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Quantitative trait loci affecting seed mineral concentrations in *Brassica napus* grown with contrasting phosphorus supplies

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• *Background and Aims* Phosphorus (P) deficiency is one of the major limitations for crop production. A significant relationship exists between plant P uptake from soils and the accumulation of P and other mineral elements in seeds. The aims of this study were to identify and characterize genetic loci (QTLs) controlling the accumulation of mineral elements in seeds of *Brassica napus* grown with contrasting P availabilities.
• *Methods* A population of 124 recombinant inbred lines derived from a cross between P-inefficient 'B104-2' and P-efficient 'Eyou Changjia' was used for phenotypic investigation and QTL analysis. Two-year field trials were conducted with two P treatments. Concentrations of mineral elements (P, Ca, Mg, Fe, Zn, Cu and Mn) in seeds were determined and QTLs were identified by composite interval mapping.
• *Key Results* There was significant genetic variation in seed concentrations of all mineral elements, and correlations between most elements were observed. A total of 78 putative QTLs (45 under the normal-P conditions and 33 under the low-P conditions) were detected, covering 17 linkage groups and accounting for 7·84–18·1 % of the phenotypic variation. Sixteen of these QTLs were identified in at least two environments, and co-location of QTLs for different mineral traits was found on several linkage groups. By *in silico* mapping, 21 genes involved in ion homeostasis in *Arabidopsis* were mapped to the QTL intervals identified in *B. napus*.
• *Conclusions* The accumulation of mineral elements in seeds is controlled by multiple genes. Common physiological and molecular mechanisms could be involved in the accumulation of several mineral elements, and genes involved in these processes in *B. napus* are suggested. These results offer insights to the genetic basis of seed mineral accumulation across different P levels in *B. napus*.

Key words: *Brassica napus*, phosphorus deficiency, seed mineral concentration, quantitative trait locus, *in silico* mapping, recombinant inbred line.

INTRODUCTION

Mineral elements such as phosphorus (P), calcium (Ca), iron (Fe), magnesium (Mg), manganese (Mn) and zinc (Zn) perform a variety of functions in plant cells, and are essential for growth and development in plants as well as in animals and humans. The acquisition of mineral elements by higher plants is primarily from the soil, while humans obtain all essential elements mostly from higher plants (Grusak and Cakmak, 2005; White and Broadley, 2009). Unfortunately, it is estimated that over three billion people are currently malnourished because of lack of minerals, especially iron and zinc, in their diet (Welch and Graham, 2004; White and Broadley, 2009). Biofortification, which aims to increase mineral concentrations in edible crops, either agronomically or genetically using both conventional breeding and modern biotechnology, has been adopted by plant scientists to address this problem, and is considered to be the most promising and cost-effective approach to alleviate mineral malnutrition (White and Broadley, 2005, 2009). However, the accumulation of minerals in seeds is a complex phenomenon, which is most likely controlled by a number of genes. The movement of mineral elements from soils to seeds involves their mobilization from soils, uptake by roots, translocation to the shoot, redistribution within the plant and deposition in seeds (Grusak and DellaPenna, 1999; White and Broadley, 2009). The genetic basis of many of these processes is unknown.

P is an essential macronutrient for plants (Marschner, 1995). Due to its high adsorption capacity in soils (e.g. adsorbed by Al- and Fe-oxides in acid soils), mineralization (e.g. to calcium phosphates such as apatite) and formation of organic P complexes, P is one of the least available plant nutrients in the rhizosphere. Indeed, P deficiency is considered to be one of the major limitations for crop production worldwide and is probably the most widely studied macronutrient deficiency (Schachtman *et al.*, 1998; Raghothama and Karthikeyan, 2005). Rapeseed (*Brassica napus*), which has high P requirement for its optimal seed yield and quality, is commonly used in animal feed or as an oil source for humans. Lack of available P in soils seriously limits the production of *B. napus* and genetic approaches to improve its P-use efficiency is considered as an efficient and promising way to maintain or increase yields whilst reducing P inputs.

Quantitative trait locus (QTL) analysis is a powerful genetic approach to dissect complex traits and to investigate natural genetic variation within a species (Paran and Zamir, 2003). In recent years, different genetic mapping populations have been developed to identify QTLs that contribute to mineral

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traits in various species. For example, in the model plant *Arabidopsis thaliana*, QTLs for phosphate accumulation in seed and leaf (Bentsink *et al.*, 2003), shoot Ca concentration (White, 2005), shoot K concentration (Harada and Leigh, 2006), seed sulfate concentration (Loudet *et al.*, 2007) and the accumulation of other mineral elements in shoots and seed (Payne *et al.*, 2004; Vreugdenhil *et al.*, 2004; Waters and Grusak, 2008*a*; Ghandilyan *et al.*, 2009*a*, *b*) have been identified. Similarly, QTLs for the accumulation of mineral elements in seeds of other plant species such as rice (Garcia-Oliveira *et al.*, 2009), wheat (Peleg *et al.*, 2009) and *Medicago truncatula* (Sankaran *et al.*, 2009) have been identified. Recently, QTLs have been identified in crop Brassicaceae that influence seed and leaf P (Zhao *et al.*, 2008), shoot Ca and Mg (Broadley *et al.*, 2008), shoot P (Hammond *et al.*, 2009), shoot Na, Mg, Mn, Fe, Al, Sr, Zn, P (Wu *et al.*, 2008), shoot B, Ca, Fe, Cu, Mg, P, Zn (Liu *et al.*, 2009) and shoot K (White *et al.*, 2010).

Arabidopsis thaliana is an excellent model system with a small and relatively simple genome. The existence of a common ancestor for *Brassica* and *Arabidopsis* enables comparative genome mapping techniques. Recent research has indicated 24 conserved chromosomal blocks in *A. thaliana* and other Brassicaceae (Schranz *et al.*, 2006). By *in silico* mapping between *A. thaliana* and *B. napus*, several genes involved in ion uptake and transport have been mapped to the QTL intervals in *B. napus* affecting shoot mineral concentrations (Liu *et al.*, 2009). Such information can provide clues for researchers to identify genes related to mineral accumulation and to finally uncover genetic networks that control plant ion homeostasis. It is well known that the major storage form for P in seeds of plants is *myo*-inositol-1,2,3,4,5,6-hexakisphosphate (IP6, or phytic acid) which often chelates metal ions, such as Ca, Mn, Zn and Fe (Raboy, 1997). However, little is known about the relationship between the accumulation of P and other mineral elements, such as Ca, Mg, Fe, Mn, Cu and Zn, in seeds of *B. napus*.

To indentify genetic loci that control the accumulation of mineral elements in seeds of *B. napus*, 2-year field trials were conducted with two P treatments using an F_{10} recombinant inbred line (RIL) population. By employing WinQTL Cartographer 2·5, a total of 78 putative QTLs were detected on 17 linkage groups. Furthermore, 21 functional genes were mapped to the interval of the QTLs affecting seed mineral concentrations by *in silico* comparative genome mapping techniques. These results improve our genetic understanding of the accumulation of mineral elements in seeds and plant responses to P availability.

MATERIALS AND METHODS

Plant materials and genetic linkage map

A set of 124 F_{10} lines of a *Brassica napus* L. RIL population was developed by single-seed descent from a cross between cv. 'B104-2' (B) and cv. 'Eyou Changjia' (E). Genotype 'Eyou Changjia' is a P-efficient cultivar, which shows a higher P efficiency coefficient (defined as the quotient of shoot dry weight at the seedling stage, or seed yield at maturity, under low P availability and that under high P availability) than 'B104-2', a P-inefficient cultivar (Duan *et al.*, 2009). The RIL population was previously genotyped with 553 molecular markers by Yang *et al.* (2010). Markers included 202 simple sequence repeat (SSR), 62 amplified fragment length polymorphism (AFLP), 234 sequence-related amplified polymorphism (SRAP) and 55 specific functional markers. Primer sequences for the SSR markers were obtained from various public sources (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; France, Piquemal *et al.*, 2005, prefixed by BRAS, CB and MR; private communications prefixed by CNU, niab and HBr). The AFLP procedure was performed according to the protocol developed by Vos *et al.* (1995), and the SRAP according to Li and Quiros (2001). The specific functional markers were developed in the laboratory according to the method described by Li *et al.* (2006), and homologous sequences in *B. napus* of *Arabidopsis thaliana* functional genes related to P homeostasis were used for primer design. In general, the genetic map covered a total length of 1592·7 cM with an average distance of 2·88 cM between adjacent markers. The genetic distance of the 19 linkage groups varied from 13·4 to 148·2 cM. The order of SSR markers in each linkage group was in agreement with previous reports (Piquemal *et al.*, 2005; Long *et al.*, 2007). Alignment between the *B. napus* linkage groups and the *A. thaliana* genome was conducted using the comparative mapping approach described by Long *et al.* (2007). Twelve syntenic blocks and 17 insertion islands were identified between the *A. thaliana* genome and the *B. napus* linkage groups (M. Yang *et al.*, unpubl. res.).

