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High-throughput root phenotyping screens identify genetic loci associated with root architectural traits in *Brassica napus* under contrasting phosphate availabilities

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• *Background and Aims* Phosphate (Pi) deficiency in soils is a major limiting factor for crop growth worldwide. Plant growth under low Pi conditions correlates with root architectural traits and it may therefore be possible to select these traits for crop improvement. The aim of this study was to characterize root architectural traits, and to test quantitative trait loci (QTL) associated with these traits, under low Pi (LP) and high Pi (HP) availability in *Brassica napus*.
• *Methods* Root architectural traits were characterized in seedlings of a double haploid (DH) mapping population ($n = 190$) of *B. napus* ['Tapidor' × 'Ningyou 7' (TNDH)] using high-throughput phenotyping methods. Primary root length (PRL), lateral root length (LRL), lateral root number (LRN), lateral root density (LRD) and biomass traits were measured 12 d post-germination in agar at LP and HP.
• *Key Results* In general, root and biomass traits were highly correlated under LP and HP conditions. 'Ningyou 7' had greater LRL, LRN and LRD than 'Tapidor', at both LP and HP availability, but smaller PRL. A cluster of highly significant QTL for LRN, LRD and biomass traits at LP availability were identified on chromosome A03; QTL for PRL were identified on chromosomes A07 and C06.
• *Conclusions* High-throughput phenotyping of *Brassica* can be used to identify root architectural traits which correlate with shoot biomass. It is feasible that these traits could be used in crop improvement strategies. The identification of QTL linked to root traits under LP and HP conditions provides further insights on the genetic basis of plant tolerance to P deficiency, and these QTL warrant further dissection.

Key words: Phosphate, phosphorus, root, *Brassica napus*, oilseed rape, QTL, biomass, genetic, heritability.

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 and mass flow is slow (Bieleski, 1973; Barber, 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).

Over 85 % of mined P is used in food production (Heffer *et al.*, 2006) and consumption of this non-renewable resource could lead to a peak P scenario (akin to peak oil; Raven, 2008; Cordell *et al.*, 2009). It is therefore likely that there will be increasing pressures on Pi fertilizer availability and, consequently, cost in the future. These pressures will be exacerbated by increasing demand on food production systems as the human population increases and by fluctuation in oil prices (Cordell *et al.*, 2009). Inappropriate use of inorganic Pi fertilizers can also perturb the nutrient balance of natural ecosystems and reduce biodiversity (White and Hammond, 2008, 2009).

Breeding crops that acquire Pi and/or use P more efficiently is one strategy to reduce the use of Pi fertilizers (White *et al.*, 2005; Veneklass *et al.*, 2012). Root traits represent a potential source of genetic variation to improve P acquisition for breeding such crops (Lynch, 2007; Römheld and Kirkby, 2010; Powlson *et al.*, 2011; De Smet *et al.*, 2012; Lynch and Brown, 2012). Assessment of root traits in crop breeding material can be slow and expensive, involving a combination of field-, glasshouse- and laboratory-based screens (Clark *et al.*, 2011). The latter of these is amenable to high-throughput screens to identify germplasm with altered root growth and morphology. Genetic loci associated with these traits have the potential for use in breeding new crop varieties with improved root phenotypes.

In arabidopsis and other plant species, root responses to low Pi availability have been well characterized (White *et al.*, 2005). Typically, a reduction in the development of the

primary root (Ticconi *et al.*, 2004; Sánchez-Calderón *et al.*, 2005; Svistoonoff *et al.*, 2007; Jain *et al.*, 2007; Fang *et al.*, 2009; Hammond *et al.*, 2009) and increases in the number and length of lateral roots are observed under low Pi availability (Williamson *et al.*, 2001; Linkohr *et al.*, 2002; López-Bucio *et al.*, 2002, 2003, 2005; Al-Ghazi *et al.*, 2003; Nacry *et al.*, 2005; Reymond *et al.*, 2006). In some species, root agravitropism or topsoil foraging, is observed (Zhu *et al.*, 2005). Both density and length of root hairs are also increased when plants are grown on a low Pi supply (Bates and Lynch, 1996; Gahoonia and Nielsen, 1997; Brown *et al.*, 2012), thus increasing the capacity for Pi acquisition. Biochemical adaptations, including the release of organic anions to release Pi bound to clay particles (Shane and Lambers, 2005) and enzymes to release Pi from organic compounds are also observed (Li *et al.*, 2002; George *et al.*, 2011; Plaxton and Tran, 2011; Richardson *et al.*, 2011).

