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ORIGINAL PAPER

Quantitative trait loci for root morphology in response to low phosphorus stress in *Brassica napus*

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Abstract Phosphorus (P) deficiency in soils is a major limiting factor for crop growth worldwide. Changes in root morphology and architecture represent as an important mechanism of adaptation of plants to low P (LP) stress. To elucidate the genetic control of tolerance to P deficiency in *Brassica napus*, quantitative trait loci (QTL) for root morphology in response to LP were identified in three independent paper culture experiments, and dissected through QTL meta-analysis. In total, 62 significant QTL for total root length, root surface area, root volume, total dry weight, and plant P uptake under high and low P conditions were detected in the three experiments. Forty-five of these QTL were clustered within four linkage groups and were integrated into eight unique QTL by two rounds of QTL meta-analysis. Three of the unique QTL, *uq.A1*, *uq.C3a* and *uq.C3b*, were specific for LP condition. *uq.C3a* and *uq.C3b* were identified specifically for root traits and P uptake under LP stress, and may contribute to the adaptability of *B. napus* to P deficiency. Two functional markers, BnIPS2-C3 and BnGPT1-C3, which were developed from the genes *AtIPS2* and *AtGPT1* in *Arabidopsis*, were located in the confidence intervals of *uq.C3a* and *uq.C3b*, respectively. And *AtGPT1* that corresponded to the interval of *uq.C3b* by in silico mapping was a possible candidate gene of *uq.C3b*. These results confirmed the importance of root traits for the adaptability of *B. napus* to LP and partially revealed the genetic basis of tolerance to P deficiency. These findings should be valuable for further study of the mechanism of P efficiency and the breeding of P-efficient cultivars by marker-assisted selection.

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Introduction

Phosphorus (P) is an essential mineral nutrient for plants. It plays important roles in plant growth and metabolism, including nucleic acid synthesis, photosynthesis, glycolysis, respiration, redox reactions, and carbohydrate metabolism (Marschner 1995). The total amount of P in soils is sufficiently high to support plant growth. However, it is largely unavailable for uptake by plants because it readily forms complexes with some metal cations, in particular, aluminum and iron in acid soils and calcium and magnesium in alkaline soils, which results in P deficiency in soils (Holford 1997). P deficiency is a major limitation to plant growth worldwide (Lynch 2007; Vance et al. 2003). It has been estimated that 5.7 billion hectares of land worldwide are deficient in P, that is, the concentration of available P does not exceed 10 μM (Batjes 1997). In China, most cultivated soils are either acidic or calcareous, which gives them a strong capability to fix P; hence, the majority of soils in China are P deficient (Yan et al. 2006). The application of P fertilizers can alleviate P deficiency, but it is not an entirely satisfactory solution, due to limited

bioavailability, potential environmental threats such as eutrophication, and the exhaustion of resources of phosphate rock (Vance et al. 2003). Therefore, it has become attractive to use an alternative approach that exploits germplasms from P-efficient crops or involves the breeding of P-efficient cultivars (Fageria et al. 2008).

Plants with an extensive root system can absorb more nutrients under conditions of nutrient stress than can plants with a less extensive system. To survive during low P (LP) stress, plants modify their root system to increase the acquisition of P by increasing the root/shoot ratio, altering root morphology and architecture, stimulating lateral root initiation, and increasing the density and elongation of root hairs (Hammond and White 2008; Hermans et al. 2006; Jain et al. 2007; Lynch 2007). However, cultivars and species show wide genetic variation with respect to changes in root morphology in response to LP stress. Thus, analysis of quantitative trait loci (QTL) has been used to elucidate the genetic mechanisms that control modification of the root system in response to P deficiency. A QTL (*qREP-6*) for root elongation under condition of P deficiency has been detected in rice that improved the adaptability of rice to LP stress (Shimizu et al. 2004, 2008; Ismail et al. 2007). Main-effect QTL that affect root hairs and lateral roots were identified in maize under P deficiency (Zhu et al. 2005a, b). In common bean, QTL for root traits, acid exudation and P uptake were shown to overlap in four linkage groups (Beebe et al. 2006; Yan et al. 2004). In *Arabidopsis*, a QTL (Atlg23010) for primary root elongation in response to LP stress has been cloned (Reymond et al. 2006; Svistoonoff et al. 2007). These studies have provided a solid basis for understanding the genetic control of tolerance to P deficiency through specific root traits.

QTL for the same or related traits that are detected in different experiments with the same population are usually located in the same regions. To identify the candidate gene, it is essential to determine whether or not these QTL represent a single locus. Appropriate statistical tools, such as QTL meta-analysis, can evaluate information about a QTL from multiple environments and refine the position of the QTL, with a reduction in the confidence interval (Arcade et al. 2004). This method has been applied successfully to the analysis of QTL for flowering time in maize and seed yield in rapeseed. Meta-analysis of these QTL revealed the genetic architecture of both flowering time and yield, and candidate genes were proposed (Chardon et al. 2004; Shi et al. 2009). However, this approach has not been used to analyse QTL for root system traits.

Brassica napus is one of main oil crops in the world and also a potential bioenergy crop. Because of a high P requirement for optimal seed yield and quality, the low concentration of available P in soils seriously limits the production of *B. napus*. Genotypic variation with respect to P efficiency has been investigated among *Brassica* species (Akhtar et al. 2008; Hammond et al. 2009; Solaiman et al. 2007; Zhang et al. 2009; Zhao et al. 2008). Compared with P-inefficient cultivars, P-efficient cultivars absorb more P and produce more biomass under LP stress due to alterations in root morphology and secretion of organic acid (Solaiman et al. 2007; Zhang et al. 2009). These results indicate that rapeseed germplasms show significant genotypic differences with respect to P uptake and utilization, and that it is possible to breed P-efficient cultivars. However, the genetic control of tolerance to P deficiency in rapeseed remains poorly understood.

The critical period for P nutrition in plants is the early growing stage. Thus, an understanding of the genetic control of P efficiency at the seedling stage should help to reveal the mechanisms of P efficiency. The aims of this study were to elucidate the genetic basis of P efficiency in *B. napus* and to provide a foundation for breeding P-efficient rapeseed cultivars by marker-assisted selection (MAS). For this purpose, we identified QTL for plant total dry weight (DW), total root length (RL), root surface area (RSA), root volume (RVol), and plant P uptake (PU), under conditions of LP and high P (HP) at the seedling stage of a recombinant inbred line (RIL) population of *B. napus* in three independent paper culture experiments. In addition, we analyzed the genetic basis of root system modification in response to P deficiency by QTL meta-analysis, and developed functional markers from genes in the P metabolic pathway in *Arabidopsis*.

Materials and methods

Plant materials

An F_{10} RIL population that consisted of 124 lines was developed from a cross between the P-efficient rapeseed cv. 'Eyou Changjia' and the P-inefficient cv. 'B104-2' using a single seed descent method. The parents, 'Eyou Changjia' and 'B104-2', were selected on the basis of their P efficiency coefficients (the ratio of shoot dry weight or seed yield under LP to that under HP) (Duan et al. 2009). In our previous studies, it was found that 'Eyou Changjia' had a larger root system and acquired more P than 'B104-2' under LP condition in pot culture, root-soil compartments, and solution culture.

Paper culture system

The paper culture experiments were conducted to investigate the root morphology of the RILs. Uniform seeds were selected from the 124 lines and the two parents,

surface-sterilized for 15 min in a 0.5% (w/v) sodium hypochlorite solution, and then washed three times with deionized water. The surface-sterilized seeds were germinated on moistened gauze that was fixed to a porcelain tray filled with deionized water. Then they were grown in an illuminated culture room for 6 days at 22–24°C until cotyledons were fully developed, but lateral roots were not differentiated from primary roots. Finally, the seedlings were transferred carefully to the paper culture system.

The paper culture system was designed according to the system described by Liao et al. (2001). It consisted of a sheet of 15.6 × 22.0-cm P-free blue germination paper (Anchor Paper Company, St Paul, MN, USA) placed inside a polyethylene bag that was punctured evenly at 1.5-cm intervals for aeration. Two seedlings were transplanted onto the top of the paper. The sheets of paper with the seedlings were suspended upright in nutrient solution pool of dimensions 200 × 40 × 22 cm (length × width × height), and 50 l of quarter-strength modified Hoagland's solution were added to submerge the bottom of the sheets (Hoagland and Arnon 1950). Two P treatments, LP (5 μM) and HP (1 mM), were designed with three replicates. The experiment was conducted in a randomized complete block design.