Field trial

The phenotypes of the RIL population were tested by 2-year field trials in a paddy soil with 12·5 mg kg^{-1} of Olsen-P located in Daye, Hubei Province, China. The field trials were conducted during 2006–2007 and 2007–2008, respectively, with two P treatments of 30 kg P_2O_5 ha^{-1} (low-P) and 90 kg P_2O_5 ha^{-1} (normal-P). Each treatment was performed in triplicate. Each replicate consisted of 124 lines as well as two parents, and each genotype was planted in two rows with nine plants per row. The application amount of N, K and B fertilizers was calculated according to the following nutrient rates: 180 kg N ha^{-1}, 83 kg K ha^{-1} and 15 kg H_3BO_3 ha^{-1}.

Mineral concentration analysis in seeds

After harvesting in May (2007 and 2008), all seed samples were weighed after drying at 60 °C to a constant mass. About 500 mg of each sample was weighed and then transferred to a digestion vessel. To each sample, 8 mL concentrated nitric acid and 2 mL of perchloric acid were added. This mixture was kept over night and then digested at 140 °C for 2 h and 220 °C for 4 h. After cooling, digested samples were diluted to 50 mL using deionized water and finally transferred to 50-mL polyethylene flasks. Concentrations of P, Ca, Mg, Cu, Fe, Mn and Zn were determined by inductively coupled

plasma-optical emission spectroscopy (VISTA-MPX; Varian, Inc., USA).

Statistical analysis

Seed mineral concentrations were calculated for all lines, and statistical analysis for all traits was conducted using the 'PROC GLM' procedure of SAS 8·1 (SAS Institute, Cary, NC, USA). Histograms and normality tests (Pearson chi-square test) were used to describe the variation of the phenotypic traits, and Pearson's correlation analysis was performed to examine their phenotypic association. The broad-sense heritability estimate (h^2) was calculated for each trait across different P environments according to the approach described by Shi *et al.* (2009).

QTL analysis and association with functional genes

QTL analysis was performed with WinQTL Cartographer 2·5 (Wang *et al.*, 2006). The composite interval mapping (CIM) model was used to scan the genetic map and estimate the likelihood of a QTL and its corresponding effect at every 2 cM. The genome wide LOD score threshold for QTL detection was determined for each trait by performing a 1000-permutation test at a significance level of $P = 0{\cdot}05$ (Churchill and Doerge, 1994). The estimated additive effect and the percentage of phenotypic variation explained by each putative QTL were obtained by the software in the CIM model. Then QTLs for the same trait, which were detected in both years and mapped to the same region of a chromosome, were integrated into one QTL by QTL meta-analysis following the method reported by Shi *et al.* (2009). All QTLs identified were named using italic trait abbreviation, followed by 'NP' or 'LP' (abbreviation of normal-P or low-P, indicating the QTL was detected under normal-P or low-P conditions), and then suffixed with the corresponding linkage group number (A1–A10 or C1–C9) and the serial letter (a, b, c . . .) in the same linkage group. For example, *CaNP-A7a* indicates a QTL for Ca concentration detected in the NP treatment at the first locus of chromosome A7. After comparative mapping between the *B. napus* linkage groups and the *A. thaliana* genome, genes affecting mineral concentrations in each syntenic block of *A. thaliana* were then aligned with the linkage map according to the position of close anchor marker(s) in the same syntenic block. If the position of an aligned gene(s) was located in the confidence interval of a QTL, the orthologous gene(s) was considered to be associated with the target QTL.

RESULTS

Phenotypic diversity for seed mineral concentrations

The mean values, ranges and heritability estimates of seven seed mineral concentrations of the two parental lines and the 124-line RIL population under four environmental conditions are presented in Table 1. Analysis of variance (ANOVA) showed significant ($P < 0{\cdot}05$) genetic variation for all the minerals analysed as well as for environmental effects (data not shown). All the mineral traits showed large variation among RILs at both P

TABLE 1. *Mean values, ranges and heritability* (h^2) *estimates of seven seed mineral concentrations* ($\mu g\ g^{-1}$) *in 124 recombinant inbred lines and two parental lines of* Brassica napus *under four P conditions*

	Normal phosphorus in 2007				Low phosphorus in 2007				Normal phosphorus in 2008				Low phosphorus in 2008				
	RILs				RILs				RILs				RILs				
Trait	Mean	Range	B	E	Mean	Range	B	E	Mean	Range	B	E	Mean	Range	B	E	h^2
P	9037	5479–12397	6723	7059	4772	3649–7014	4557	4900	8171	6349–10341	6576	7785	5389	3508–8283	4736	5900	0·70
Ca	2847	2302–3431	2697	2791	2622	1986–3705	1970	2201	3135	2328–3995	3015	3398	2698	1610–3991	2625	2859	0·56
Mg	3736	2853–4749	3743	2589	3143	1932–4830	3497	3770	3749	3053–4480	3525	3707	2918	2011–3754	2845	2936	0·29
Zn	50·96	40·35–70·71	64·19	57·75	39·01	22·59–55·63	54·64	55·01	57·71	40·00–73·57	58·15	53·28	43·14	25·89–61·36	55·69	58·15	0·41
Cu	5·03	3·68–6·85	4·03	5·08	4·77	3·54–6·48	4·87	5·28	4·91	3·14–7·31	4·97	5·10	4·52	3·34–6·41	4·41	5·21	0·34
Fe	56·49	41·21–74·23	47·76	60·29	40·94	21·53–74·23	48·56	61·72	58·26	42·86–78·92	59·39	57·50	50·12	31·92–71·12	47·23	55·90	0·32
Mn	77·68	55·56–103·66	63·73	74·57	59·94	41·79–85·84	54·65	73·36	77·07	55·11–97·45	70·34	74·03	63·96	47·75–86·40	56·25	64·91	0·46

B and E denote the parental lines 'B104-2' and 'Eyou Changjia', respectively.

supplies, but wider ranges were observed under low-P conditions than that under normal-P conditions, except for P in 2007 and Cu in both years. Among the elements studied, P was the most abundant element ranging from 3508 μg g^{-1} to 12397 μg g^{-1} (Table 1). Among the micro-nutrients, Mn was most abundant ranging from 41·79 μg g^{-1} to 103·66 μg g^{-1}. For the two parental lines, higher concentrations were observed for all mineral elements except Cu under normal-P conditions than under low-P conditions. The P-efficient genotype 'Eyou Changjia' accumulated more P in seeds in low-P environments than the P-inefficient genotype 'B104-2'. Seed concentrations of nearly all minerals assayed in the RIL population fell into relatively normal distributions under both normal-P (Fig. 1) and low-P supply (Fig. 2). Considerable transgressive segregations for seed mineral concentrations were generally observed in both directions, suggesting multiple genes act on these traits. However, there were some exceptions, such as seed P concentrations in plants with normal-P supply and seed Ca concentrations in plants with low-P supply, for which the transgression was solely towards higher concentrations. To estimate the proportion of phenotypic variation in the population that can be attributed to genetic variation, the h^2 values for all traits were calculated. These varied from 0·29 for Mg to 0·70 for P (Table 1).

Correlation analysis showed that positive correlations existed among mineral traits (Table 2). Macro-nutrients (P, Ca and Mg) showed relatively high and significant correlations with each other in all environments with the highest value (0·86) between P and Mg in 2007. Among the micro-nutrients (Zn, Cu, Fe and Mn), the correlations between Zn and Cu were