Root traits affecting Pi acquisition are complex and regulated by multiple genetic loci. In rice, a major quantitative trait locus (QTL) for P-deficiency tolerance, P uptake 1 (*Pup1*) was mapped to a 150-kb region of chromosome 12 containing 60 predicted genes (Wissuwa *et al.*, 2002; Chin *et al.*, 2011). Subsequently the gene conferring the phenotype (PSTOL1) was identified as a protein kinase (Gamuyao *et al.*, 2012). Among Brassicaceae species, QTL have been associated with leaf and seed P and phytate concentrations, and primary root growth responses to low Pi availability (Bentsink *et al.*, 2003; Vreugdenhil *et al.*, 2004; Reymond *et al.*, 2006; Svistoonoff *et al.*, 2007; Zhao *et al.*, 2007, 2008; Hammond *et al.*, 2009, 2011; Ding *et al.*, 2010, 2012; Yang *et al.*, 2010). Significant QTL associated with shoot-P and measures of P-use efficiency (PUE) on chromosome C03 and C07 of *B. oleracea* have previously been identified, and a significant correlation between these shoot traits and root morphological traits was observed (Hammond *et al.*, 2009). The locations of these QTL have been confirmed using substitution lines. Recently, a new mapping population of *B. napus* has been developed, utilizing parents with contrasting PUE characteristics (Duan *et al.*, 2009) and used to identify QTL associated with seed elemental concentrations (Ding *et al.*, 2010), seed yield (Ding *et al.*, 2012) and root morphological traits (Yang *et al.*, 2010) under contrasting Pi supplies. The latter of these studies identified significant QTL associated with root length, volume and surface area on chromosomes A01, A03, C03 and C02.

Here we used a mapping population, derived from a cross between a winter and a semi-winter cultivar of oilseed rape (OSR), to characterize the component traits of root architectural adaptations to low Pi availability, and identify QTL associated with them. Root architectural traits were scored using an agar-based high-throughput root phenotyping system to assess the roots of over 6600 individual seedlings.

MATERIALS AND METHODS

Plant material and growth conditions

Plant material consisted initially of 190 double haploid (DH) lines representing the 'Tapidor' × 'Ningyou 7' (TNDH) mapping population (Qiu *et al.*, 2006), plus the parents. The TNDH mapping population was generated through anther culture of the F_1 generation of a cross between *Brassica napus* cultivar 'Tapidor' (a European winter OSR) and *B. napus* cultivar 'Ningyou 7' (a Chinese semi-winter OSR). A new genetic linkage map was developed combining 53 gene-based markers (Ding *et al.*, 2011) with an existing well-defined genetic map (Long *et al.*, 2011). The new genetic linkage map had a total of 798 molecular markers and an average distance between two adjacent markers of 2·6 cM. The linkage map was constructed using JoinMap 4·0 (Van Ooijen, 2006) and the mapping procedure followed the method of Long *et al.* (2011) using RFLP, SSR and STS with default parameters and linkage groups distinguished at LOD (logarithm of the ratio of likelihoods) values between 8 and 19. The order of the markers on the new linkage map agreed well with our published maps (Shi *et al.*, 2009; Long *et al.*, 2011).

Seeds were first surface sterilized in 70 % (v/v) ethanol, rinsed in deionized water and then surface sterilized for 1 min using NaOCl (2·5 % active chlorine). Seeds were rinsed three times in sterile deionized water, before being placed in sterile deionized water at 4 °C for 24 h to imbibe. Imbibed seeds were sown into vented polystyrene trays (QTray; 240 × 240 × 20 mm; Molecular Devices, Hampshire, UK) containing 300 mL 0·8 % (w/v) agar and a modified basal salt mix (Murashige and Skoog, 1962) with either 0·625 mM P (HP) added as KH_2PO_4 or 0 mM P (LP), with 0·625 mM KCl added to provide K. The mean (± s.e.m., $n = 3$) total P concentration was 0·652 ± 0·011 mM P for HP agar and 0·082 ± 0·002 mM P for LP agar. Root responses of parental genotypes to external Pi concentrations ($[P]_{ext}$) were also assessed at 0, 0·006, 0·312, 0·625 and 1·25 mM P, with KCl added to balance K. Seeds were sown 3 cm from the top edge of the tray, with four seeds per line and two lines per tray. Trays were sealed with Nescofilm and placed 10 ° from vertical in a growth room under a 16-h photoperiod at a constant temperature of 24 °C. Illumination was provided by a bank of 84 100-W cool fluorescent tubes (Philips, Eindhoven, Netherlands), giving a photon flux density between 400 and 700 nm of 80–100 μmol photons m^{-2} s^{-1} at plant height.

For each line, 16 seeds were sown across four independent replicates, at both LP and HP. Trays were placed randomly within the growth room. However, due to variation in germination rate, the total number of observations for each line varied between 4 and 16, with the average number of observations per line per treatment being 11.