The quarter-strength nutrient solution was replaced with half-strength solution after 5 days, which was replaced in turn with full-strength solution after a further 5 days. The full-strength modified Hoagland's solution consisted of 4 mM KNO_3, 1 mM KH_2PO_4, 2 mM $MgSO_4{\cdot}7H_2O$, 4.5 mM $Ca(NO_3)_2{\cdot}4H_2O$, 46 μM H_3BO_3, 0.32 μM $CuSO_4{\cdot}5H_2O$, 0.77 μM $ZnSO_4{\cdot}7H_2O$, 9.14 μM $MnCl_2{\cdot}4H_2O$, 0.37 μM $NaMoO_4{\cdot}2H_2O$, and 50 μM EDTA-Fe. In the LP nutrient solution, 1 mM KH_2PO_4 was replaced by 0.5 mM K_2SO_4. The pH value of the nutrient solution was adjusted to 5.7 ± 0.2 with 2 M NaOH or HCl every 2 days. The three independent paper culture experiments were all conducted in the same greenhouse at Huazhong Agricultural University, Wuhan, China. The first experiment (Exp. 1) was carried out from February 1 to March 6, 2008, the second (Exp. 2) from March 4 to 31, 2008, and the third (Exp. 3) from November 13 to December 15, 2008. During the three experiments, the minimum temperature in the greenhouse ranged from 5 to 8°C, 10 to 14°C, and 8 to 12°C, respectively, and the maximum temperature ranged from 18 to 21°C, 25 to 28°C, and 22 to 26°C, respectively.

Measurement of phenotypic traits

The intact root systems grown on the germination paper were scanned as a digital image with a scanner after harvesting. The RL, RSA and RVol were determined from the root images using the root image analysis software WinRHIZO Pro (Regent Instruments, QC, Canada).

After they had been scanned, the plants were detached from the paper sheets. They were then oven-dried at 105°C for 30 min and then at 60°C to constant weight to determine the plant DW. The dried plants were ground to fine powder and digested by adding 5 ml of a mixture of concentrated H_2SO_4:$HClO_4$ (19:1, v:v). P concentration in plant dry matter in the digested solution was analyzed by P vanadate–molybdate yellow colorimetric method using a flow injection analysis instrument (FIAstar 5000 analyzer; FOSS, Hilleroed, Denmark). Plant PU was calculated as plant DW × P concentration.

Analysis of molecular markers and construction of a linkage map

Genomic DNA was extracted from plant leaf tissues by the Cetyltrimethylammonium Bromide (CTAB) method (Doyle and Doyle 1990). The genotypes of the RILs were analyzed using simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP), sequence related amplified polymorphism (SRAP), and functional markers (FM) to enable the construction of a genetic linkage map.

Primer sequences for SSR markers were obtained from various sources as follows: UK (http://www.brassica.bbsrc.ac.uk/BrassicaDB, prefixed by OL and Na), Australia (http://www.hornbill.cspp.latrobe.edu.au, prefixed by sA), Canada (http://www.brassica.agr.gc.ca/index_e.shtml, prefixed by sR and sN), Japan (Suwabe et al. 2002, prefixed by BRMS), and France (Piquemal et al. 2005, prefixed by BRAS, CB and MR). The remainder of SSR primer pairs were obtained from private communications (prefixed by CNU, niab and HBr). The protocol for SSR analysis followed the method described by Lowe et al. (2004).

The AFLP analysis was performed according to the protocol developed by Vos et al. (1995). Polymorphisms were detected using genomic DNA digested with the restriction enzymes *Eco*RI and *Mse*I. One hundred primer combinations, which involved 10 *Eco*RI and 10 *Mse*I primers, were used. The AFLP markers were named using codes for each *Eco*RI and *Mse*I primer, for example e2m4.

The analysis of SRAP markers followed the protocol of Li and Quiros (2001), and the polymorphic primer pairs were named by combining the names of forward and reverse primers (e.g., em5me3).

FM were obtained from the functional genes that are related to P transporters and from regulatory factors that are induced in response to P starvation in *Arabidopsis* (http://www.arabidopsis.org/). The primers were designed from sequences that are conserved between the homologous genes from *Arabidopsis* and *Brassica*. The FM were

named using the prefix Bn and the gene name in *Arabidopsis*, for example BnIPS2.

All PCR products were separated by PAGE and stained with $AgNO_3$. When a primer pair detected more than one polymorphic locus, the different loci were distinguished by small letters after the name of the marker. For example, the primer pair BRMS056 generated three polymorphic loci, which were named BRMS056a, BRMS056b and BRMS056c, respectively.

The genetic linkage map was constructed using the software program Joinmap4.0 (Van Ooijen 2006), which is based on the Kosambi mapping function. The mapping procedure followed the method of Qiu et al. (2006). Firstly, a framework linkage map was constructed using 269 SSR markers. The threshold for goodness of fit was set to ≤5.0 with logarithm of the odds ratio (LOD) scores >1.0 and a recombination frequency <0.4. Secondly, using the order of the SSR markers in the framework map as a basis, the AFLP, SRAP, and FM markers were joined to construct a map with a recombination level equal to 0.45 and minimum LOD scores equal to 0. Markers with a χ^2 value > 3.0 were removed in every genetic group.

Statistical analysis and QTL detection

Statistical analysis of the traits was conducted using the ANOVA procedure of SAS 8.1 (SAS Institute, Cary, NC, USA). Pearson's correlation analysis was performed using the CORR procedure of SAS.

QTL were detected by the composite interval method (CIM) using WinQTL Cartographer 2.5 software (Wang et al. 2006; Zeng 1994). CIM was used to scan the genetic map and estimate the likelihood of a QTL and its corresponding effect at every 2 cM. For each trait, the threshold for the detection of a significant QTL ($P < 0.05$) was estimated by 1,000 permutations (Churchill and Doerge 1994).

The two-round strategy of QTL meta-analysis was performed according to the method described by Shi et al. (2009). The coincidental significant QTL for the same traits from the three experiments were first integrated into consensus QTL using the BioMercator2.1 software (Arcade et al. 2004). The consensus QTL for different traits that overlapped were integrated into unique QTL in the second round of QTL meta-analysis.

For the consensus QTL, the QTL nomenclature corresponded to "q" (abbreviation of QTL), followed by "HP" or "LP" (to indicate whether the QTL was identified under HP or LP), then an abbreviation of the trait (e.g., RL), the linkage group number (A1–A10 and C1–C9), and finally the serial letter (a, b, c…), for example *qHP-RL-A3a* and *qLP-RVol-C3b*. For the unique QTL, the nomenclature corresponded to "uq.", followed by the linkage group number, and then a small serial letter, for example *uq.C3b*.

Results

Genetic linkage map

Four types of molecular marker, SSR, AFLP, SRAP and FM, were used to construct a genetic linkage map. Firstly, SSR markers were used to construct a framework map that was based on the anchored markers reported by Long et al. (2007) and Piquemal et al. (2005). The framework map contained 176 SSR markers (Supplementary Table S1a). These SSR markers were assigned to 23 linkage groups, which corresponded to 19 chromosomes, named as A1–A10 and C1–C9. Chromosomes A4, A5, A9 and C7 were divided into two smaller linkage groups, which were named A4a and A4b, A5a and A5b, A9a and A9b, and C7a and C7b, respectively.

Secondly, a new linkage map was constructed by adding the AFLP, SRAP and FM markers to the framework map. This linkage map consisted of 553 markers: 202 SSR, 62 AFLP and 234 SRAP markers and 55 FM (Supplementary Table S1b). The total length of the 19 linkage groups was 1592.7 cM, with an average distance of 2.9 cM between adjacent markers. In general, the order of the SSR markers in the map agreed with that reported previously (Long et al. 2007; Piquemal et al. 2005; Qiu et al. 2006). This map was employed in subsequent QTL analysis.

Phenotypic variation and correlation analysis among traits

Plant DW, root traits and PU were investigated in the two parents under conditions of HP and LP. Under HP, the two parents showed no significant difference in plant DW or root traits, including RL, RSA, and RVol. However, they consistently showed significant differences under LP condition. The P-efficient parent 'Eyou Changjia' yielded a higher plant DW and developed a larger root system (longer RL, and larger RSA and RVol) than 'B104-2' (Tables 1, 2). Moreover, 'Eyou Changjia' acquired more P than 'B104-2' under LP condition in all three experiments (Table 1). 'Eyou Changjia' accumulated 41.3% more P than 'B104-2' in Exp. 1, 37.6% more in Exp. 2, and 38.4% more in Exp. 3. ANOVA revealed that significant effects of environment, P level, genotype, and interactions of E × P and G × P were detected for most traits (Table 2).