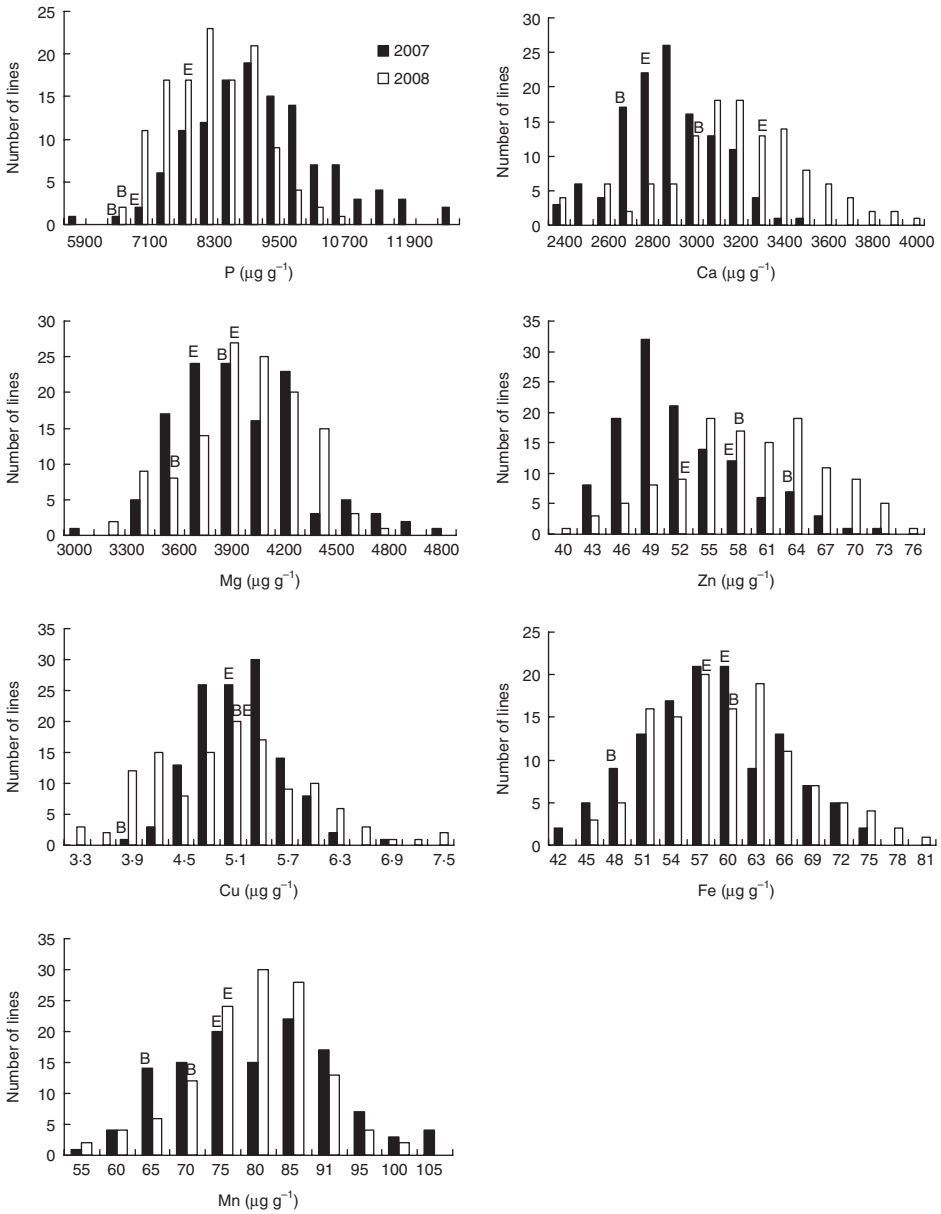

FIG. 1. Frequency distributions of seed mineral concentrations in a *Brassica napus* 'B104-2' × 'Eyou Changjia' RIL population grown under normal-phosphorus conditions in 2-year field trials. B and E indicate the position of parents 'B104-2' and 'Eyou Changjia', respectively.

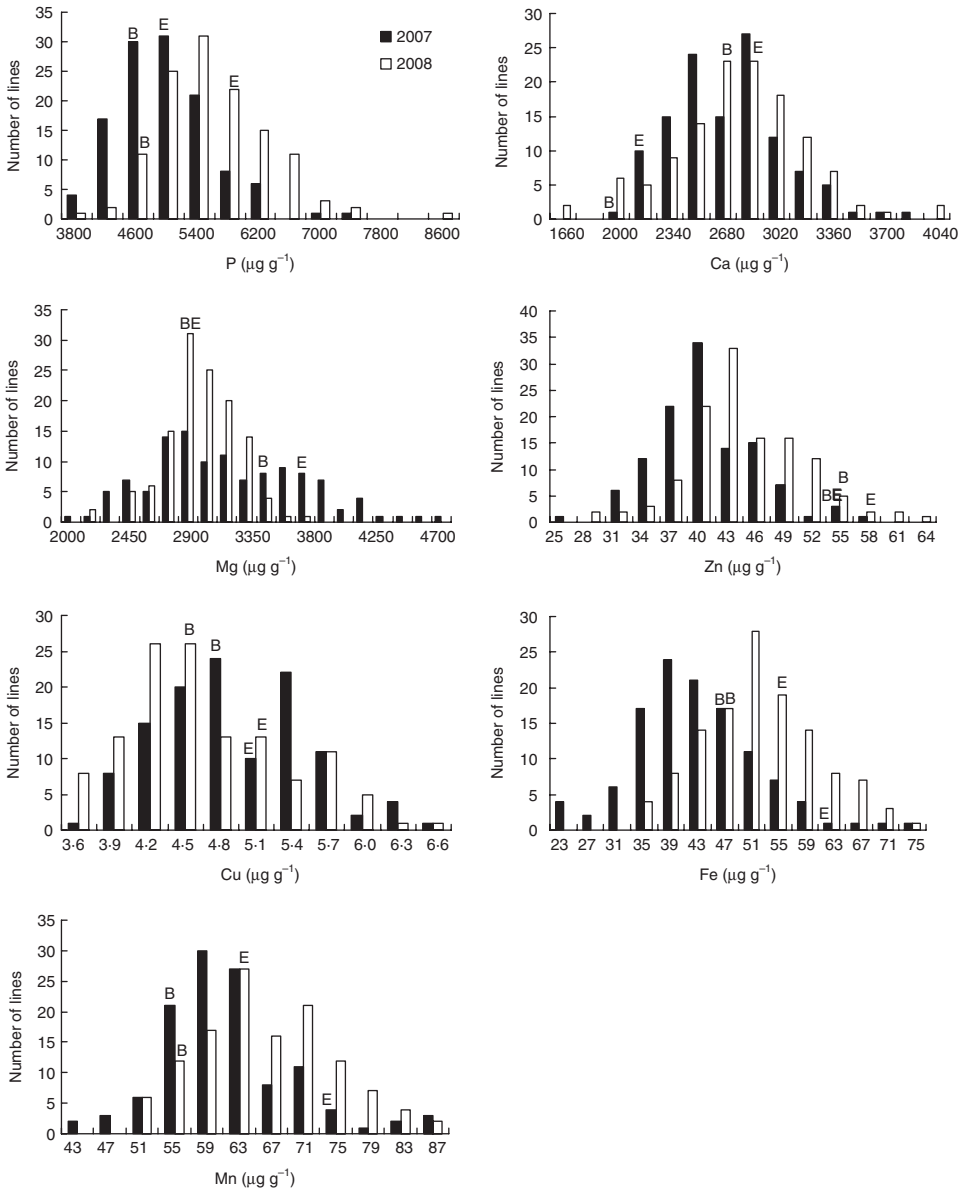

FIG. 2. Frequency distributions of seed mineral concentrations in a *Brassica napus* 'B104-2' × 'Eyou Changjia' RIL population grown under low-phosphorus conditions in 2-year field trials. B and E indicate the position of parents 'B104-2' and 'Eyou Changjia', respectively.

significant under both P levels in both years, but correlations between other elements were often either non-significant or inconsistent between years and/or P treatments. Significant positive correlations were observed between some macro-nutrients and micro-nutrients, such as P and Zn, and Ca and Zn, and weak positive correlations were observed in others, especially under low-P conditions. Correlations between seed mineral traits and seed yield were weak, or even negative, except between Mg and seed yield in 2007 under normal-P conditions (Table 2).

General characteristics of the QTLs detected

A total of 78 putative QTLs, including 45 under normal-P conditions and 33 under low-P conditions, were detected in the 2-year field trials (Table 3). These QTLs were mainly located on 17 linkage groups, explaining 7·84–18·1 % of the phenotypic variation. About 65 % of these QTLs were co-located with at least one other QTL. Alleles from the P-efficient cultivar 'Eyou Changjia' contributed to increase seed mineral concentrations at 44 QTLs (57 %), while alleles from the P-inefficient cultivar 'B104-2' contributed to increase seed mineral concentrations at 34 QTLs (43 %). Chromosome C6 had the largest number of QTLs (a total of 11 QTLs), including QTLs for all nutrients except Mn, whereas the smallest number of QTLs was observed on C5 and C9 (one QTL each). For each mineral trait, at least two QTLs were located on different chromosomes. Sixteen QTLs were identified in at least two environments, but the rest were detected in only one environment. The co-location of QTLs occurred on most chromosomes, except A8, C5 and C9 (Fig. 3).