Images of the root systems were captured using a flatbed scanner (Scanjet 3670; Hewlett-Packard, Palo Alto, CA, USA) 12 d after sowing. At harvest, shoot and root fresh weight (SFW and RFW, respectively) were determined. Tissue samples were dried at 80 °C and dry weights (shoot, SDW; root, RDW) determined. Tissue samples were digested by the addition of 2 mL nitric acid to 0·3 g dried ground material and processed in a closed vessel acid digestion microwave (MARSXpress; CEM Corporation, Matthews, NC, USA). Digested samples were diluted with 23 mL of deionized water and analysed using inductively coupled plasma emission spectrometry (JY Ultima 2; Jobin Yvon Ltd, Stanmore, Middlesex, UK) to determine tissue P concentrations.

Image analysis

Images were loaded into ImageJ (Abramoff *et al.*, 2004). Primary root length (PRL, cm) and lateral root length (LRL, cm) were measured. Lateral root numbers were counted and used to calculate lateral root density (LRD, cm^{-1}). Total root length (TRL, cm) was calculated as the sum of PRL and LRL.

Data analysis

Raw data were entered into GenStat (Release 13·1·0·4470; VSN International, Oxford, UK). Data for parent lines grown at different $[P]_{ext}$ were analysed using a two-way ANOVA. Due to variability in germination of lines within the mapping population, data for the mapping population grown at two $[P]_{ext}$ were analysed using REML (residual maximum likelihood) procedures to allocate sources of variation and estimate individual line means (Patterson and Thompson, 1971; Robinson, 1987). Prior to analysis SDW and RDW were ln transformed and LRN, LRD and TRL were square root transformed to improve the normality and variance of the data. PRL and LRL did not require transformation. A random term [(Replicate/Run/Plate/Position) + ($[P]_{ext}$ × Line)] and no defined fixed factors was used to allocate sources of variation for individual traits. Subsequently, line means were estimated using the [($[P]_{ext}$ × Line)] term as a fixed factor, retaining [(Replicate/Run/Plate/Position)] as a random factor.

QTL positions were estimated using the zmapQTL model 6 CIM (composite interval mapping) option in WinQTL cartographer 2·5 software (Wang *et al.*, 2011*a*) and the estimated line means obtained from the REML procedures with the [($[P]_{ext}$ × Line)] as a fixed factor. For each trait, the threshold LOD value for the detection of a significant QTL ($P < 0·05$) was estimated from 1000 permutations (Churchill and Doerge, 1994). Thresholds for the LOD ranged between 3·04 and 3·43.

RESULTS AND DISCUSSION

Parents of the TNDH mapping population show differences in root architecture

To determine optimal assay conditions for screening the large TNDH mapping population, root responses to different $[P]_{ext}$ were quantified in the parents of the mapping population (Fig. 1). The effect of $[P]_{ext}$ was significant ($P < 0·01$) for both RDW and SDW. Root and shoot DW was greatest in both parents at 0·625 mM $[P]_{ext}$, with SDW declining with declining $[P]_{ext}$ and also being lower at 1·25 mM $[P]_{ext}$ compared with 0·625 mM $[P]_{ext}$, and RDW declining with declining $[P]_{ext}$ and remaining similar at 1·25 mM $[P]_{ext}$ compared with 0·625 mM $[P]_{ext}$ (Fig. 1A, B). There was a significant difference between lines for both RDW ($P = 0·012$) and SDW ($P < 0·001$), with 'Ningyou 7' accumulating a greater biomass than 'Tapidor' at all $[P]_{ext}$ (Fig. 1A, B).

Root traits differed significantly between the parents of the TNDH mapping population and showed characteristic changes in root architectural traits with declining $[P]_{ext}$ (Fig. 1). There was a significant decrease in PRL with declining $[P]_{ext}$, with the PRL of 'Tapidor' seedlings declining from 10·45 cm at 1·25 mM $[P]_{ext}$ to 5·11 cm at 0 mM $[P]_{ext}$, and 'Ningyou 7' seedlings declining from 6·27 cm at 1·25 mM $[P]_{ext}$ to 3·68 cm at 0 mM $[P]_{ext}$ (Fig. 1C). This is consistent with primary root responses of other *B. napus* (Akhtar *et al.*, 2008; Yang *et al.*, 2010) and *B. oleracea* (Hammond *et al.*, 2009) cultivars observed previously. At all $[P]_{ext}$, the PRL of 'Tapidor' was greater than the PRL of 'Ningyou 7' seedlings. In contrast, the LRL of 'Ningyou 7' seedlings was consistently greater than the LRL of 'Tapidor' seedlings at all $[P]_{ext}$ (Fig. 1D). Whilst the LRL of 'Ningyou 7' seedlings increased up to 0·625 mM $[P]_{ext}$, before declining again at 1·25 mM $[P]_{ext}$, the LRL of 'Tapidor' seedlings did not change significantly with $[P]_{ext}$ (Fig. 1D). The increase in LRL with $[P]_{ext}$ for 'Ningyou 7' seedlings is consistent with the root responses of other *B. napus* (Solaiman *et al.*, 2007; Hu *et al.*, 2010; Yang *et al.*, 2010) and *B. oleracea* (Hammond *et al.*, 2009) cultivars observed previously, but the LRL response of 'Tapidor' seedlings is atypical. The reduction in LRL of 'Ningyou 7' seedlings when $[P]_{ext}$ is reduced contrasts with the increase in LRL observed for arabidopsis when $[P]_{ext}$ is reduced (Williamson *et al.*, 2001; Linkohr *et al.*, 2002; López-Bucio *et al.*, 2002, 2003, 2005; Al-Ghazi *et al.*, 2003; Nacry *et al.*, 2005; Reymond *et al.*, 2006). Also, in a comparison between two *B. napus* cultivars with either high or low physiological PUE, Akhtar *et al.* (2008) showed a significant increase in LRL in both cultivars when supplied with low $[P]_{ext}$ or rock phosphate. Consequently, this phenotype may be specific to the parental lines used in this study, or may represent a more general phenotype within *B. napus* species. The number of lateral roots was greatest in both parents at 0·312 and 0·625 mM $[P]_{ext}$, but decreased significantly with both increasing and decreasing $[P]_{ext}$ (Fig. 1E). LRD increased with declining $[P]_{ext}$ for 'Tapidor', and increased for 'Ningyou 7' up to 0·312 mM $[P]_{ext}$ before declining slightly at lower $[P]_{ext}$ (Fig. 1F).

