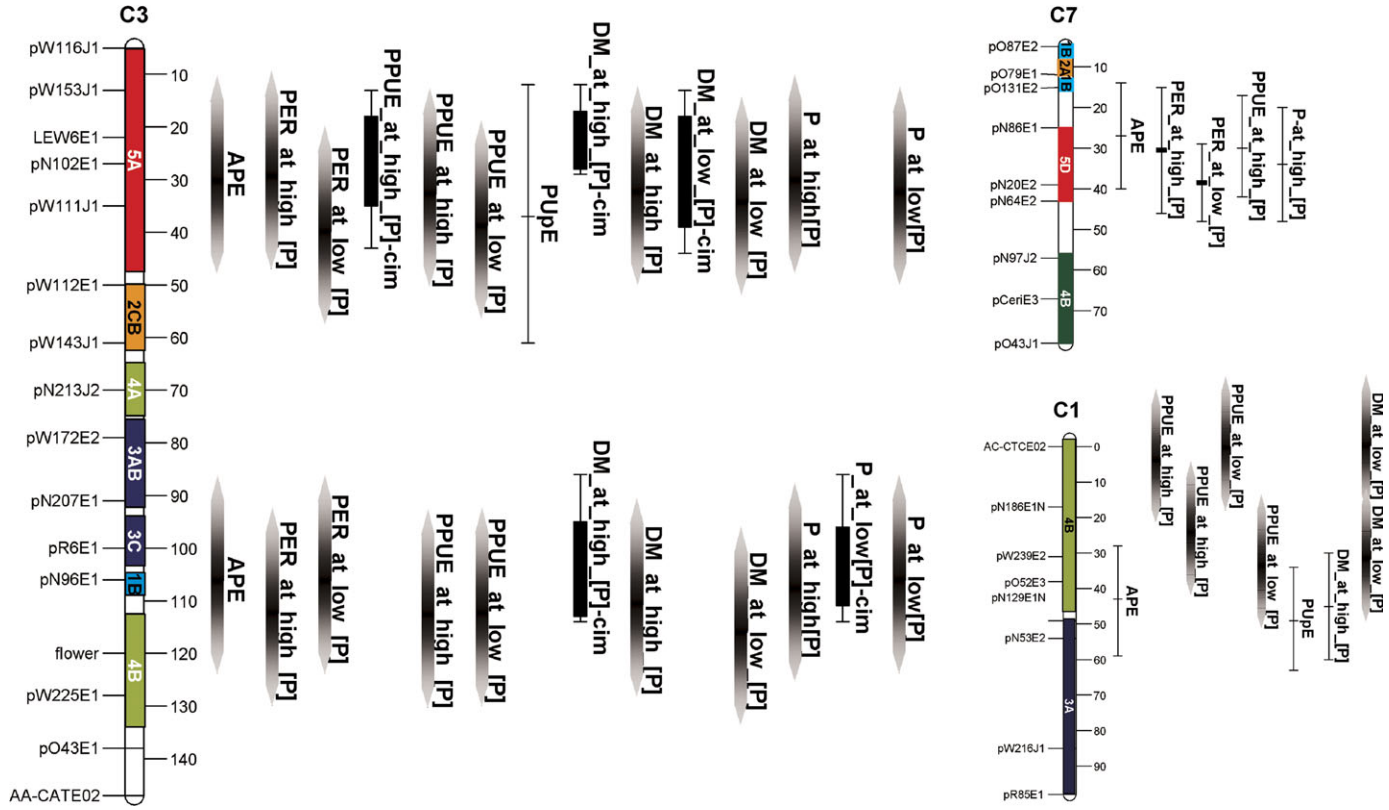

Fig. 8. QTL associated with shoot-P and measures of phosphorus use efficiency (PUE) on chromosomes C1, C3, and C7 in *Brassica oleracea.* Shoot DM, shoot-P, and measures of PUE were determined in 90 DH accessions of the AG mapping population (GE2). QTL associated with these traits were identified by multiple marker regression in the QTL Café program [continuous 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).

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 utilize applied inorganic Pi fertilizers 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 with 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 $[P]_{ext}$ (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, a subset of extreme phenotypes (Fig. 5) was selected and their root systems scored for root growth and architectural traits (Figs 6, 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 $[P]_{ext}$ (Groups 3 and 4) had a greater number of lateral roots, which were longer and grew faster compared with accessions that had average or low yields at high $[P]_{ext}$ (Fig. 7). Accessions that had higher yields at low $[P]_{ext}$

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 program (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; a 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. For line values see Table S5 in Supplementary data available at *JXB* online. nc=region not covered by the AGSL lines tested.

(Groups 2–5) also had a greater number of lateral roots, which were longer compared with 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 with 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 with the low PPUE cultivar. Similarly, Solaiman *et al.* (2007) demonstrated that a P-efficient canola cultivar had a greater total root length compared with a P-inefficient cultivar. Lateral root traits also had the greatest correlations with various measures of PUE (Table 3). In *Phaseolus vulgaris*, low P conditions result 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, 2008; Rubio *et al.*, 2003). 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.

Several regions of the *B. oleracea* genome associated with shoot-P and responsiveness to $[P]_{ext}$ were detected (Fig. 8; Table 4). The loci associated with several of these traits co-localize, including a significant QTL for shoot DM, shoot-P, and measures of PUE at ~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-localize 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 and 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 and 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-localize 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-localize with QTL for leaf-P identified on A3 (Zhao *et al.*, 2008). Alignment with QTL identified in *Arabidopsis* for shoot-P reveals some co-localization between loci. QTL mapped to the top of C1 and C3 in this study co-localize 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

The species-wide diversity in *B. oleracea* for shoot-P and various measures of PUE has been successfully characterized. 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 fertilizer, providing environmental and financial benefits by reducing the use of inorganic Pi fertilizers.

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-P at low and high $[P]_{ext}$ and measures of PUE for *Brassica oleracea* grown in glasshouse (GE1, GE2) experiments

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.

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