TABLE 2. *Correlations among the concentrations of seed minerals and seed yield (SY) in 124 RI lines of* Brassica napus *grown under low-phosphorus (below diagonal) and normal-phosphorus (above diagonal) conditions*

	P	Ca	Mg	Zn	Cu	Fe	Mn	SY
2007								
P	–	0·49***	0·86***	0·61***	0·40***	0·32***	0·18	−0·03
Ca	0·23*	–	0·53***	0·30**	0·18	0·19	0·37***	0·06
Mg	0·63***	0·13	–	0·60***	0·41***	0·29**	0·22*	0·26*
Zn	0·37***	0·28**	0·07	–	0·51***	0·22*	−0·08	0·02
Cu	0·22*	0·18	0·01	0·37***	–	0·35***	0·15	−0·08
Fe	0·06	0·14	0·21	−0·10	0·23*	–	0·10	0·03
Mn	0·22*	0·19	−0·12	0·32**	0·11	0·24*	–	0·11
SY	0·02	0·08	0·16	−0·06	−0·07	−0·27**	0·09	–
2008								
P	–	0·38***	0·39***	0·23*	0·16	0·49***	0·06	−0·07
Ca	0·23*	–	0·55***	0·24*	0·37***	0·42***	0·30**	−0·15
Mg	0·65***	0·48***	–	0·34***	0·28**	0·29**	0·39***	−0·05
Zn	0·34***	0·41***	0·24*	–	0·57***	0·59***	0·53***	−0·10
Cu	0·17	−0·07	0·10	0·43***	–	0·61***	0·37***	−0·18*
Fe	0·02	0·28**	0·24*	0·07	0·13	–	0·29**	−0·02
Mn	0·18	0·59***	0·34***	0·50***	0·20	−0·01	–	0·15
SY	0·05	0·10	−0·01	−0·30**	−0·18	−0·04	−0·06	–

*$P < 0·05$, **$P < 0·01$, ***$P < 0·001$.

QTLs detected for each mineral across different P levels

Phosphorus. In total, 12 putative QTLs explaining 7·84–15·94 % of the phenotypic variation for seed P concentration were detected. These were located on ten different chromosomes and had LOD scores ranging from 3·27 to 5·30. Seven QTLs were detected under normal-P conditions, and five were detected under low-P conditions. Three QTLs (*PLP-A1*, *PLP-C3b* and *PNP-C6a*), locating on A1, C3 and C6, respectively, were identified in both years and accounted for a high percentage of phenotypic variation (15·94 %). However, no QTLs were common to both P conditions. Favourable alleles for increasing seed P concentration were contributed by the P-efficient cultivar 'Eyou Changjia' at seven loci and from the P-inefficient cultivar 'B104-2' at five loci (Table 3).

Calcium. For seed Ca concentration, a total of 14 significant QTLs explaining 8·44–18·10 % of the phenotypic variation were identified. These were located on nine chromosomes and had LOD scores ranging from 3·19 to 6·35 (Table 3). Two QTLs, *CaNP-C6a* and *CaLP-C2b*, were detected in both years. One QTL (*CaLP-A1*) explaining the highest phenotypic variation (18·10 %) was only detected under low-P conditions in 2007. Several QTLs on different chromosomes (A1, A4, A5, A7, A9, C2 and C6) co-located with the QTLs controlling seed concentrations of other mineral elements. Favourable alleles for increasing seed Ca concentration were contributed by 'Eyou Changjia' at ten loci and by 'B104-2' at four loci.

Magnesium. A total of 12 significant QTLs, scattered across nine chromosomes with LOD scores ranging from 3·28 to 6·23, were identified for seed Mg concentration. These QTLs accounted for a range of 9·11–16·49 % of the total phenotypic variation. Six of the QTLs were detected under low-P conditions. Two QTLs, *MgNP-C6b* and *MgLP-C8*, were detected in both years. The QTL, *MgNP-A7*, located on A7 accounted for the largest proportion of phenotypic variation (16·49 %). Most QTLs for seed Mg concentration overlapped with QTLs affecting the concentrations of other mineral elements, especially P and Ca. A higher Mg concentration was conferred by the 'Eyou Changjia' allele at seven loci and by the 'B104-2' allele at five loci (Table 3).

Zinc. A total of ten significant QTLs, with LOD scores ranging from 3·76 to 4·84, distributed across eight chromosomes were associated with seed Zn concentration. Seven QTLs were detected under normal-P conditions and three QTLs were found under low-P conditions. One QTL, *ZnLP-C3*, detected in both years under low-P conditions, explained a high percentage of phenotypic variation (13·39 %), while another QTL, *ZnNP-A10*, was detected in both years under normal-P conditions. Six QTLs on five chromosomes (A10, C1, C3, C6 and C7) co-located with QTLs affecting seed concentrations of the other mineral elements. Higher seed Zn concentration was associated with the 'Eyou Changjia' alleles at six QTLs and by the 104-2 alleles at four QTLs. Interestingly, favourable alleles for increasing seed Zn concentration under low-P conditions were all contributed by 'Eyou Changjia'.

Copper. Eleven putative QTLs explaining 7·84–16·82 % of the total phenotypic variation in seed Cu concentration were identified on five chromosomes (A6, A9, A10, C6 and C7) with LOD scores ranging from 3·35 to 6·50. One QTL, *CuNP-A10a*, was detected in both years under normal-P conditions, while another QTL, *CuLP-C6*, was detected in both years under low-P conditions. Three QTLs, *CuNP-A10a*, *CuLP-A10* and *CuNP-A10b*, co-located on A10 under different P conditions, with QTL *CuNP-A10b* explaining the highest percentage of phenotypic variation (16·82 %). Similarly, QTLs *CuNP-C7b* and *CuLP-C7* co-located on C7. Higher seed Cu concentration was conferred by 'Eyou Changjia' alleles at five loci and by 'B104-2' at six loci.

Iron. In total, nine significant QTLs, which were located on seven chromosomes with LOD scores ranging from 3·38 to

TABLE 3. *Significant QTLs for seed mineral concentrations in a* Brassica napus *'B104-2' × 'Eyou Changjia' RIL population grown under two phosphorus conditions*