Given the significant differences observed between the two parental lines for key root traits under low $[P]_{ext}$, the TNDH mapping population was screened for root architectural traits at low (0 mM added P, LP) and high (0·625 mM added P, HP) $[P]_{ext}$ to identify genomic regions (QTL) associated with these traits.

Root traits show transgressive segregation in the TNDH mapping population

Seeds from 190 lines of the TNDH mapping population were sown on plates containing either LP or HP agar, to facilitate high-throughput phenotyping of root traits. Root architectural traits were scored for all lines at 12 d (Fig. 2 and Table 1). Transgressive segregation was observed for all traits. At the population level, variance attributed to line, $[P]_{ext}$, and their interaction was highly significant ($P < 0·001$) for all traits (Table 1), and correlations between LP and HP treatments for individual traits were all significant ($P < 0·001$; Fig. 2). Mean SDW and RDW for the population did not change significantly when plants were grown on HP agar compared with LP agar, although the individuals within the population showed large responses to $[P]_{ext}$ with the difference in SDW between LP and HP varying from −2·2 to 4·4 mg, and the difference in RDW between LP and HP varying from −0·6 to 0·9 mg (Fig. 2A, B).

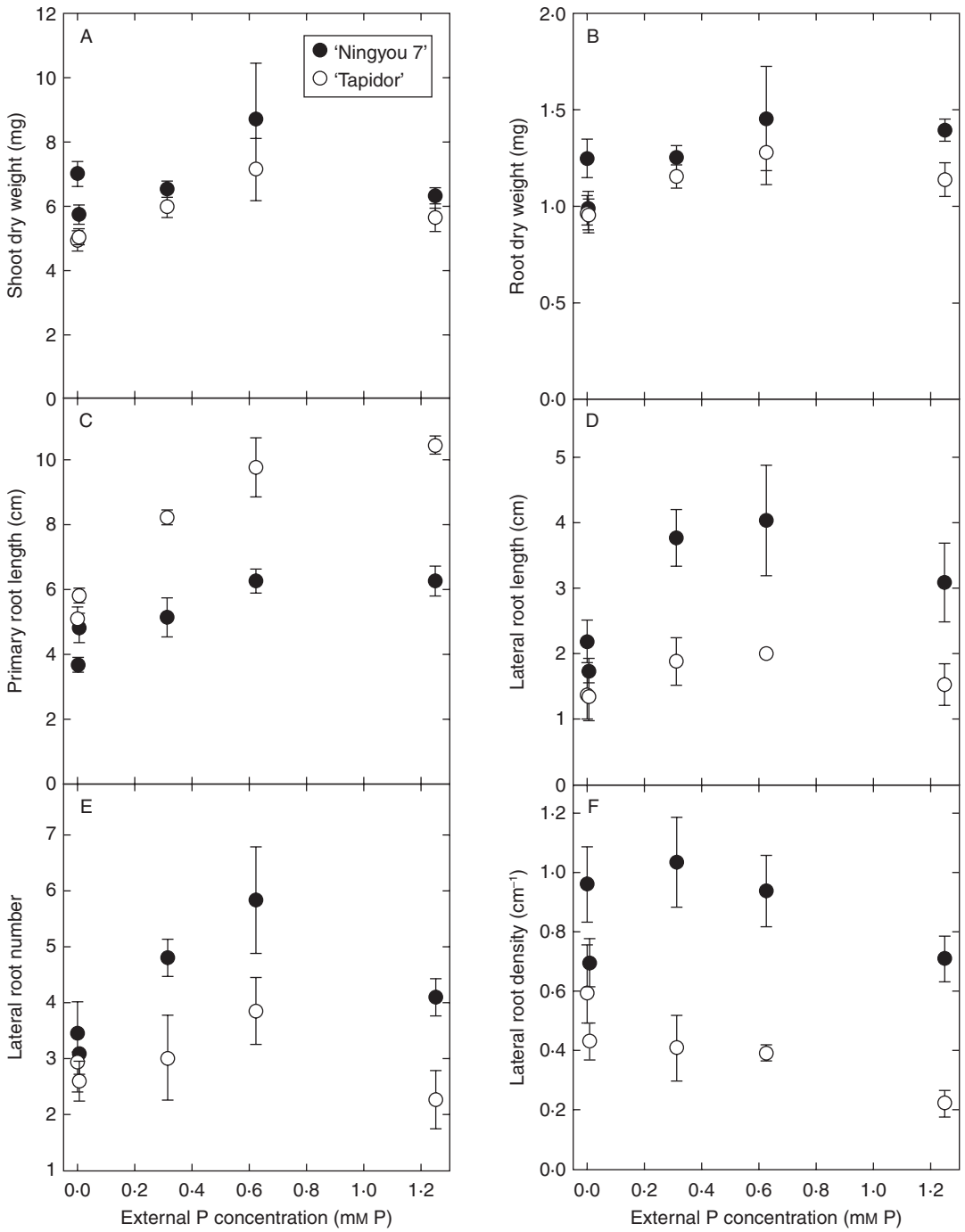

FIG. 1. Changes in shoot dry weight (A), root dry weight (B), primary root length (C), lateral root length (D), lateral root number (E) and lateral root density (F) in 'Tapidor' and 'Ningyou 7' at different external Pi concentrations. Seedlings were grown on trays containing 300 mL 0·8 % (w/v) agar and a modified basal salt mix with 0, 0·006, 0·312, 0·625 or 1·25 mM P for 8 d and images of root systems analysed for root traits. Symbols represent means ± s.e.m. ($n = 4$).

Across the TNDH population, mean PRL and LRL were both significantly lower at LP compared with HP; however, there were a range of responses within the population with some individuals increasing their LRL or PRL by 1·0 and 0·4 cm, respectively, at LP compared with HP. 'Tapidor' had a longer primary root, and shorter lateral roots, compared with 'Ningyou 7' (Fig. 2C, D). The mean LRN and LRD were both higher at LP compared with HP, with a greater range in values for LRD when plants were grown at LP compared with plants grown at HP (Fig. 2E, F). Overall, TRL (data not shown) was greater at HP (mean 17·60 cm, $n = 4536$) compared with LP (mean 13·76 cm), with a greater range in values observed at HP (5·13–34·22 cm) than at LP (5·35–25·12 cm).