In all three experiments, all five traits showed a continuous distribution in the RIL population (Table 1; Fig. 1). The coefficients of variation (CVs) for these traits ranged from 20.5 to 40.6%. Significant transgressive segregation was observed for each trait under both P conditions. The transgressive lines showed more extreme phenotypes than 'B104-2' or 'Eyou Changjia'. For example, in the three individual experiments, 21, 11 and 10 lines, respectively,

Table 1 Mean values and ranges of the five traits in the parents and the RIL population

Traits	Exp	Parents				RILs			
		HP		LP		HP		LP	
		B[a]	E[b]	B	E	Mean (min–max)	CV	Mean (min–max)	CV
DW (mg plant^{-1})	1	24.2	23.1	13.2	17.4	23.5 (10.6–43.0)	26.9	16.5 (8.7–25.7)	22.4
	2	29.1	30.9	16.9	21.5	24.9 (10.9–40.8)	25.9	22.4 (12.6–34.1)	20.5
	3	36.1	36.9	14.6	20.7	22.3 (11.6–48.1)	28.4	17.2 (8.4–28.9)	23.2
RL (cm)	1	115.0	114.5	70.1	105.8	76.2 (30.7–168.1)	32.7	66.6 (29.1–120.9)	26.9
	2	191.8	185.4	94.4	122.9	154.5 (65.6–290.6)	30.9	90.2 (44.0–150.5)	25.8
	3	209.2	213.2	104.4	142.9	122.7 (62.1–229.1)	29.3	87.5 (43.9–161.3)	27.9
RSA (cm^2)	1	16.5	16.8	9.9	12.8	16.0 (6.1–27.6)	27.9	10.6 (4.2–18.7)	26.0
	2	21.1	22.0	10.7	14.8	20.6 (9.8–34.6)	26.9	13.9 (7.2–23.1)	24.7
	3	23.4	23.8	10.4	14.3	17.0 (7.8–29.6)	26.5	13.2 (6.9–22.7)	25.1
RVol (cm^3)	1	0.17	0.18	0.10	0.14	0.29 (0.10–0.52)	31.4	0.15 (0.05–0.31)	36.1
	2	0.21	0.20	0.10	0.12	0.23 (0.10–0.47)	31.4	0.17 (0.08–0.34)	32.2
	3	0.22	0.22	0.09	0.12	0.20 (0.09–0.34)	31.0	0.16 (0.06–0.32)	32.7
PU (μg plant^{-1})	1	102.1	100.7	16.2	27.6	91.9 (43.8–179.9)	29.7	24.7 (11.8–57.7)	28.6
	2	95.5	110.6	15.3	24.2	85.2 (39.4–152.2)	27.2	26.2 (11.6–50.6)	28.0
	3	65.5	176.6	21.5	34.9	60.1 (33.9–164.9)	40.6	27.1 (15.1–53.0)	24.1

Data are the means of the six replicates

[a] B104-2, P-inefficient parent, [b] Eyou Changjia, P-efficient parent

Table 2 Significance of three-way ANOVA analysis for the five traits among the parents and RILs in HP and LP treatments in three experiments

Source	DF	DW	RL	RSA	RVol	PU
Parents						
Environment	2	***	***	***	*	***
P level	1	***	***	***	***	***
Genotype	1	***	**	***	**	***
E × P	2	***	***	***	***	*
E × G	2	ns	ns	ns	ns	***
P × G	1	**	***	**	***	***
RILs						
Environment	2	***	***	***	***	–
P level	1	***	***	***	***	–
Genotype	123	***	***	***	***	–
E × P	2	***	***	***	***	–
E × G	246	***	***	***	***	–
P × G	123	***	***	***	***	–

DF degrees of freedom, *E* × P environment × P level, *E* × G environment × genotype, *P* × G P level × genotype, *ns* not significant, "–" no analyzed

*** $P < 0.001$, ** $0.01 > P > 0.001$; * $0.05 > P > 0.01$

showed a significantly lower RSA than 'B104-2' under LP condition, whereas 13, 23 and 24 lines, respectively, had a significantly higher RSA than 'Eyou Changjia' (data not shown). The results of ANOVA showed that environment, P level, genotype and the interactions between these factors had highly significant effects on the four traits (except for PU) in the RIL population (Table 2).

Pearson's correlation coefficients between traits were calculated (Supplementary Table S2). Significant positive correlations ($P < 0.01$) were found among the five traits under either HP or LP condition in every experiment. In any two of the three independent experiments, the same traits showed highly significant positive correlations at the same P level.

QTL detection and the analysis of consensus QTL

From the three experiments, a total of 62 significant QTL was identified for the five traits under the two P conditions. These QTL were distributed on 10 linkage groups (Fig. 2; Supplementary Table S3). In the three independent experiments, 12, 28 and 22 QTL were detected, respectively. Of the 62 significant QTL, 26 were detected specifically in one experiment under one P condition, 24 were detected in two experiments, and 12 were identified in all three experiments. The majority of the QTL (72.6%) were clustered on A1, A3, C2 and C3 (Fig. 2). So QTL meta-analysis was performed to integrate the QTL clusters. Significant QTL that were identified for the same traits on the four linkage groups were integrated into consensus QTL by first round meta-analysis. As a result, 26 consensus QTL were identified on A1, A3, C2 and C3 (Table 3).

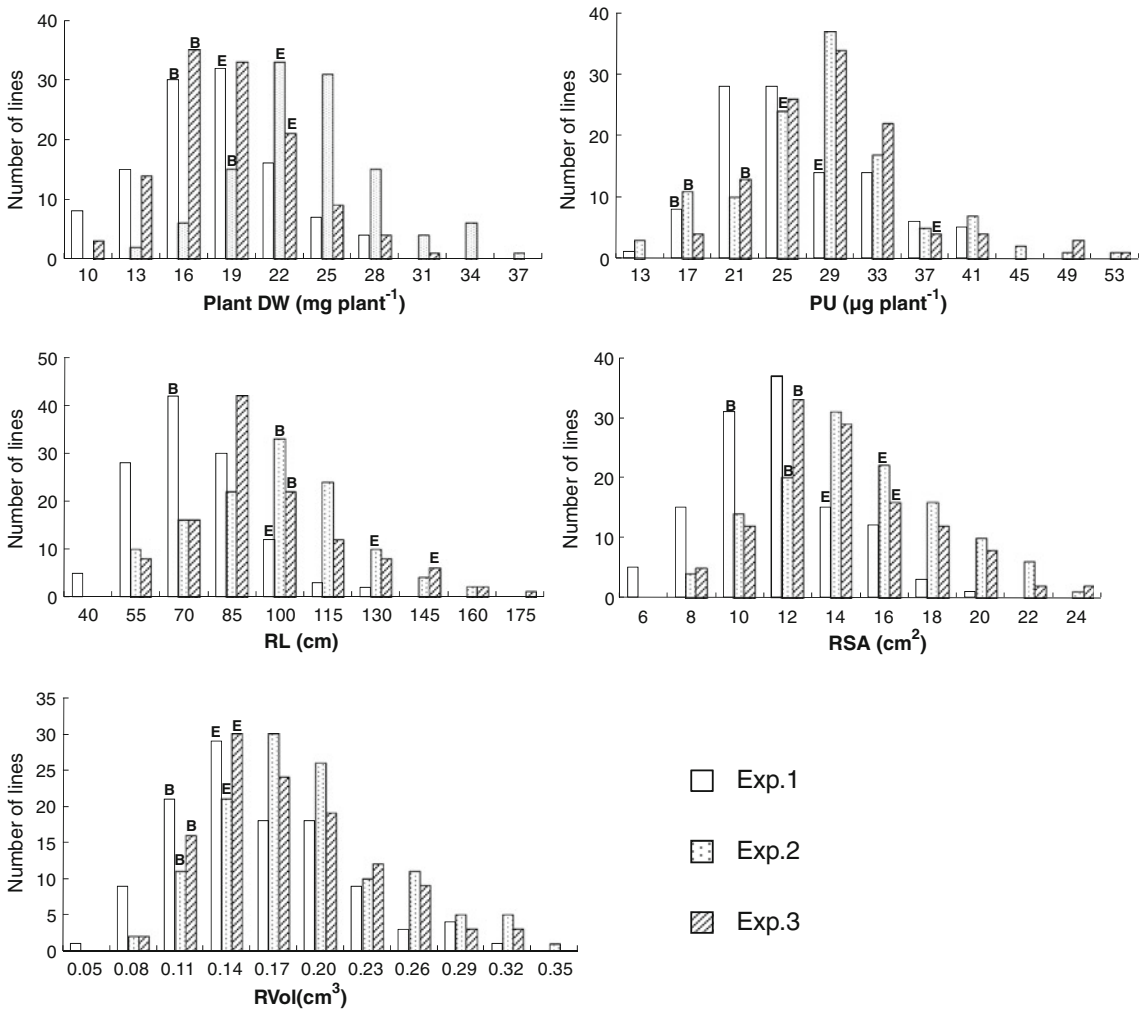


Fig. 1 Frequency distribution of plant DW, PU, RL, RSA and RVol in the RIL population under LP condition in the three experiments. The letters '*B*' and '*E*' indicate the values for the parents 'B104-2' and 'Eyou Changjia', respectively, in the three experiments