QTL	Chromosome	Trait	CI (peak)*	LOD†	R2(%)‡	Add.§	Year¶	Reference#
QTLs detected under normal-P conditions								
PNP-A4	A4	P	59·6–64·2 (62·21)	3·91	10·59	+	2007	
PNP-A5	A5	P	44·1–56·1 (50·61)	5·02	13·86	−	2008	
PNP-A6	A6	P	31·9–33·5 (32·41)	3·37	7·84	−	2008	Wu *et al.*, 2008
PNP-A8	A8	P	6·7–8·7 (7·31)	3·37	9·20	−	2007	Zhao *et al.*, 2008
PNP-C1	C1	P	52·8–58·9 (54·61)	3·59	10·09	+	2007	Hammond *et al.*, 2009
PNP-C6a	C6	P	8·9–14·3 (11·61)	5·30	15·94	−	2007/2008	
PNP-C6b	C6	P	128·8–131·6 (129·21)	3·27	8·63	−	2008	
CaNP-A4	A4	Ca	39·3–41·0 (40·41)	3·33	9·83	+	2007	
CaNP-A5	A5	Ca	49·6–54·5 (52·51)	3·77	12·91	−	2008	
CaNP-A7a	A7	Ca	12·8–15·3 (13·21)	3·41	10·41	−	2007	
CaNP-A7b	A7	Ca	15·3–22·2 (18·81)	3·47	10·92	−	2007	
CaNP-C2	C2	Ca	0·0–5·5 (0·01)	4·07	13·04	−	2008	
CaNP-C6a	C6	Ca	0·0–1·2 (0·01)	4·27	13·12	−	2007/2008	Broadley *et al.*, 2008
CaNP-C6b	C6	Ca	6·9–10·9 (8·81)	3·19	10·72	−	2008	Broadley *et al.*, 2008
MgNP-A5	A5	Mg	51·9–62·1 (58·11)	4·92	15·92	−	2007	Liu *et al.*, 2009
MgNP-A7	A7	Mg	13·2–16·8 (15·21)	6·23	16·49	−	2008	Liu *et al.*, 2009
MgNP-C5	C5	Mg	0·9–16·3 (4·01)	3·66	14·48	+	2008	
MgNP-C6a	C6	Mg	0·0–3·3 (0·01)	5·44	14·10	−	2008	Broadley *et al.*, 2008
MgNP-C6b	C6	Mg	6·9–10·8 (8·81)	3·85	10·83	−	2007/2008	Broadley *et al.*, 2008
MgNP-C7	C7	Mg	49·5–57·6 (53·51)	3·75	12·87	−	2007	
ZnNP-A8	A8	Zn	11·2–18·0 (12·71)	4·84	12·32	−	2008	
ZnNP-A10	A10	Zn	15·0–17·3 (16·41)	4·22	12·56	+	2007/2008	
ZnNP-C1	C1	Zn	52·4–55·9 (54·61)	3·92	10·89	+	2007	
ZnNP-C6a	C6	Zn	4·3–10·9 (8·81)	4·02	12·43	−	2007	
ZnNP-C6b	C6	Zn	132·2–134·9 (133·31)	4·20	10·32	+	2008	
ZnNP-C7	C7	Zn	47·6–53·5 (49·51)	4·03	9·69	−	2008	
ZnNP-C8	C8	Zn	23·7–31·0 (24·51)	3·94	8·86	+	2008	
CuNP-A6	A6	Cu	31·9–39·3 (35·81)	3·57	8·07	−	2008	
CuNP-A9	A9	Cu	104·5–108·2 (106·21)	3·35	7·84	+	2007	
CuNP-A10a	A10	Cu	19·3–21·8 (20·51)	4·69	12·35	+	2007/2008	Liu *et al.*, 2009
CuNP-A10b	A10	Cu	23·8–29·3 (25·91)	6·50	16·82	+	2007	Liu *et al.*, 2009
CuNP-C6	C6	Cu	8·5–12·9 (10·81)	5·58	14·00	−	2007	
CuNP-C7a	C7	Cu	48·8–52·9 (49·51)	5·02	11·51	−	2008	
CuNP-C7b	C7	Cu	55·8–59·6 (57·61)	3·92	9·27	−	2008	
FeNP-A3	A3	Fe	0·0–5·1 (0·01)	3·94	10·29	+	2007	
FeNP-A5	A5	Fe	20·0–41·1 (22·51)	3·42	8·97	+	2007	
FeNP-A6	A6	Fe	31·4–33·5 (32·41)	3·68	10·08	−	2008	
FeNP-A10a	A10	Fe	24·9–26·9 (25·91)	3·65	10·16	+	2008	
FeNP-A10b	A10	Fe	46·3–60·0 (54·51)	4·26	11·56	+	2007	
FeNP-C6	C6	Fe	129·2–130·7 (130·01)	3·75	10·59	+	2008	
FeNP-C7	C7	Fe	46·2–51·1 (48·63)	4·18	11·63	−	2007/2008	
MnNP-A1	A1	Mn	0·4–8·3 (3·31)	3·34	9·06	−	2008	
MnNP-A9	A9	Mn	75·3–79·5 (76·61)	4·10	11·17	−	2007	
MnNP-A10a	A10	Mn	6·8–12·8 (9·31)	3·59	10·61	+	2008	
MnNP-A10b	A10	Mn	17·4–19·4 (18·40)	4·65	13·14	+	2007/2008	
QTLs detected under low-P conditions								
PLP-A1	A1	P	74·9–79·0 (76·96)	3·88	10·46	+	2007/2008	Zhao *et al.*, 2008
PLP-A9	A9	P	8·1–15·9 (9·91)	3·90	10·82	+	2008	Zhao *et al.*, 2008
PLP-C2	C2	P	38·5–55·7 (46·81)	3·36	11·37	+	2007	
PLP-C3a	C3	P	4·2–12·3 (8·91)	3·69	13·94	−	2007	Hammond *et al.*, 2009
PLP-C3b	C3	P	51·6–55·2 (53·41)	4·19	11·05	−	2007/2008	Hammond *et al.*, 2009
CaLP-A1	A1	Ca	39·1–43·8 (42·51)	6·35	18·10	−	2007	Liu *et al.*, 2009
CaLP-A3	A3	Ca	69·7–73·7 (71·91)	3·55	8·62	−	2007	Liu *et al.*, 2009
CaLP-A4	A4	Ca	113·6–117·8 (115·11)	3·72	9·83	−	2008	
CaLP-A9	A9	Ca	98·3–108·2 (105·71)	3·40	9·66	+	2008	
CaLP-C2a	C2	Ca	38·0–44·8 (41·91)	3·21	8·44	+	2008	
CaLP-C2b	C2	Ca	46·9–53·0 (49·86)	4·59	12·73	+	2007/2008	
CaLP-C9	C9	Ca	2·7–6·8 (4·31)	3·53	8·46	−	2007	Broadley *et al.*, 2008
MgLP-A1a	A1	Mg	37·1–45·0 (38·31)	3·28	9·82	+	2008	
MgLP-A1b	A1	Mg	74·5–80·1 (76·11)	3·95	12·30	+	2007	
MgLP-A5	A5	Mg	20·3–24·6 (22·51)	3·36	9·11	+	2007	Liu *et al.*, 2009
MgLP-C1	C1	Mg	65·5–69·6 (67·61)	3·33	9·85	+	2008	
MgLP-C3	C3	Mg	24·5–33·0 (28·01)	3·78	11·30	−	2008	
MgLP-C8	C8	Mg	138·2–142·6 (140·38)	3·80	11·39	−	2007/2008	Liu *et al.*, 2009

Continued

TABLE 3. *Continued*

QTL	Chromosome	Trait	CI (peak)*	LOD†	R2(%)‡	Add.§	Year¶	Reference#
ZnLP-A4	A4	Zn	99·0–105·2 (100·21)	4·26	13·19	−		Wu *et al.*, 2008
ZnLP-C3	C3	Zn	16·8–23·2 (21·01)	4·10	13·39	−	2007/2008	
ZnLP-C7	C7	Zn	63·0–76·3 (67·91)	3·76	12·85	−		
CuLP-A9	A9	Cu	6·5–13·9 (9·91)	3·46	9·26	+	2007	
CuLP-A10	A10	Cu	18·6–25·9 (21·31)	3·35	8·96	+	2008	Liu *et al.*, 2009
CuLP-C6	C6	Cu	138·1–140·9 (139·51)	4·34	14·30	+	2007/2008	
CuLP-C7	C7	Cu	51·9–57·0 (55·11)	5·85	16·29	−	2008	
FeLP-A3	A3	Fe	50·2–54·8 (52·51)	3·38	10·38	−	2007/2008	
FeLP-A9	A9	Fe	58·5–63·1 (60·11)	4·26	11·95	+	2008	
MnLP-A3	A3	Mn	77·8–84·8 (82·81)	4·60	14·74	−	2007	
MnLP-A4	A4	Mn	107·2–120·3 (117·11)	4·31	14·14	−	2008	
MnLP-A6	A6	Mn	0·0–4·5 (0·01)	6·55	16·43	−	2007	Wu *et al.*, 2008
MnLP-A10	A10	Mn	5·2–9·3 (7·61)	4·63	11·80	+	2007	
MnLP-C3	C3	Mn	12·1–15·5 (13·81)	4·23	11·46	−	2007/2008	
MnLP-C8	C8	Mn	1·6–21·3 (14·01)	3·64	13·40	+	2008	

*Confidence interval (CI) of QTL ($P < 0·05$), given in centimorgans. The peak position is denoted by the number in parentheses.
†LOD score as calculated by WinQTLCart 2·5.
‡Percentage of phenotypic variation explained by each identified QTL.
§Additive effect. Positive (+) effect indicates 'B104-2' alleles that increase the levels of seed minerals, negative (−) effect indicates 'Eyou Changjia' alleles that increase levels of seed minerals.
¶Numbers indicate the year in which the QTLs were detected, and '2007/2008' indicates that the QTL was detected in both years.
#Reference indicates that the QTL was detected on the same chromosome using another mapping population in a *Brassica* species.

4·26, were associated with seed Fe concentration. These QTLs accounted for a range of 8·97–11·95 % of the total phenotypic variation. Two QTLs, *FeNP-C7* and *FeLP-A3* were detected in both years under normal- and low-P conditions, respectively. Six of the nine QTLs for Fe overlapped with QTLs affecting seed concentrations of other mineral elements (P, Mg, Zn and Cu). Favourable alleles for increasing seed Fe concentration were contributed by 'Eyou Changjia' at three loci and by 'B104-2' at six loci.

Manganese. A total of ten QTLs, with LOD scores ranging from 3·34 to 6·55, distributed across eight chromosomes were associated with seed Mn concentration, explaining between 9·06 % and 16·43 % of the total phenotypic variation. Two QTLs, *MnNP-A10b* and *MnLP-C3*, were detected in both years under normal and low-P conditions, respectively. The largest proportion of phenotypic variation (16·43 %) could be attributed to the QTL, *MnLP-A6*, which was observed under the low-P condition in 2007. Two QTLs (*MnNP-A10a* and *MnLP-A10*) detected under different P conditions co-located on A10 with the same allelic effects of 'B104-2'. Higher seed Mn concentration was conferred by 'Eyou Changjia' alleles at six loci and by 'B104-2' alleles at four loci.