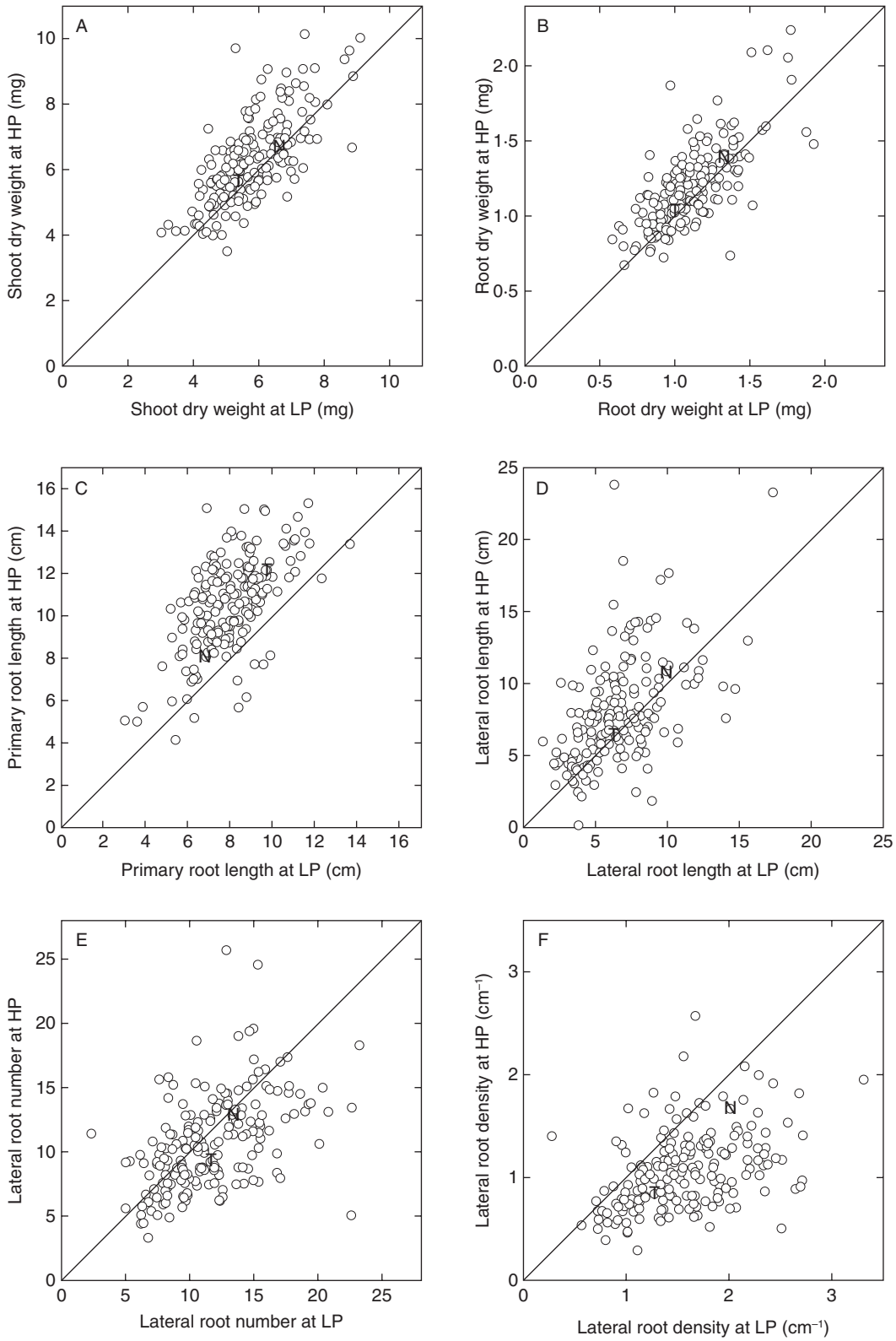

FIG. 2. Variation in shoot dry weight (A), root dry weight (B) primary root length (C), lateral root length (D), lateral root number (E) and lateral root density (F) in the 'Tapidor' × 'Ningyou 7' double haploid (TNDH) mapping population. Seedlings were grown on trays containing 300 mL 0·8 % (w/v) agar and a modified basal salt mix with 0 (LP) or 0·625 (HP) mM P for 12 d. Data are REML-estimated means (n varies between 4 and 16, average 11 observations per line per treatment). Symbols 'T' and 'N' represent the mapping population parental values for 'Tapidor' and 'Ningyou 7', respectively. The continuous line represents the 1 : 1 line.

TABLE 1. *Percentage contributions for individual variance components derived from the REML analyses of biomass and root architectural data measured in the* Brassica napus *TNDH mapping population*

Trait	Replicate	Run	Plate	Position	Line†	$[P]_{ext}^{\dagger}$	Line × $[P]_{ext}^{\dagger}$	Residual
SDW	0·32	2·97	10·62	2·74	30·70***	3·98***	4·72***	43·94
RDW	1·64	3·53	13·19	0·84	28·01***	2·67***	3·47***	46·64
PRL	2·29	9·95	9·64	3·27	13·08***	23·52***	4·15***	34·10
LRL	0·53	6·11	13·91	1·56	16·06***	3·12***	5·08***	53·63
LRN	0·67	9·98	10·93	1·17	15·92***	1·30***	4·54***	55·48
LRD	1·11	6·43	8·41	1·28	13·47***	17·77***	4·88***	46·65
TRL	0·79	12·14	9·88	1·46	14·81***	14·78***	4·65***	41·48

Data were analysed in GenStat using the REML procedures with a random term [(Replicate/Run/Plate/Position) + ($[P]_{ext}$ × Line)] and no defined fixed factors.