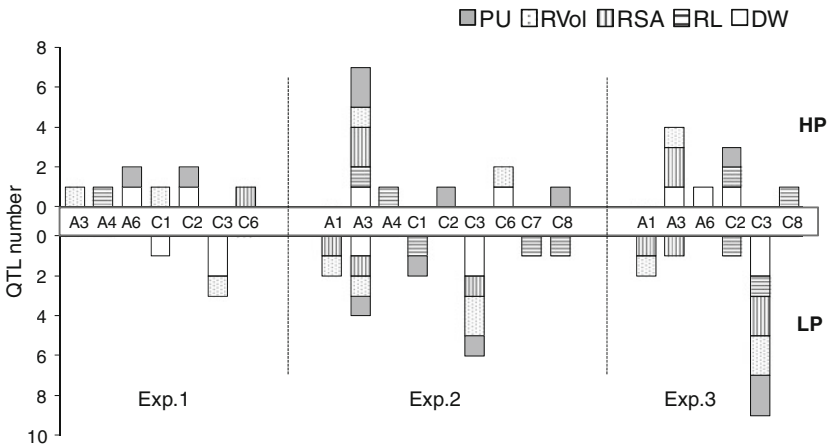


Fig. 2 Distribution of total QTL for plant DW, RL, RSA, RVol, PU on ten linkage groups under HP (*above* abscissa) and LP (*below* abscissa) conditions in the three experiments. The three experiments are differentiated by the two *long dotted lines*

Sixteen consensus QTL were detected under LP condition. Three consensus QTL for plant DW were detected in the three experiments. *qLP-DW-A3a* was detected only in Exp. 2 and accounted for 13.7% of the phenotypic variation. *qLP-DW-C3a* was identified in Exps. 2 and 3, and accounted for 13.8 and 19.7% of the phenotypic variation,

Table 3 Overview of consensus QTL and unique QTL obtained from QTL meta-analysis on linkage groups A1, A3, C2, and C3 of *B. napus*

Unique QTL					Consensus QTL							FM[d]	Gene name[e]
Name	P level	Linkage	Position	CI[a]	Name	Position	CI	LOD	R^{2b}	A[c]	Exp		
uq.A1	LP	A1	16.0	14.6–17.5	*qLP-RSA-A1a*	16.0	14.1–17.9	4.54	11.8	+	2,3		
					qLP-RVol-A1a	16.0	13.7–18.4	3.81	9.46	+	2,3		
uq.A3a	LP	A3	22.9	14.9–27.6	*qLP-PU-A3a*	22.9	14.9–27.6	3.59	9.53	+	2		
uq.A3b	LP & HP	A3	33.3	32.3–34.2	*qHP-PU-A3a*	29.9	27.0–35.6	3.21	8.11	+	2		
					qHP-DW-A3a	34.7	30.1–39.3	3.62	10.8	+	2,3		
					qHP-RSA-A3a	31.7	28.2–35.1	6.32	17.1	+	2,3		
					qHP-RVol-A3a	33.3	31.9–34.9	5.58	15.3	+	1,2,3		
					qLP-DW-A3a	34.3	32.3–36.5	4.10	13.7	+	2		
					qLP-RSA-A3a	32.6	30.4–34.9	4.71	14.4	+	2,3		
					qLP-RVol-A3a	34.3	29.7–36.5	4.35	12.2	+	2		
uq.A3c	HP	A3	44.0	41.9–48.0	*qHP-RL-A3a*	44.0	41.9–48.0	3.53	9.36	+	2		
uq.A3d	HP	A3	87.4	86.4–88.6	*qHP-PU-A3b*	85.3	80.6–89.4	4.36	11.3	–	2		
					qHP-RSA-A3b	87.6	86.3–88.9	4.16	11.2	–	2,3		
uq.C2	LP & HP	C2	42.2	41.0–43.3	*qHP-DW-C2a*	44.8	38.6–51.0	3.67	11.6	+	1,3	BnGPT1-C2	*AtGPT1*
					qHP-PU-C2a	42.0	40.8–43.2	3.72	10.3	+	1,2,3		
					qHP-RL-C2a	45.7	37.2–56.9	4.46	11.4	+	3		
					qLP-RL-C2a	43.3	37.9–57.0	3.37	12.4	+	3		
uq.C3a	LP	C3	10.3	9.3–11.4	*qLP-DW-C3a*	10.3	7.6–13.0	5.51	16.8	–	2,3	BnIPS2-C3	*AT4*
					qLP-PU-C3a	12.5	7.1–15.4	5.09	16.7	–	2,3		
					qLP-RL-C3a	10.3	9.3–12.3	3.52	11.3	–	3		
					qLP-RSA-C3a	9.5	6.9–12.1	4.46	15.2	–	2,3		
					qLP-RVol-C3a	10.3	7.4–13.2	4.21	11.8	–	2,3		
uq.C3b	LP	C3	44.8	43.5–46.0	*qLP-DW-C3b*	45.4	43.7–47.1	4.69	13.1	+	1,2,3	BnGPT1-C3	*AtGPT1*
					qLP-PU-C3b	45.9	41.3–48.3	3.88	11.33	+	3		
					qLP-RSA-C3b	43.3	41.5–48.0	3.61	9.37	+	3		
					qLP-RVol-C3b	43.3	40.4–46.3	4.75	12.5	+	1,2,3		

[a] Confidence interval at $P = 0.05$

[b] Represents the phenotypic variation accounted for

[c] Additive effects, positive value (+) means the allele came from the parent 'B104-2', negative value (−) means the allele came from the parent 'Eyou Changjia'

[d] Functional markers developed from genes in the P metabolic pathway in *Arabidopsis* that are located in the confidence interval

[e] Gene name in *Arabidopsis*

respectively. *qLP-DW-C3b* was identified in all three experiments, and accounted for 13.3% in Exp. 1, 9.9% in Exp. 2 and 10.1% in Exp. 3 of the phenotypic variation, respectively. Two consensus QTL for RL were identified in Exp. 3. *qLP-RL-C2a* explained 12.4% of the phenotypic variation with a positive additive effect, whereas *qLP-RL-C3a* contributed 11.3% of the variation with a negative effect. A total of four consensus QTL for RSA were identified. Three of them, *qLP-RSA-A1a*, *qLP-RSA-A3a* and *qLP-RSA-C3a*, were found in Exps. 2 and 3, each explaining 12.9, 16.1 and 10.1% of the phenotypic variation in Exp. 2, and 10.7, 12.6 and 20.3% in Exp. 3, respectively. An additional QTL, *qLP-RSA-C3b*, was identified only in Exp. 3 and contributed 9.4% of the phenotypic variation. Four consensus QTL for RVol were identified on A1, A3 and C3. *qLP-RVol-A1a* and *qLP-RVol-C3a* were identified in Exps. 2 and 3, each explaining 8.0 and 9.6% of the phenotypic variation in Exp. 2, and 10.9% and 14.0% in Exp. 3, respectively. *qLP-RVol-A3a* was identified only in Exp. 2 and contributed 12.2% of the phenotypic variation. *qLP-RVol-C3b* was detected in all three experiments, and explained 17.0% in Exp. 1, 8.1% in Exp. 2 and 12.5% in Exp. 3 of the phenotypic variation, respectively. Three consensus QTL for PU were detected in Exp. 2 and Exp. 3. *qLP-PU-A3a* was detected in Exp. 2 and accounted for 9.5% of the phenotypic variation. *qLP-PU-C3a* explained 14.6% of the phenotypic variation in Exp. 2 and 18.9% in Exp. 3. *qLP-PU-C3b* was detected in Exp. 3 and accounted for 11.3% of the variation.