Association of QTLs with functional genes by in silico *mapping*

Twelve syntenic blocks and 17 insertion islands have been identified between the *A. thaliana* genome and *B. napus* linkage groups by *in silico* mapping (Fig. 3). In total, 21 orthologous genes, which are involved in ion homeostasis in *A. thaliana* (Table S1 in Supplementary data, available online), were mapped to the QTL intervals identified in *B. napus* (Fig. 3). Three PAP family members, *PAP16*, *PAP17* and *PAP28*, which have acid phosphatase activity (Li *et al.*, 2002), overlapped well with QTLs on A5 and C2. Three YSL family members, *YSL1*, *YSL5* and *YSL7*, and one ZIP family member, *ZIP1*, which are all thought to be involved in metal homeostasis (Colangelo and Guerinot, 2006; Waters and Grusak, 2008*b*; White and Broadley, 2009), were located in the QTL intervals on four chromosomes (A1, A3, A5 and C6). The co-localized QTL intervals for Ca, Mg and P on A5 contained two CAX family genes, *CAX2* and *CAX9*, and four other genes, *MT3*, *MT2A, MRS2-3* and *IPS1*. One of the CAX family genes, *CAX1*, was also located in the QTL intervals for Ca and Mn on A4. Three P transporter genes, *GPT1*, *PHT4;5* and *PHT3;1*, were mapped to the QTL intervals on C2 and C3. Another four genes, *UGP*, *IRT1*, *NRAMP5* and *AZF2*, were mapped to the QTL intervals for Mn and P on A1 and A3, respectively.

DISCUSSION

Concentrations of seed mineral nutrients are a key factor for vigorous germination and successful seedling establishment (Welch, 1999). Mineral contents of plant seeds depend on both environmental and genetic factors, and a number of researchers have identified QTLs affecting seed mineral concentrations in different plant species in various environments. In this research, QTLs affecting the concentrations of mineral elements in seeds of *Brassica napus* have been identified under two P conditions in an effort to reveal the genetic complexity of seed mineral accumulation.

Genetic variation is essential for achieving significant increases in seed mineral concentrations. Recent studies have shown significant genetic variation in seed mineral concentrations in *Brassica* species (Wu *et al.*, 2007; Broadley *et al.*, 2008; Liu *et al.*, 2009). In the research presented here, a 1·5- to 3·4-fold variation was observed in the concentrations of all mineral elements studied (P, Ca, Mg, Fe, Mn, Cu and Zn) in seeds of a *B. napus* 'B104-2' × 'Eyou Changjia' RIL population grown at two P supplies, even though the two

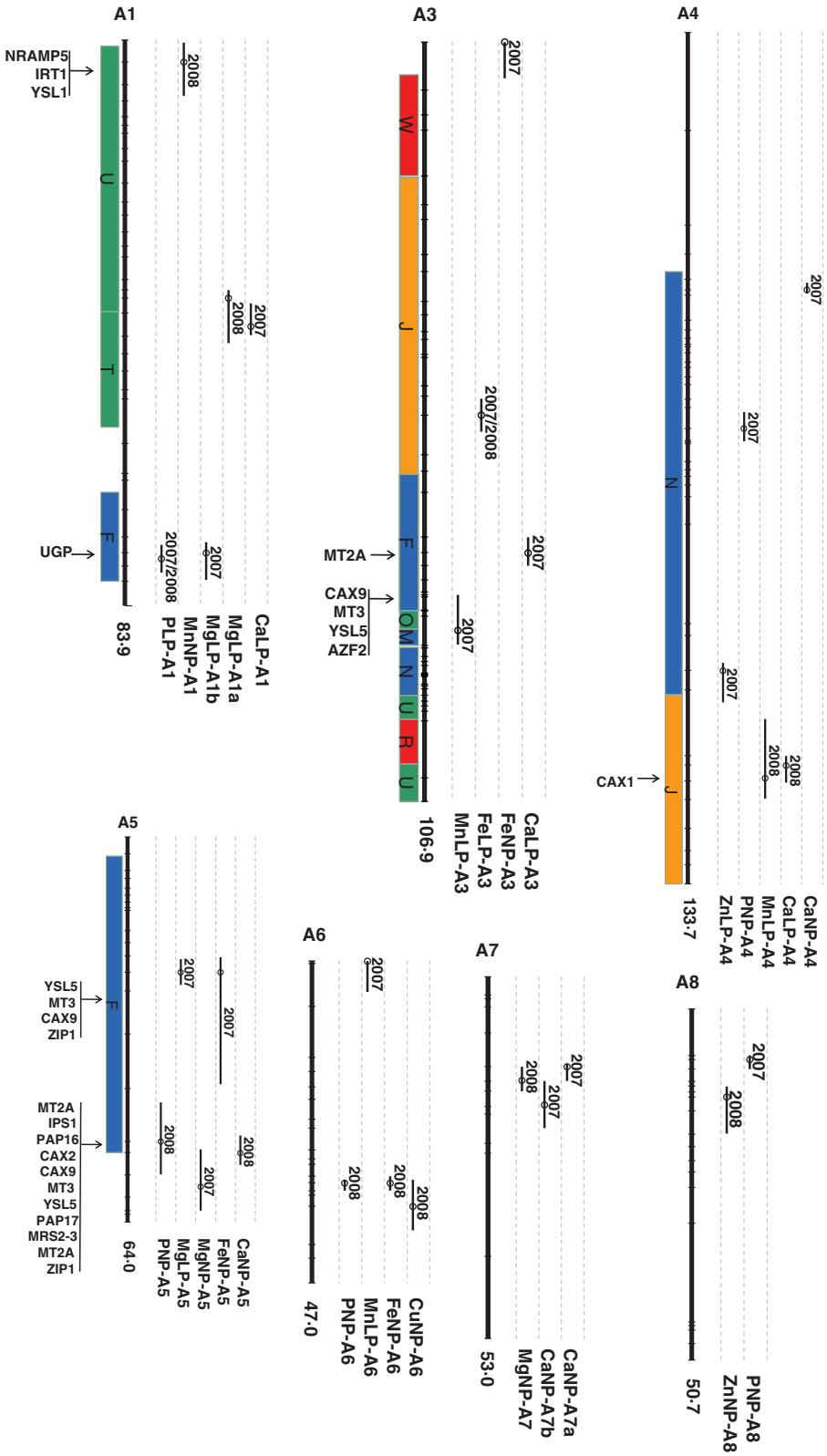

FIG. 3. Chromosomal locations of putative QTLs for seed mineral concentrations in a *Brassica napus* 'B104-2' × 'Eyou Changjia' RIL population and genes aligned with the pseudo chromosomes of *Arabidopsis thaliana*. The length of the vertical black lines to the right of the chromosomes indicate the two-LOD support intervals, circles on the lines indicate the peak position, and the numbers to the right of the lines indicate the year in which the QTL was detected. The coloured bars to the left of the chromosomes represent different pseudochromosomes of *A. thaliana* that have been aligned to the linkage map of *B. napus.* The positions of putative candidate genes are indicated by arrows to the left of the chromosome bars. Numbers at the bottom of each linkage group indicate the map length (cM).