† A Wald test statistic was calculated so that significant sources of variation could be identified using a χ^2 function (degrees of freedom for Line = 191, $[P]_{ext}$ = 1, Line × $[P]_{ext}$ = 191; *** = $P < 0{\cdot}001$).

Root architectural traits are heritable in Brassica napus

Breeding new cultivars with improved acquisition and/or utilization of Pi through selection for root architectural traits requires them to be heritable. Within the variance components in the REML analyses, the Line component represents variance attributed to genetic factors. This variance component approximates the population wide additive genetic variation, or narrow-sense heritability. Shoot dry weight and RDW had the highest heritabilities, of 30·70 and 28·01, respectively (Table 1). The root architectural traits had heritabilities ranging from 13·08 to 16·06, suggesting the underlying genetic control of these traits can be selected for, and used in breeding new cultivars with improved root traits. Interestingly, the variance attributed to the $[P]_{ext}$ treatment varied between the root traits. For LRL and LRN, the trait variance attributed to $[P]_{ext}$ was relatively small, compared with the trait variance attributed to $[P]_{ext}$ for PRL, LRD and TRL (Table 1). This implies that PRL, LRD and TRL traits are more responsive to $[P]_{ext}$ in this mapping population, and in *B. napus* may represent key adaptive root architectural traits to low Pi availability.