Ten consensus QTL were detected under HP condition. Two consensus QTL for plant DW were identified on A3 and C2, respectively. *qHP-DW-A3a* explained 11.0% of the phenotypic variation in Exp. 2 and 10.6% in Exp. 3. *qHP-DW-C2a* accounted for 12.7% of the phenotypic variation in Exp. 1 and 10.5% in Exp. 3. Two consensus QTL for RL, *qHP-RL-A3a* and *qHP-RL-C2a*, were detected in Exp. 2 and Exp. 3, respectively. Two consensus QTL for RSA, *qHP-RSA-A3a* and *qHP-RSA-A3b*, were detected in Exps. 2 and 3. The consensus QTL for RVol, *qHP-RVol-A3a*, was identified in all three experiments. Three consensus QTL were identified for PU. *qHP-PU-A3a* and *qHP-PU-A3b* were detected only in Exp. 2, whereas *qHP-PU-C2a* was detected in all three experiments.

Analysis of unique QTL identified under two P conditions

Most of the consensus QTL for each trait overlapped with those for other traits. Under LP condition, the consensus QTL for the different traits were clustered on A1, A3 and C3. For example, consensus QTL were detected for all five traits in the 6.9–15.4 cM region of C3. Similarly, the consensus QTL identified under HP condition clustered on A3 and C2 (Table 3; Fig. 3). Therefore, the consensus QTL were subjected to a second round of QTL meta-analysis and were integrated into eight unique QTL (Fig. 3). Among these eight QTL, four and two unique QTL were specific for LP and HP conditions, respectively. The remaining two unique QTL were detected under both P conditions. Thus, the eight unique QTL could be classified into three categories, namely, LP-specific, HP-specific, and constitutive QTL.

Fig. 3 Unique QTL integrated from consensus QTL on linkage groups A1, A3, C2 and C3 of *B. napus* by the second round of QTL meta-analysis. The *symbols* '┤' in *gray* to the left of the linkage groups represent the positions of consensus QTL for different traits, and those in *black* represent the positions of unique QTL. The length of the *vertical bar* in the '┤' *symbol* indicates the confidence interval of the QTL, and the position of the *horizontal bar* indicates the peak position of the QTL. The names of the QTL are labeled above the '┤' *symbol*. The *black arrows* to the right of the linkage groups represent the positions of functional markers (FM) developed from genes in the P metabolic pathway in *Arabidopsis*

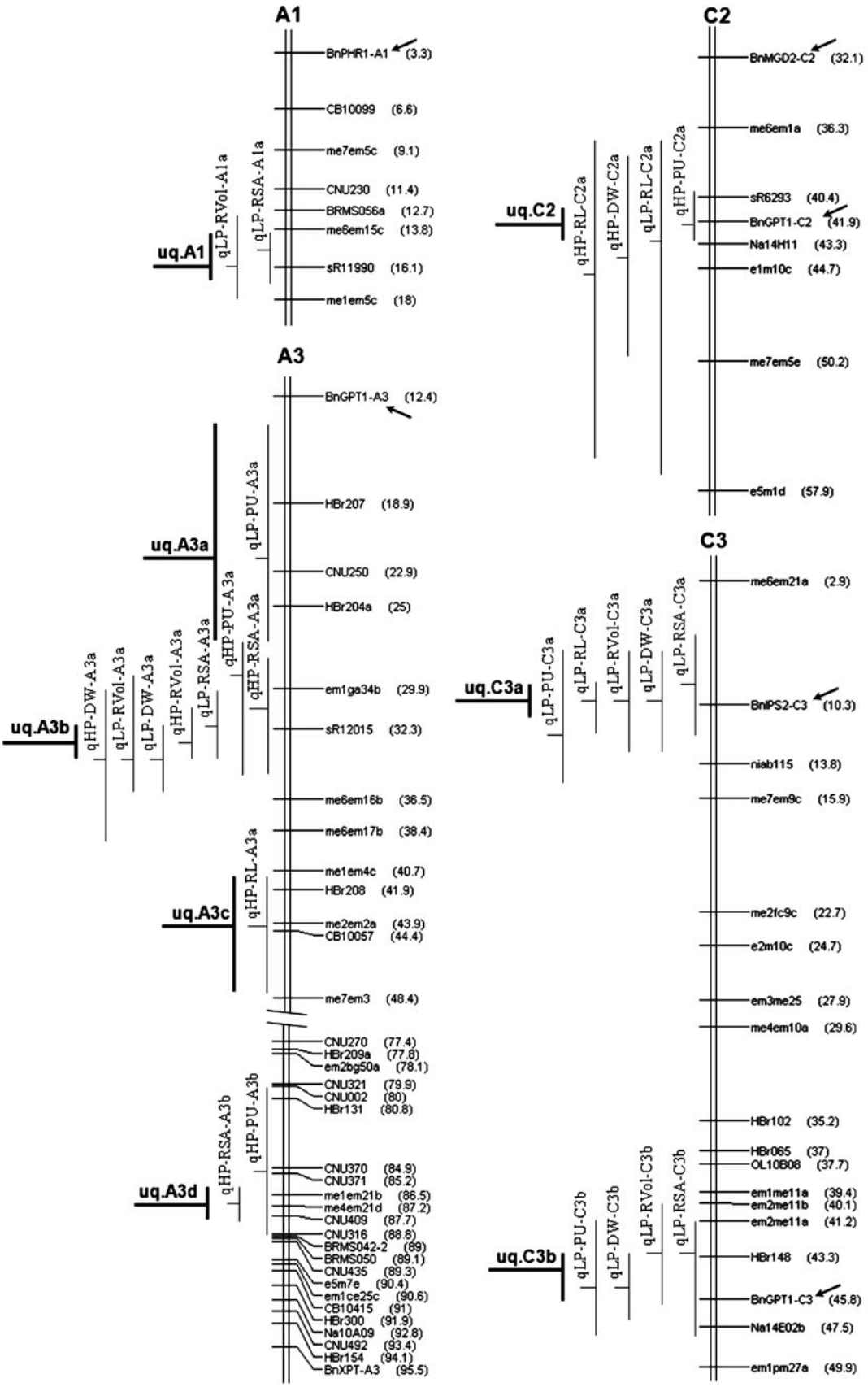
The four LP-specific unique QTL were *uq.A1*, *uq.A3a*, *uq.C3a* and *uq.C3b*. *uq.A1* was integrated from two consensus QTL, *qLP-RSA-A1a* and *qLP-RVol-A1a* that were identified under LP condition. *uq.A3a* corresponded to one consensus QTL *qLP-PU-A3a*, which could therefore be considered as a single unique QTL. *uq.C3a* and *uq.C3b* were detected for all five traits, and were located at intervals of 9.3–11.4 cM and 43.5–46.0 cM, respectively. *uq.C3a* was integrated from five consensus QTL, *qLP-DW-C3a*, *qLP-RL-C3a*, *qLP-RSA-C3a*, *qLP-RVol-C3a* and *qLP-PU-C3a*, whereas *uq.C3b* was integrated from four consensus QTL, *qLP-DW-C3b*, *qLP-RSA-C3b*, *qLP-RVol-C3b* and *qLP-PU-C3b*.

uq.A3c and *uq.A3d* were HP-specific unique QTL. *uq.A3c* was related to *qHP-RL-A3a* and was considered as a single unique QTL. *uq.A3d* was integrated from two consensus QTL, *qHP-RSA-A3b* and *qHP-PU-A3b*.

The remaining two unique QTL, *uq.A3b* and *uq.C2*, were constitutive QTL. *uq.A3b* was integrated from seven consensus QTL, *qLP-DW-A3a*, *qLP-RSA-A3a* and *qLP-RVol-A3a*, which were obtained under LP condition, and *qHP-DW-A3a*, *qHP-RSA-A3a*, *qHP-RVol-A3a*, *qHP-PU-A3a*, which were obtained under HP condition. *uq.C2* was integrated from one consensus QTL obtained under LP (*qLP-RL-C2a*) and three obtained under HP (*qHP-DW-C2a*, *qHP-RL-C2a*, and *qHP-PU-C2a*).