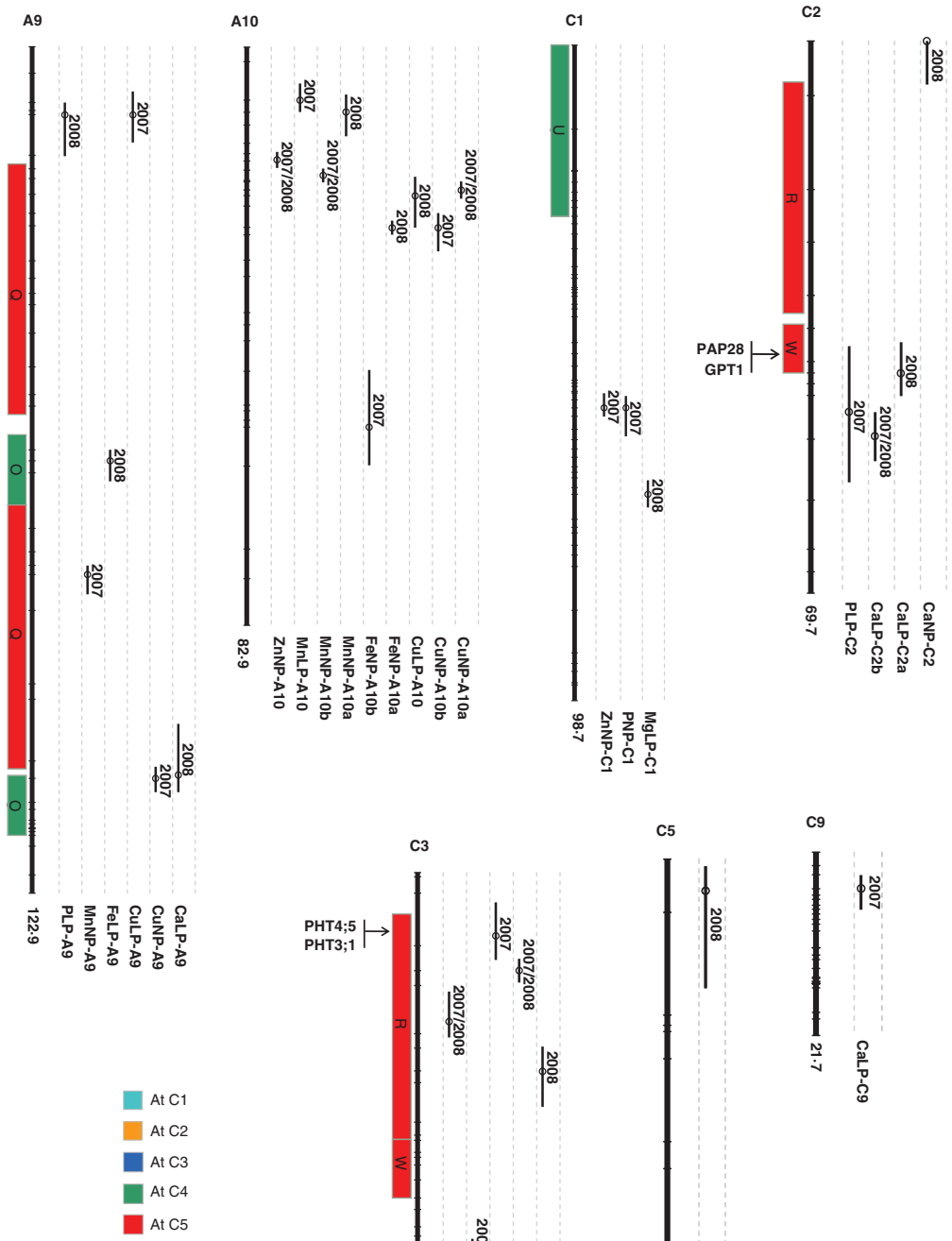

FIG. 3. Continued

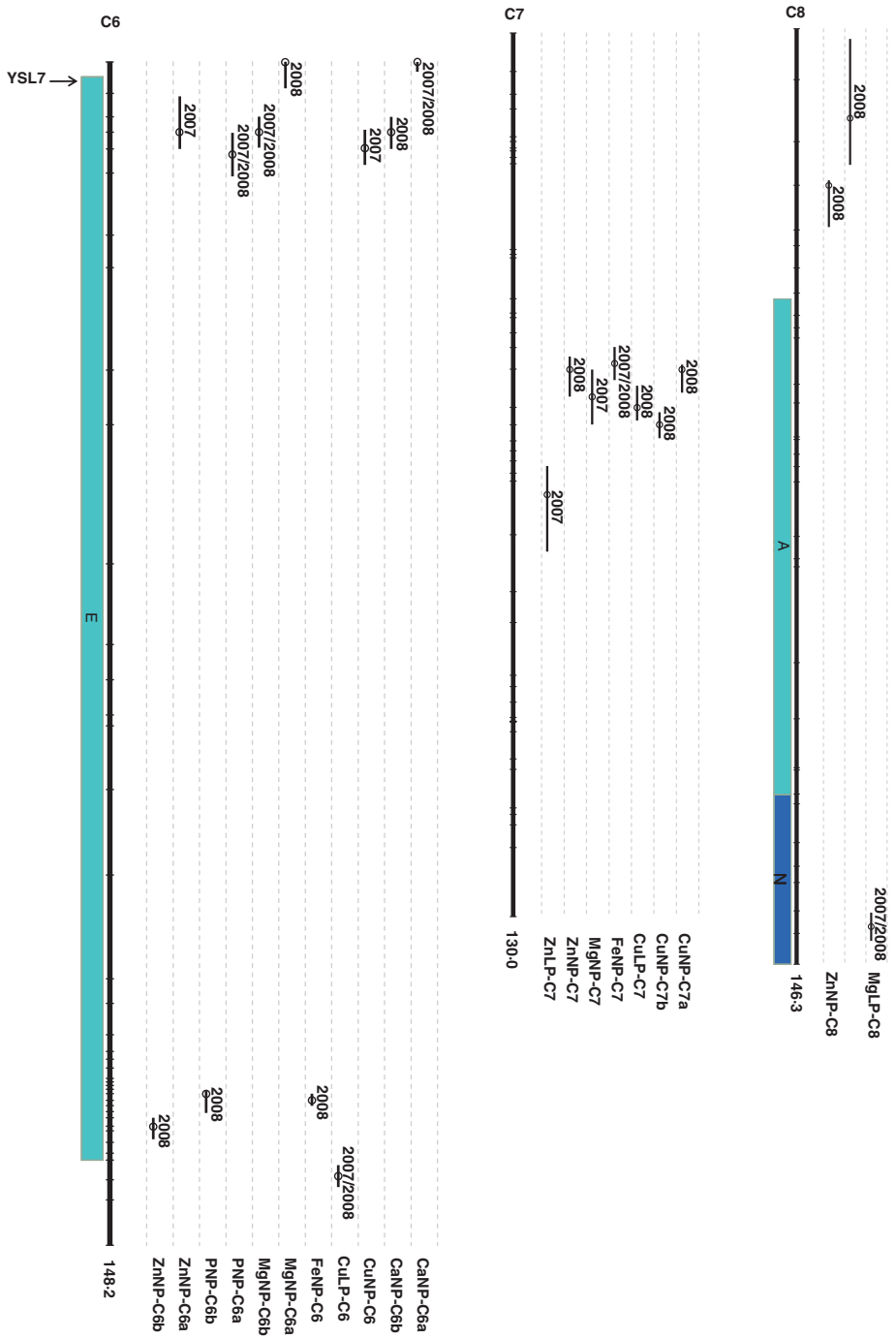

FIG. 3. Continued

parental lines were not very different for most traits except for their seed P concentrations. Wider ranges of phenotypic variation in the seed concentrations of most mineral elements studied were observed in low-P environments than in high P environments, except seed P concentration in 2007 and seed Cu concentrations in both years. Estimates of heritability for these mineral traits varied greatly (Table 1). The wide phenotypic variation and the low level of heritability indicate the influence of environmental factors on quantitative traits and/or significant G × E interactions, since a number of epistatic interaction pairs for shoot mineral concentrations were identified by whole genome analysis of QTL and epistatic interactions in *B. napus* (Liu *et al.*, 2009). The frequency distributions in the RIL population of nearly all mineral elements assayed in plants grown under both P conditions were relatively normal, and considerable transgressive segregation was observed (Figs 1 and 2). This suggests that these are quantitatively inherited traits controlled by multiple genes. Hence, this population can be used to identify QTLs affecting concentrations of mineral elements in seeds and the genes underpinning these traits.

Correlations among different seed mineral traits were observed across P levels. Seed concentrations of macro-nutrients (P, Ca and Mg) were significantly positively correlated, as were seed concentrations of some micro-nutrients, such as Zn and Cu, suggesting that similar transport and chelation processes affected the accumulation of both elements in seeds (Table 2). Most mineral traits were correlated under normal-P conditions, and better correlations were observed under normal-P conditions than under low-P conditions. Similar results were reported by Liu *et al.* (2009) for the accumulation of mineral elements in shoots of *B. napus*. This may point to common molecular mechanisms controlling the uptake and transport of several mineral elements in plants under normal-P supply. However, when plants lack P, different adaptive strategies are required for the plants to survive. High yield capacity is a major requirement for any crop cultivar. Despite positive correlations between concentrations of many mineral elements in seeds, very weak or even negative correlations between seed mineral concentrations and seed yield were observed, except between seed Mg concentration and seed yield in 2007 under normal-P conditions. This result suggests that seed yield may have exerted a 'dilution effect' on seed mineral concentrations. Moreover, it may indicate that QTLs for seed mineral concentrations are not directly helpful in breeding crops with enhanced seed yield under reduced input agriculture. However, it is possible that QTLs affecting leaf mineral concentrations have more effect on seed yield than QTLs for seed mineral concentrations.