QTL associated with biomass and root architectural traits are conserved within the Brassicaceae

A total of 38 QTL, associated with root architectural and biomass traits, were identified across nine of the 19 chromosomes (Table 2). Significant QTL associated with SDW at both LP and HP co-localized to 44·2 cM on chromosome A03, explaining 14·9 and 8·5 % of the genetic variation for SDW at LP and HP, respectively. With the exception of one QTL on chromosome A02 (SDW_HP_C02a), all shoot biomass QTL had a negative additive effect, indicating that the allele from 'Ningyou 7' increased the trait value. QTL associated with LRN at LP and RDW at LP also co-localized at 44·2 cM on A03, again with alleles from 'Ningyou 7' increasing the trait value. Also on chromosome A03, three markers between 60·3 and 76·8 cM were significantly associated with LRD at LP, accounting for 23·9 % of the genetic variation associated with this trait.

Primary root length at LP and HP was associated with nine loci within the *B. napus* genome. At LP, PRL was associated with three loci on A07 between 28·8 and 54·6 cM, accounting for 27·9 % of the genetic variation for PRL at LP (Table 2). QTL associated with PRL at LP and HP, and also TRL at HP, were located between 10·8 and 18·8 cM on A03, and were associated with the functional markers BnWRKY-A3 and BnPHT3-A3 (Ding *et al.*, 2011; Table 2). The remaining three loci associated with PRL at LP and HP co-located to chromosome C06 at 16 cM (support interval from 17·6 to 33·6 cM), together with a QTL associated with LRD at LP. No QTL were identified for LRL traits at either LP or HP within this population, despite the large variation between the parents in the initial experiment (Fig. 1D).

The ancestral genome segments of Brassicaceae species have been relatively well defined in terms of rearrangements and duplication events (Parkin *et al.*, 2005; Mun *et al.*, 2009; Wang *et al.*, 2011*b*). These segments facilitate comparative genomics between Brassicaceae species, including other *Brassica* species and the model plant arabidopsis. QTL associated with plant responses to low Pi availability have been identified in both *Brassica* species and arabidopsis (Bentsink *et al.*, 2003; Vreugdenhil *et al.*, 2004; Reymond *et al.*, 2006; Svistoonoff *et al.*, 2007; Zhao *et al.*, 2007, 2008; Hammond *et al.*, 2009, 2011; Ding *et al.*, 2010, 2012; Yang *et al.*, 2010, 2011) and share common genomic regions to those identified in this study. For example, QTL identified here, associated with SDW at LP and HP, and RDW and LRN at LP, and located on chromosome A03 between 36·8 and 46 cM, co-locate with QTL associated with SDW, RDW, root volume and root surface area at LP, and plant height at LP and HP, determined in a cross between P-efficient and P-inefficient *B. napus* cultivars (Ding *et al.*, 2010, 2012; Yang *et al.*, 2010, 2011). This corresponds to a pleiotropic QTL associated with multiple measures of biomass and flowering time and seed weight (Shi *et al.*, 2009) and overlaps with a region of chromosome C03 in *B. oleracea* which has previously been associated with shoot biomass and PUE traits (Hammond *et al.*, 2009). This region is syntenous with ancestral block J on arabidopsis chromosome 2, where QTL for SDW (Loudet *et al.*, 2003) and rosette and root weight (Prinzenberg *et al.*, 2010) have previously been identified, further supporting the presence of a pleiotropic gene in this region.

The unique QTL associated with PRL at LP between 28·8 and 54·6 cM on chromosome A07, corresponds to two

TABLE 2. *Significant QTL associated with biomass and root architectural traits in the* Brassica napus *TNDH mapping population*