Functional markers and unique QTL

As shown in Fig. 3, six functional markers (FM) were distributed in or near the confidence intervals of unique QTL. Three of these markers, BnGPT1-C2, BnIPS2-C3 and BnGPT1-C3, were located in the confidence intervals of the unique QTL, *uq.C2*, *uq.C3a* and *uq.C3b*,


A1
BnPHR1-A1 (3.3)
CB10099 (6.6)
me7em5c (9.1)
CNU230 (11.4)
BRMS056a (12.7)
me6em15c (13.8)
sR11990 (16.1)
me1em5c (18)
uq.A1
qLP-RVol-A1a
qLP-RSA-A1a
A3
BnGPT1-A3 (12.4)
HBr207 (18.9)
CNU250 (22.9)
HBr204a (25)
em1ga34b (29.9)
sR12015 (32.3)
me6em16b (36.5)
me6em17b (38.4)
me1em4c (40.7)
HBr208 (41.9)
me2em2a (43.9)
CB10057 (44.4)
me7em3 (48.4)
CNU270 (77.4)
HBr209a (77.8)
em2bg50a (78.1)
CNU321 (79.9)
CNU002 (80)
HBr131 (80.8)
CNU370 (84.9)
CNU371 (85.2)
me1em21b (86.5)
me4em21d (87.2)
CNU409 (87.7)
CNU316 (88.8)
BRMS042-2 (89)
BRMS050 (89.1)
CNU435 (89.3)
e5m7e (90.4)
em1ce25c (90.6)
CB10415 (91)
HBr300 (91.9)
Na10A09 (92.8)
CNU492 (93.4)
HBr154 (94.1)
BnXPT-A3 (95.5)
uq.A3a
qLP-PU-A3a
qHP-PU-A3a
qHP-RSA-A3a
uq.A3b
qHP-DW-A3a
qLP-RVol-A3a
qLP-DW-A3a
qHP-RVol-A3a
qLP-RSA-A3a
uq.A3c
qHP-RL-A3a
uq.A3d
qHP-RSA-A3b
qHP-PU-A3b
C2
BnMGD2-C2 (32.1)
me6em1a (36.3)
sR6293 (40.4)
BnGPT1-C2 (41.9)
Na14H11 (43.3)
e1m10c (44.7)
me7em5e (50.2)
e5m1d (57.9)
uq.C2
qHP-RL-C2a
qHP-DW-C2a
qLP-RL-C2a
qHP-PU-C2a
C3
me6em21a (2.9)
BnlPS2-C3 (10.3)
niab115 (13.8)
me7em9c (15.9)
me2fc9c (22.7)
e2m10c (24.7)
em3me25 (27.9)
me4em10a (29.6)
HBr102 (35.2)
HBr065 (37)
OL10B08 (37.7)
em1me11a (39.4)
em2me11b (40.1)
em2me11a (41.2)
HBr148 (43.3)
BnGPT1-C3 (45.8)
Na14E02b (47.5)
em1pm27a (49.9)
uq.C3a
qLP-PU-C3a
qLP-RL-C3a
qLP-RVol-C3a
qLP-DW-C3a
qLP-RSA-C3a
uq.C3b
qLP-PU-C3b
qLP-DW-C3b
qLP-RVol-C3b
qLP-RSA-C3b

respectively. BnGPT1-C2 and BnGPT1-C3, which were developed from *AtGPT1*, were located in the region of the constitutive QTL *uq.C2* and the LP-specific QTL *uq.C3b*, respectively. BnIPS2-C3, which was developed from *AtIPS2*, mapped within the interval of the LP-specific QTL *uq.C3a*. BnPHR1-A1, which was developed from *AtPHR1*, was located near to the region of the LP-specific QTL *uq.A1*. BnGPT1-A3, which was developed from *AtGPT1*, mapped near to the interval of the LP-specific QTL *uq.A3a*. The final marker, BnMGD2-C2, which was developed from *AtMGD2*, mapped near to the region of the constitutive QTL *uq.C2*.

Discussion

Root system traits are very important for the adaptation of plant to P deficiency (Ismail et al. 2007; Jain et al. 2007; Lynch 2007). Given that root traits are influenced readily by the growth environment and are difficult to measure accurately, the selection of root traits as a phenotype for QTL analysis presents certain challenges (De Dorlodot et al. 2007). Many techniques that mimic field conditions have been developed to investigate root morphology and root architecture, for example, the gel chamber (Bengough et al. 2004; Reymond et al. 2006), the root-compartment chamber (Zhang et al. 2009), the stratified sand culture system, and the paper culture growth system (Hammond et al. 2009; Liao et al. 2001; Zhu et al. 2005a, b). In the present study, the root system, which was grown naturally on the surface of P-free blue paper, could be investigated visually and was not destroyed when harvested. This should have improved the accuracy of the data obtained, which was demonstrated by the highly significant correlation ($P < 0.001$) of the data for the same root traits from the three independent experiments (Supplementary Table S2). The lower correlation coefficients between Exp. 1 and Exp. 2 or Exp. 3 might have been due to the lower environmental temperature in the greenhouse when Exp. 1 was conducted (minimum temperature: 5–8°C in Exp. 1, 10–14°C in Exp. 2 and 8–12°C in Exp. 3). This finding also demonstrates the plasticity of the root traits.

Although plants grown on paper do differ from plants grown in soil, they can reflect the responses of plants in soil to a certain extent. Research on common bean has indicated that the effects of P on basal roots are consistent in paper, sand, and soil culture experiments (Liao et al. 2001). Previously, we showed that similar results were obtained in paper culture, pot culture and root-soil compartment chamber with respect to the root system and PU efficiency of P-inefficient and P-efficient lines from the RIL population, together with those of the two parents, the P-inefficient cultivar 'B104-2' and P-efficient cultivar 'Eyou Changjia'. The seed yield of RILs from 2-year field trials showed a highly significant correlation with RL ($R = 0.45$–0.56, $P < 0.001$, $n = 124$), RSA ($R = 0.60$–0.72, $P < 0.001$, $n = 124$), and RVol ($R = 0.52$–0.63, $P < 0.001$, $n = 124$) when grown on paper under LP condition (unpublished data). Furthermore, three root traits (RL, RSA and RVol) investigated in this study were mathematical interdependent, but were mathematical independent with PU. The following results from two parents, 'B104-2' and 'Eyou Changjia', and RILs proved that root traits with PU were physiologic correlated.

In general, plants develop three main mechanisms to adapt to LP stress: root interception of P, P acquisition efficiency, and internal P use efficiency (Ismail et al. 2007). Modifications in root architecture that are related to plant PU have been identified as an efficient adaptation to P deficiency (Lynch 2007). Therefore, we used root morphology and PU to assess tolerance to P deficiency in *B. napus*. The cultivar 'Eyou Changjia' absorbed more P and had a higher uptake efficiency than 'B104-2' (Table 1), but had a lower P use efficiency than 'B104-2' (data not shown). This suggested that the high P efficiency of 'Eyou Changjia' could be attributed mainly to uptake efficiency, which was consistent with our previous results on the two cultivars (Duan et al. 2009). The data obtained in the present study showed that 'Eyou Changjia' was superior to 'B104-2' in root traits under the condition of P deficiency (Table 1). This indicated that the high PU efficiency of 'Eyou Changjia' was related closely to the development of an extensive root system. Thus, 'Eyou Changjia' could acquire more P from an LP environment and produce more biomass than 'B104-2', which represented higher tolerance to LP stress. Similar findings from earlier studies have demonstrated that the P-efficient cultivars of *B. napus* intercept more available P and have a higher phosphatase activity and greater total RL than P-inefficient ones (Solaiman et al. 2007; Zhang et al. 2009).

QTL mapping using the data from multi-environment experiments enables the identification of reliable QTL. Sixty-two significant QTL were identified in the three experiments performed (Fig. 2; Supplementary Table S3). Twenty-six of these QTL were identified in only one experiment, which suggested that these QTL were influenced readily by environmental factors. This was consistent with the significant effect of environment on the phenotype of the RIL population (Table 2). The remaining 36 QTL were detected repeatedly in at least two experiments, which suggested that these QTL were reliable in the RIL population.

The majority of the significant QTL clustered within six regions of four linkage groups (Table 3; Fig. 3). Under LP condition, two QTL clusters, which included QTL for plant DW, root traits and PU, were identified on C3. These clusters were then integrated into two unique QTL, *uq.C3a*

and *uq.C3b*, respectively. Another QTL cluster for LP condition including QTL for RSA and RVol was located on A1 and was integrated into *uq.A1*. QTL for plant DW and root traits, which were identified under HP condition, coincided with QTL for PU on linkage groups A3 and C2, and were integrated into further two unique QTL, *uq.A3d* and *uq.C2*. These indicated that different mechanisms were involved in the response of *B. napus* to different P environments. In addition, *uq.A3b* was detected consistently for root traits and P content under both P conditions; hence it corresponded to a constitutively expressed QTL. Both *uq.C3a* and *uq.C3b* were specific QTL for root traits and PU under LP stress, which suggested that the two unique QTL corresponded to a single gene or gene clusters. These genes or gene clusters are likely to be involved in the metabolic pathway that is involved in the response of *B. napus* to P deficiency.