Movement of mineral elements from soil through the plant to the seeds involves a series of transport processes that are tightly regulated by element-specific and element-non-specific transcriptional and post-transcriptional controls (Dunlop and Phung, 2002; Amtmann *et al.*, 2006; Schachtman and Shin, 2007). However, not much is known about the genetic control and molecular-physiological mechanisms that contribute to the high accumulation of mineral elements in the seeds of different crop species, especially under abiotic stresses such as drought and mineral toxicity or deficiency. With the development of molecular marker technology, QTL analysis has been widely used as a powerful tool to dissect complex agronomic traits. A number of QTLs associated with seed mineral concentrations under abiotic stress have been identified in plants such as *A. thaliana* (Ghandilyan *et al.*, 2009*a*) and wheat (Peleg *et al.*, 2009). In the present study, a previously constructed linkage map was employed to detect QTLs affecting seed mineral concentrations in *B. napus* grown at two P supplies. A total of 78 putative QTLs were identified covering 17 linkage groups (Table 3 and Fig. 3). For each trait assayed, at least two QTLs were identified in different chromosomal regions contributing to small percentages of the phenotypic variation under different P conditions. This suggests that the accumulation of mineral elements in seeds of *B. napus* is a complex trait involving several genes. Although 16 QTLs were identified in both years, the majority were observed in only one year. This has also been observed for QTLs affecting mineral traits in other plant species such as *A. thaliana* (Waters and Grusak, 2008*a*) and *Medicago truncatula* (Sankaran *et al.*, 2009). Many factors such as genetic variation in individuals, environmental variation, population size, number of markers and experimental error can influence the detection of QTLs in a segregating population (Collard *et al.*, 2005). In this research, most of the single-occurrence QTLs have small effects as well as low, although significant, LOD scores. Thus, the detection of these QTLs may vary according to environmental conditions. Moreover, because of the limited molecular markers and their unbalanced distribution, the genetic map used here was only 1592·72 cM and individual linkage groups ranging from 13·42 to 148·16 cM. Thus, many QTLs could remain undetected due to the relatively small linkage map which did not cover the whole genome of *B. napus*.

One of the main objectives of this research was to detect QTLs affecting seed concentrations of mineral elements in *B. napus* and their response to P supply. A total of 33 significant QTLs were identified under low-P conditions (Table 3). Most of these QTLs differed from the QTLs identified for the same mineral elements under normal-P conditions. Similar observations were made by Liu *et al.* (2009) for QTLs affecting shoot mineral concentrations in *B. napus* grown with different boron availabilities. Considering the large number of solute-specific and non-specific transport proteins and chelates in plants (Maser *et al.*, 2001), it is not surprising that different molecular mechanisms might be expressed under different P conditions, especially since P supply can alter the bio-availabilities of mineral elements in the soil. Analysis of QTLs affecting mineral accumulation in *A. thaliana* has also indicated that there is a strong dependence of QTLs on environment (Waters and Grusak, 2008*a*; Ghandilyan *et al.*, 2009*a*, *b*). In addition, the fusion of *B. oleracea* (CC genome) and *B. rapa* (AA genome) progenitors has resulted in the genome of *B. napus* (AACC) being more than six times the size of the *A. thaliana* genome (Johnston *et al.*, 2005) and it is suggested that *B. napus* might have two to eight orthologous genes corresponding to each single-copy gene in *A. thaliana* (Cavell *et al.*, 1998). Some members of these gene families might have become specialized during the process of evolution to function optimally under different growth conditions, such as mineral deficiency. Thus, different family members distributing randomly in different chromosomal regions might account for differences in the QTLs identified under contrasting environmental conditions.

Co-localization of QTLs affecting different traits suggests either a single pleiotropic locus is involved in controlling of multiple traits or that several separate loci affecting independent traits are in close proximity. In this research, many QTLs (51 out of 78) co-localized with at least one other QTL. In particular, QTLs affecting seed P concentration co-located with QTLs affecting seed concentrations of other mineral elements. This is consistent with correlations between seed concentrations of P and those of other mineral elements in plants grown under different P conditions, suggesting a strong genetic association between mechanisms affecting seed P concentrations and concentrations of other mineral elements. Cations in seeds are often stored in a complex with phytic acid (1,2,3,4,5,6 hexakisphosphate) which is well known as the main storage compound for phosphorus in seeds (Raboy, 1997). The association of QTLs affecting seed P concentration with QTLs affecting the concentrations of other mineral elements in seeds could indicate a physiological process affecting several mineral elements similarly or physiological coupling in sink or source tissues.

Co-localization of QTLs involved in the accumulation of different mineral elements, such as Ca/Mg and Fe/Mn/Cu/Zn, were also detected in this mapping population, suggesting a possible relationship at the molecular level among these traits. Similar phenomena have also been reported for QTLs affecting mineral traits in other plant species (Vreugdenhil *et al.*, 2004; Broadley *et al.*, 2008; Waters and Grusak, 2008*a*; Garcia-Oliveira *et al.*, 2009; Ghandilyan *et al.*, 2009*a*, *b*; Liu *et al.*, 2009; Peleg *et al.*, 2009; Sankaran *et al.*, 2009). Co-localization of QTLs was not always consistent with the correlations observed between mineral traits. For instance, although seed concentrations of most mineral elements were correlated under normal-P supply, the QTLs identified for these traits were not always co-located. Similar results have been reported by Ghandilyan *et al.* (2009*b*). Correlations among seed concentrations of mineral elements reflect the association of total phenotypic variation which may be due to various genetic effects. However, because of the quantitative nature of seed mineral traits, weak statistical significance of QTLs is prevalent and co-location of QTLs may be obscured. QTLs for seed concentrations of several mineral elements, including P, Ca, Mg, Cu and Zn, overlapped on the top of C6, and two of these QTLs, *MgNP-C6b* and *PNP-C6a*, were detected under normal-P conditions in both years (Fig. 3), suggesting it was a hotspot locus for mineral traits under normal-P conditions. However, under low-P conditions most of the QTLs explaining a small percentage of the phenotypic variation were detected in only one year and were located on different chromosomal regions, suggesting that it maybe difficult to set up an efficient breeding programme for increasing seed mineral concentrations in rapeseed grown in a low-P environment.

QTLs should be robust enough for further fine mapping or a breeding scheme. Hence, it is important to compare the present results with those from other research groups. Due to its amphidiploid nature, *B. napus* (AACC genome) contains the diploid CC genome of *B. oleracea* and AA genome of *B. rapa* and the QTLs identified in this research can be related to those found by other research groups studying other Brassicaceae. Twenty-three QTLs controlling different aspects of mineral accumulation were found on the same chromosomes of different *Brassica* species (Table 3). However, because no common anchoring markers were used in these studies, it is difficult to determine whether these QTLs were located on the same chromosomal regions. Hence, further comparative QTL research should be conducted to confirm these QTLs using common molecular markers.

The existence of a common ancestor for *Brassica* and *Arabidopsis* makes comparative genome mapping possible. Twenty-four conserved genomic blocks have been identified in different Brassicaceae species by comparing the sequences of molecular markers with the annotated genome sequence of *A. thaliana* (Schranz *et al.*, 2006). By *in silico* mapping, 21 genes involved in ion homeostasis in *A. thaliana* (Table S1 in Supplementary data, available online) were mapped to the QTL intervals of *B. napus*, including three PAP family members, three YSL family members, one ZIP family member, three P transporter genes, three CAX family genes and several other genes (Fig. 3). Our hypothesis was that QTLs were characterized by orthologous genes in both *A. thaliana* and *B. napus*. The approach of associating QTLs with functional genes in *Arabidopsis* by *in silico* mapping may provide more information for QTL analysis in *B. napus*. However, further fine mapping and analysis of near isogenic lines or mutants will be needed to confirm the involvement of a candidate gene on the identified quantitative trait. Moreover, there were no orthologous genes associated with the hotspot locus on C6, offering the possibility of identifying unknown genes that affect mineral uptake and translocation to seeds in *B. napus*.

CONCLUSIONS

Several QTLs for seed mineral concentrations were identified in *Brassica napus* under two P conditions. In total, 78 putative QTLs were identified affecting seed concentrations of diverse mineral elements (P, Ca, Mg, Fe, Mn, Cu and Zn). For each mineral trait, at least two QTLs were identified on different chromosomes, indicating that these mineral traits are quantitatively inherited and controlled by multiple genes. Often QTLs were clustered within a linkage group, suggesting that common mechanisms might control the accumulation of several mineral elements in seeds. Frequently, different chromosomal regions affecting the same mineral trait were identified under normal-P and low-P conditions, indicating different molecular mechanisms were employed for seed mineral accumulation in these two environments. Several genes involved in ion homeostasis in *A. thaliana* were mapped to the QTL intervals affecting seed mineral concentrations in *B. napus*, but further studies need to be conducted to determine whether these genes are true candidates for further investigation. These results may offer insights to the genetic basis of mineral accumulation in seeds of *B. napus* grown under different P supplies and help to unravel the genetic complexity of mineral accumulation in seeds of *B. napus*. However, the low robustness of the QTLs identified could make it difficult to focus on a particular QTL for cloning, and also difficult to set up an efficient breeding programme for increasing seed mineral concentrations in rapeseed in low-P environments.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org, and consist of Table S1: Orthologous genes associated with mineral QTLs detected in the 'B104-2' × 'Eyou Changjia' RIL population by *in silico* mapping between *Arabidopsis thaliana* and *Brassica napus*.

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