Trait	QTL name	Chromosome	Marker	Position (cM)	LOD score	2 LOD support interval (cM)	Additive effect	R^2 (%)
SDW at LP	SDW_LP_A02a	A02	znS16M07-1-230	80·3	3·46	77·8–83	−0·30	6·2
	SDW_LP_A03a	A03	BRMS-043	44·2	7·68	42–44·6	−0·47	14·9
	SDW_LP_A04a	A04	JICB0283	7·2	5·15	5·3–17·2	−0·36	9·9
SDW at HP	SDW_HP_A03a	A03	BRMS-043	44·2	4·28	43·2–46	−0·43	8·5
	SDW_HP_C02a	C02	sN3761b	11·9	3·33	8·5–15	0·36	7·2
RDW at LP	RDW_LP_A03a	A03	BRMS-043	44·2	3·89	43·2–46	−0·07	7·8
RDW at HP	RDW_HP_A03a	A03	CNU098	61·3	3·13	60·3–62·4	−0·07	6·4
LRN at LP	LRN_LP_A03a	A03	HBr082	37·5	4·11	36·8–38	−1·19	8·9
	LRN_LP_A03b	A03	BRMS-043	44·2	4·81	43·2–46	−1·30	10·4
	LRN_LP_A03c	A03	B068E07-2	51·9	3·71	50·8–52·5	−1·13	8·4
LRN at HP	LRN_HP_C09a	C09	CB10064	36·0	3·51	34·6–43·6	1·06	8·2
LRD at LP	LRD_LP_A02a	A02	em12me31-320	73·3	3·92	66·6–75·5	−0·14	7·5
	LRD_LP_A03a	A03	CNU098	61·5	4·55	60·3–62·4	−0·16	8·7
	LRD_LP_A03b	A03	H034E17-1	69·3	3·83	67·3–70·7	−0·14	7·4
	LRD_LP_A03c	A03	BnPYK10-A3b	76·7	3·89	70·7–76·8	−0·14	7·8
	LRD_LP_A09a	A09	B019F12-3	37·9	5·37	34·9–40·6	−0·23	11·5
	LRD_LP_C06a	C06	JBnB061J08	29·0	3·74	25·7–35·2	−0·13	7·1
LRD at HP	LRD_HP_A04a	A04	JICB0283	16·2	5·98	7·6–21	−0·15	13·2
	LRD_HP_C04a	C04	sN12353c	50·6	3·36	49·9–52·3	−0·10	6·8
	LRD_HP_C04b	C04	Na10C01a	62·2	3·64	59·6–62·7	−0·10	7·5
TRL at HP	TRL_HP_A03a	A03	BnWRKY-A3	14·4	3·64	10·8–17	−1·33	7·5
PRL at LP	PRL_LP_A03a	A03	BnPHT3-A3	15·5	3·36	13·5–18·8	−0·40	5·8
	PRL_LP_A07a	A07	BRAS023	29·8	4·76	28·8–36·3	0·48	9·5
	PRL_LP_A07b	A07	HR-Tp4-305	42·6	5·70	39·5–46·4	0·50	10·2
	PRL_LP_A07c	A07	sR7223	50·6	4·46	48–54·3	0·44	8·2
	PRL_LP_C06a	C06	em18me23-350	27·5	6·07	17·6–34·7	0·53	12·0
PRL at HP	PRL_HP_A03a	A03	BnPHT3-A3	15·5	4·59	14·4–17	−0·67	8·3
	PRL_HP_A03b	A03	H003M07-4	21·9	3·88	20·9–27·8	−0·64	8·0
	PRL_HP_C06a	C06	CNU053a	21·4	6·82	20·6–25·7	0·82	14·8
	PRL_HP_C06b	C06	em18me23-350	27·5	8·00	25·7–33·6	0·85	16·3

REML estimated means for biomass and root architectural traits for 176 lines in the TNDH mapping population were used to estimate QTL positions associated with these traits using the zmapQTL model 6 composite interval mapping function (Wang *et al.*, 2011*a*). Significant ($P < 0·05$) LOD thresholds for individual traits were determined using 1000 permutations. A positive additive effect indicates a positive contribution of the 'Tapidor' allele to the trait value.

ancestral blocks, E and N, on the bottom of arabidopsis chromosomes 1 and 3, respectively. The region containing QTL associated with PRL and LRD on chromosome C06 (Table 2), is also syntenous with the ancestral block E, suggesting these regions may contain paralogues of genes involved in the regulation of PRL. Arabidopsis genes identified as regulating primary root development under low Pi availability have previously been identified in block E. Arabidopsis *LPR2* (At1g71040) encodes multicopper oxidases in arabidopsis, and is a paralogue of arabidopsis *LPR1* (At1g23010). These proteins are critical for the reduction in primary root growth observed when the root tips are in contact with low-Pi media (Reymond *et al.*, 2006; Svistoonoff *et al.*, 2007). An *AINTEGUMENTA*-like gene, named *PRD* (At1g79700), the mutant of which was identified as having reduced primary and lateral root development under low Pi availability compared with the wild-type, also co-locates to this region (Camacho-Cristóbal *et al.*, 2008). The latter of these ancestral blocks also co-locates with a QTL for PRL in arabidopsis (Loudet *et al.*, 2005).

Conclusions

The development of crops that can acquire and/or utilize P more efficiently is essential for the sustainability of future crop production. Plant adaptations to low Pi availability include alterations in the allocation of resources to roots and changes in the distribution of those roots in the soil. Since the assessment of root traits in plants can be slow and expensive, we employed an agar based high-throughput root phenotyping screen to characterize the root traits of a large *B. napus* mapping population and identify genetic loci controlling these traits under low Pi availability (Fig. 2 and Table 2). Significant QTL associated with biomass and root architectural traits were identified on A03, and co-locate with QTL for biomass traits in *B. napus*, *B. rapa* and arabidopsis. Significant QTL associated with root architectural traits were also identified, including one for PRL at LP on A07, which co-locates with several arabidopsis genes implicated in primary root development. Identification of the genetic elements associated with these traits will provide targets for the future development of crops adapted to growth in low Pi soils. The use of high-throughput root phenotyping assays has the potential to advance the breeding and selection of these cultivars, but requires cross validation with root characteristics and yield determined under field conditions.

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