Arabidopsis has been exploited as a standard in comparative genomics analyses between *Arabidopsis* and Brassicaceae species. A set of 24 conserved chromosomal blocks (labeled A–X) has been identified among the ancestral karyotype, *A. thaliana* and *B. rapa* (Schranz et al. 2006). In the present study, we mapped the alignment between the *B. napus* linkage groups and *Arabidopsis* genome using the comparative mapping approach described by Long et al. (2007). One syntenic island (R) and one syntenic block (W) were identified between C3 of *B. napus* and chromosome 5 of *Arabidopsis*. *uq.C3a* and *uq.C3b* were located within the regions R and W, respectively (Fig. 4a). In *B. oleracea*, QTL for shoot DW, shoot P content and P use efficiency under LP condition have been identified in two regions of chromosome C3. The positions of these QTL aligned with blocks 5A, and 3C, 1B and 4B of *Arabidopsis* (Hammond et al. 2009). Comparison of the positions of *uq.C3a* with QTL on C3 in *B. oleracea* revealed that they were located within the same genomic block of *Arabidopsis.* We speculated the QTL on C3 of *B. napus* and *B. oleracea* co-localized. Furthermore, in silico mapping revealed that a number of orthologous genes for root development, auxin transport, and P metabolism in *Arabidopsis* were located in the confidence intervals of *uq.C3a* and *uq.C3b*; six genes corresponded to *uq.C3a* and eight to *uq.C3b* (Fig. 4b). Near-isogenic lines should be developed to allow further fine-mapping of these QTL and the cloning of potential candidate genes.

Two functional markers, BnIPS2-C3 and BnGPT1-C3, were located in the confidence intervals of two unique QTL (Table 3; Fig. 3). BnIPS2-C3, which was developed from *AtIPS2*, was located in the interval of the LP-specific QTL *uq.C3a*. *AtIPS2* is up-regulated in roots in response to LP stress in *Arabidopsis* (Shin et al. 2006), and *OsIPS2* in rice exhibits a similar pattern of expression (Hou et al. 2005). Therefore, *BnIPS2* was a possible candidate gene for *uq.C3a* and might be involved in the P-deficiency-response pathway in *B. napus*. BnGPT1-C3, which was developed from *AtGPT1*, was located in the interval of the LP-specific QTL *uq.C3b*. *AtGPT1* belongs to the P translocator family and apparently functions as an importer of glucose 6-phosphate (Niewiadomski et al. 2005). BnGPT1-C2 was also developed from *AtGPT1* but was located in the interval of the constitutive unique QTL *uq.C2*. The results of in silico mapping demonstrated that *AtGPT1* was located in the intervals of *uq.C2* and *uq.C3b*. Therefore, *AtGPT1* might be one of the candidate genes that underlie *uq.C2* and *uq.C3b* (Fig. 4). From the information about the possible candidate genes, we assumed that the gene(s) that underlie *uq.C3a* might promote lateral root development under P starvation, resulting in improved PU, whereas the

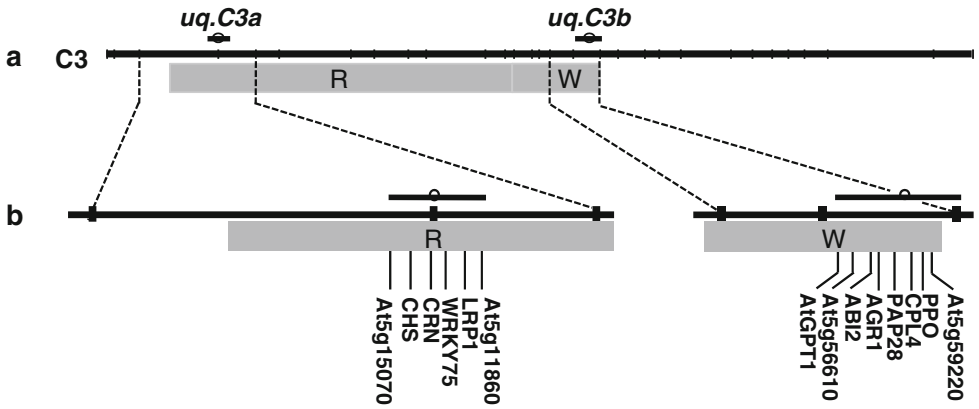


Fig. 4 The alignment between the linkage group C3 of *B. napus* and chromosome 5 of *Arabidopsis*. **a** The distribution of two unique QTL, *uq.C3a* and *uq.C3b*, on C3 and the alignment between C3 and *Arabidopsis* genome. C3 is shown as a *black thick line* with *vertical lines* to indicate the positions of the molecular markers. The unique QTL, *uq.C3a* and *uq.C3b*, are drawn with *horizontal bars* where the length indicates the confidence interval and the circle indicates the peak position. The two blocks with "*R*" and "*W*" represent the two genomic regions of *Arabidopsis* that aligned to C3 of *B. napus* by comparative mapping approach. **b** Genes for root development, auxin transport, and P metabolism in *Arabidopsis* located in the confidence intervals of *uq.C3a* and *uq.C3b* by in silico mapping approach

gene(s) that underlie *uq.C3b* might be responsible for P translocation from roots to shoots and P utilization in shoots under LP stress. This information could assist in identifying and cloning of the genes that underlie *uq.C3a* and *uq.C3b*, and that contribute to adaptability to P deficiency.

MAS is an efficient system for the selection of traits of interest than traditional phenotypic-based selection techniques. It relies on the availability of markers that are linked closely for traits or target QTL. For example, molecular markers that are linked tightly with QTL, *Sub1*, for submergence tolerance and with QTL, *Pup1*, for PU were developed in rice, and could be used to breed cultivars with high tolerance to submergence and P deficiency, respectively, by MAS (Neeraja et al. 2007; Heuer et al. 2009; Wissuwa et al. 2002). These previous studies have demonstrated that MAS can be used effectively in a molecular breeding program for abiotic stress. Therefore, another major goal of this study was to develop markers linked closely to target traits or QTL for MAS. In the study, two LP-specific unique QTL, *uq.C3a* and *uq.C3b*, were detected, which accounted for 9.4–16.8% of the phenotypic variation for plant DW, PU, RSA and RVol (Table 3). In addition, QTL for seed yield co-localized with *uq.C3b* under P deficiency (unpublished data). Therefore, *uq.C3a* and *uq.C3b* were represented important targets for MAS. Two FM, BnIPS2-C3 and BnGPT1-C3, which were located in the confidence intervals of these two QTL were linked significantly by single-marker analysis with plant DW, PU, RSA and RVol ($P < 0.005$) under LP conditions in at least two experiments (data not shown). The RI lines with favorable alleles related to BnIPS2 and BnGPT1 could develop larger RSA, produce higher DW, and acquire more P under LP condition, and yield more seed in the field trials (Supplementary Table S4). These suggested that two functional markers may be potential markers for MAS to breed P-efficient rapeseed cultivars.

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References

Akhtar MS, Oki Y, Adachi T (2008) Intraspecific variations of phosphorus absorption and remobilization, P forms, and their internal buffering in *Brassica* cultivars exposed to a P-stressed environment. J Integr Plant Biol 50:703–716

Arcade A, Labourdette A, Falque M, Mangin B, Chardon F, Charcosset A, Joets J (2004) BioMercator: integrating genetic maps and QTL towards discovery of candidate genes. Bioinformatics 20:2324–2326

Batjes NH (1997) A world dataset of derived soil properties by FAO-UNESCO soil unit for global modelling. Soil Use Manage 13:9–16

Beebe SE, Rojas-Pierce M, Yan XL, Blair MW, Pedraza F, Munoz FM, Tohme J, Lynch JP (2006) Quantitative trait loci for root architecture traits correlated with phosphorus acquisition in common bean. Crop Sci 46:413-423

Bengough AG, Gordon DC, Al-Menaie H, Ellis RP, Allan D, Keith R, Thomas WTB, Forster BP (2004) Gel observation chamber for rapid screening of root traits in cereal seedlings. Plant Soil 262:63–70

Chardon F, Virlon B, Moreau L, Falque M, Joets J, Decousset L, Murigneux A, Charcosset A (2004) Genetic architecture of flowering time in maize as inferred from quantitative trait loci meta-analysis and synteny conservation with the rice genome. Genetics 168:2169–2185

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

De Dorlodot S, Forster B, Pagès L, Price A, Tuberosa R, Draye X (2007) Root system architecture: opportunities and constraints for genetic improvement of crops. Trends Plant Sci 12:474–481

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Duan HY, Shi L, Ye XS, Wang YH, Xu FS (2009) Identification of phosphorous efficient germplasm in oilseed rape. J Plant Nutr 32:1148–1163

Fageria NK, Baligar VC, Li YC (2008) The role of nutrient efficient plants in improving crop yields in the twenty-first century. J Plant Nutr 31:1121–1157

Hammond JP, White PJ (2008) Sucrose transport in the phloem: integrating root responses to phosphorus starvation. J Exp Bot 59:93–109

Hammond JP, Broadley MR, White PJ, King GJ, Bowen HC, Hayden R, Meacham MC, Mead A, Overs T, Spracklen WP, Greenwood DJ (2009) Shoot yield drives phosphorus use efficiency in *Brassica oleracea* and correlates with root architecture traits. J Exp Bot 60:1953–1968

Hermans C, Hammond JP, White PJ, Verbruggen N (2006) How do plants respond to nutrient shortage by biomass allocation? Trends Plant Sci 11:610–617

Heuer S, Lu XC, Chin JH, Tanaka JP, Kanamori H, Matsumoto T, De Leon T, Ulat VJ, Ismail AM, Yano M, Wissuwa M (2009) Comparative sequence analyses of the major quantitative trait locus *phosphorus uptake 1* (*Pup1*) reveal a complex genetic structure. Plant Biotechnol J 7:456–471

Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. California Department of Agriculture experimental station circular 347, Berkeley

Holford ICR (1997) Soil phosphorus: its measurement, and its uptake by plants. Aust J Soil Res 35:227–239

Hou XL, Wu P, Jiao FC, Jia QJ, Chen HM, Yu J, Song XW, Yi KK (2005) Regulation of the expression of *OsIPS1* and *OsIPS2* in rice via systemic and local Pi signalling and hormones. Plant Cell Environ 28:353–364

Ismail AM, Heuer S, Thomson MJ, Wissuwa M (2007) Genetic and genomic approaches to develop rice germplasm for problem soils. Plant Mol Bio 65:547–570

Jain A, Vasconcelos MJ, Raghothama KG, Sahi SV (2007) Molecular mechanisms of plant adaptation to phosphate deficiency. In: Janick J (ed) Plant breeding reviews, vol 29. Wiley, NJ, pp 359–419

Li G, Quiros CF (2001) Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction:

its application to mapping and gene tagging in *Brassica*. Theor Appl Genet 103:455–461

Liao H, Rubio G, Yan XL, Cao A, Brown KM, Lynch JP (2001) Effect of phosphorus availability on basal root shallowness in common bean. Plant Soil 232:69–79

Long Y, Shi JQ, Qiu D, Li RY, Zhang CY, Wang J, Hou JN, Zhao JW, Shi L, Park BS, Choi SR, Lim YP, Meng JL (2007) Flowering time quantitative trait Loci analysis of oilseed Brassica in multiple environments and genomewide alignment with Arabidopsis. Genetics 177:2433–2444

Lowe AJ, Moule C, Trick M, Edwards KJ (2004) Efficient large-scale development of microsatellites for marker and mapping applications in *Brassica* crop species. Theor Appl Genet 108: 1103–1112

Lynch JP (2007) Roots of the second green revolution. Aust J Bot 55:493–512

Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic Press, London

Neeraja C, Maghirang-Rodriguez R, Pamplona A, Heuer S, Collard B, Septiningsih E, Vergara G, Sanchez D, Xu K, Ismail A, Mackill D (2007) A marker-assisted backcross approach for developing submergence-tolerant rice cultivars. Theor Appl Genet 115:767–776

Niewiadomski P, Knappe S, Geimer S, Fischer K, Schulz B, Unte US, Rosso MG, Ache P, Flugge UI, Schneider A (2005) The Arabidopsis plastidic glucose 6-phosphate/phosphate translocator GPT1 is essential for pollen maturation and embryo sac development. Plant Cell 17:760–775

Piquemal J, Cinquin E, Couton F, Rondeau C, Seignoret E, doucet I, Perret D, Villeger MJ, Vincourt P, Blanchard P (2005) Construction of an oilseed rape (*Brassica napus* L.) genetic map with SSR markers. Theor Appl Genet 111:1514–1523

Qiu D, Morgan C, Shi JQ, Long Y, Liu J, Li RY, Zhuang XF, Wang YH, Tan XL, Dietrich E, Weihmann T, Everett C, Vanstraelen S, Beckett P, Fraser F, Trick M, Barnes S, Wilmer J, Schmidt R, Li JY, Li DR, Meng JL, Bancroft I (2006) A comparative linkage map of oilseed rape and its use for QTL analysis of seed oil and erucic acid content. Theor Appl Genet 114:67–80

Reymond M, Svistoonoff S, Loudet O, Nyssaume L, Desnos T (2006) Identification of QTL controlling root growth response to phosphate starvation in *Arabidopsis thaliana*. Plant Cell Environ 29:115–125

Schranz ME, Lysak MA, Mitchell-Olds T (2006) The ABC's of comparative genomics in the Brassicaceae: building blocks of crucifer genomes. Trends Plant Sci 11:535–542

Shi JQ, Li RY, Qiu D, Jiang CC, Long Y, Morgan C, Bancroft I, Meng JL (2009) Unraveling the complex trait of crop yield with QTL mapping in *Brassica napus*. Genetics 182:851–861

Shimizu A, Yanagihara S, Kawasaki S, Ikehashi H (2004) Phosphorus deficiency-induced root elongation and its QTL in rice (*Oryza sativa* L.). Theor Appl Genet 109:1361–1368

Shimizu A, Kato K, Komatsu A, Motomura K, Ikehashi H (2008) Genetic analysis of root elongation induced by phosphorus deficiency in rice (*Oryza sativa* L.): fine QTL mapping and multivariate analysis of related traits. Theor Appl Genet 117:987–996

Shin H, Shin HS, Chen RJ, Harrison MJ (2006) Loss of *At4* function impacts phosphate distribution between the roots and the shoots during phosphate starvation. Plant J 45:712–726

Solaiman Z, Marschner P, Wang D, Rengel Z (2007) Growth, P uptake and rhizosphere properties of wheat and canola genotypes in an alkaline soil with low P availability. Biol Fert Soils 44:143–153

Suwabe K, Iketani H, Nunome T, Kage T, Hirai M (2002) Isolation and characterization of microsatellites in *Brassica rapa* L. Theor Appl Genet 104:1092–1098

Svistoonoff S, Creff A, Reymond M, Sigoillot-Claude C, Ricaud L, Blanchet A, Nussaume L, Desnos T (2007) Root tip contact with low-phosphate media reprograms plant root architecture. Nat Genet 39:792–796

Van Ooijen JW (2006) JoinMap®4.0: software for the calculation of genetic linkage maps in experimental populations. Kyazma BV, Wageningen, Netherlands

Vance CP, Uhde-Stone C, Allan DL (2003) Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytol 157:423–447

Vos P, Hogers R, Bleeker M, Reijans M, Vandelee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA-fingerprinting. Nucleic Acids Res 23:4407–4414

Wang SC, Bastern J, Zeng ZB (2006) Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC, USA

Wissuwa M, Wegner J, Ae N, Yano M (2002) Substitution mapping of *Pup1*: a major QTL increasing phosphorus uptake of rice from a phosphorus-deficient soil. Theor Appl Genet 105:890–897

Yan XL, Liao H, Beebe SE, Blair MW, Lynch JP (2004) QTL mapping of root hair and acid exudation traits and their relationship to phosphorus uptake in common bean. Plant Soil 265:17–29

Yan XL, Wu P, Ling HQ, Xu GH, Xu FS, Zhang QF (2006) Plant nutriomics in China: An overview. Ann Bot 98:473–482

Zeng ZB (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468

Zhang HW, Huang Y, Ye XS, Shi L, Xu FS (2009) Genotypic differences in phosphorus acquisition and the rhizosphere properties of *Brassica napus* in response to low phosphorus stress. Plant Soil 320:91–102

Zhao JJ, Jamar DC, Lou P, Wang YH, Wu J, Wang XW, Bonnema G, Koornneef M, Vreugdenhil D (2008) Quantitative trait loci analysis of phytate and phosphate concentrations in seeds and leaves of *Brassica rapa*. Plant Cell Environ 31:887–900

Zhu JM, Kaeppler SM, Lynch JP (2005a) Mapping of QTL controlling root hair length in maize (*Zea mays* L.) under phosphorus deficiency. Plant Soil 270:299–310

Zhu JM, Kaeppler SM, Lynch JP (2005b) Mapping of QTLs for lateral root branching and length in maize (*Zea mays* L.) under differential phosphorus supply. Theor Appl Genet 111:688–695