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Quantitative trait loci for seed mineral concentrations and root morphology responding to low phosphorus stress in *Brassica napus*

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Introduction

The mineral concentrations in plants often change due to different soil nutrient stresses. The development of ionomics, the study of the mineral nutrient and trace element composition of an organism, provides a new approach for the detection of mineral element profiles in plant tissues (Clemens 2001; Lahner et al., 2003; Hirschi 2003). It means that, rather than a single element, many elements can be detected at one time.

Quantitative trait loci (QTL) analysis is a powerful genetic approach that enables the dissection of complex traits in order to unravel the natural genetic variations within species or cultivars (Mauricio 2001). Different genetic mapping populations have been developed to dissect mineral nutrient-related traits in *Brassica* and *Arabidopsis*, and some QTL for natural variations in qualitative traits or with large effect have been identified. QTL mapping has been performed in *A. thaliana* recombinant inbred line populations to identify loci that influence shoot nitrogen (N) (Loudet et al. 2003), seed and leaf phosphorus (P) (Bentsink et al. 2003), shoot potassium (K) (Harada and Leigh 2006), shoot caesium (Cs) (Payne et al. 2004) and other mineral characteristics (Vreugdenhil et al. 2004; Waters and Grusak 2008). The recent identification of QTL affecting mineral concentrations discovered homologous genetic loci on *Brassica oleracea* (Broadley et al. 2008) and on *Brassica rapa* (Wu et al. 2008).

Phosphorus is frequently the most limiting element for plant growth and development because of its low bioavailability in soils. However, genotype differences exist in uptake and utilization of nutrients among cultivars of *Brassica napus*. Phosphorus is stored in the form of phytate, which is the salts of phytic acid, myoinositol, 1,2,3,4,5,6-hexakisphosphate. The sparingly soluble Ca-Mg salt of phytic acid is termed phytin, and phytate is main storage site of K, Mn, Zn, and Fe (Marschner 1995). So there is tight relationship among P, phytate and other minerals concentration. Uptake and transport of mineral nutrients in plants is depended on root development and activity. The objectives of this study are to map QTL for mineral concentrations in seeds and root system morphology in a *B. napus* recombinant inbred (RI) population under two P conditions to increase genetic understanding of the mineral ion storage in seeds responding to P-deficiency.

Materials and Methods

Plant material

An F₁₀ recombinant inbred line (RIL) population consisting of 124 lines developed from a cross between *Brassica napus* P-efficient cv. 97081 and P-inefficient cv. 97009 using a single seed descent method was used in field trials and paper culture experiments.

Field Trial

The field trials located at Daye, Hubei, China were conducted in 2006 and 2007, and the experiment soil was paddy soil with Olsen-P 12.5 mg P kg⁻¹. Two P treatments, 30 kg P₂O₅ ha⁻¹ (low-P) and 90 kg P₂O₅ ha⁻¹ (high-P), were designed with three replications. Each replication consisted of 124 IR lines and both parents, and each material was planted in two rows with a total of 18 individuals in one replicate. 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

H₃BO₃ ha⁻¹. After harvesting seed yield, seeds were used to determine P, Ca, Mg, Fe, Zn, Mn, and Cu concentration with ICP-AES after digested by density HNO₃-HClO₄.

Paper culture experiment

Paper culture experiments were conducted to investigate root morphology change under low-P starvation. The surface-sterilized seeds of two parents and 124 lines were germinated on moistened gauze and then the uniform plants with fully developed cotyledons were transferred to blue P-free paper (Anchor Paper Company, USA). Each paper was transplanted with two plants on each side, which were suspended upright into Hoagland and Arnon nutrient solution with low P (5 µM P) or high P (1 mM P) in a greenhouse. The experiment was a randomized complete block design with three replications. Plants were harvested 28 days after transplanting and the intact root system on the paper was scanned by the scanner, which was further analyzed for total root length, root surface area, root volume, and root average diameter with root image analysis software (WinRhizo Pro, Canada).

Statistical analysis and QTL mapping

The investigated data for the mineral nutrient concentrations in seeds and root morphology traits from the RI population were first analyzed with ANOVA using a general linear model to determine the significance of the effect of P availability and the genotype × P interaction and correlation among the investigated traits. Based on the construction of a genetic linkage map, composite interval mapping (CIM) was then used to determine putative QTL for each trait and the closest marker to each local LOD score peak, and estimate the likelihood of a QTL and its corresponding phenotypic effect (R²) at every 2 cM using Model 6 of QTL Cartographer 2.50.

Results

Genetic linkage map

Three molecular markers, simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP) and sequence related amplified polymorphism (SRAP), were used to construct a linkage map with the RI population using JoinMap 4.0 (Van 2006) with Kosambi function. The linkage map consisted of 19 linkage groups with a total of 527 molecular markers, which included 198 SSR markers, 56 AFLP markers, 237 SRAP markers and 36 functional markers. A total length of the 19 linkage groups was 1,855.7 cM with an average distance of 3.5 cM between adjacent two markers. The function markers were developed in our lab according to the sequences of EST or genes related to P transport and regulation in *Brassica* species.

QTL for mineral concentration in seed

Concentrations of seven mineral nutrients in seeds of the *Brassica napus* RI population were determined by ICP-AES. The range of variation in nutrients concentration in the RI lines varied from 1.5 to 4-fold under two different P condition in both two years' field trails. Concentrations of mineral nutrients were higher under high-P environment generally, but wider variation was observed under low-P environment except Mn, Cu, and Zn. The distribution of trait values

among individual lines were normally distributed as expected, except P, and transgressive segregation was also observed which suggests multiple gene action for these traits. Significantly positive correlations between nutrients, for example P and Mg under both P-condition were examined, but most minerals had weak positive correlations, with a few exhibiting weak negative correlations, such as P and Fe.

By employing complex interval mapping, a total of 49 putative QTL, including 28 under high-P conditions and 21 under low-P conditions, were identified to be significant for seed mineral nutrients content in oilseed, which mainly located on 15 linkage groups, explaining the phenotypic variation from 8.59% to 36.48%. Furthermore, QTL-overlapping was detected between minerals on several linkage groups, and in both two years' field trails.

QTL for root morphology

The Pearson's correlation coefficients between traits were calculated. Root dry weight, root length, root surface area and root volume had positive correlation with each other in both high P and low P environments in the two experiments. While under high P condition, root diameter only had positive correlation with root volume and negative correlation with root length in two experiments. Under low P condition, root diameter was positively correlated with root dry weight, root surface area, and root volume, and was negatively correlated with root length in experiment 1.

A total of 61 significant QTL for root traits at two P treatments in two experiments were identified and located at 10 chromosomes. Some QTL regions for the same traits in two experiments overlapped. At low P, there were two and one coincident QTL for SDW and RDW in two experiments which located on A3. One QTL for RL on C8 in two experiments was detected and shared the same peak position with same interval markers. One QTL for RSA in two experiments was coincided on A3 which was contributed by P-inefficient parent 97009. One QTL for RD were detected on C8 which regions largely overlapped in both experiments. Two QTL for RVol in two experiments were located on the same region of A3, which explained 35% and 20% of total phenotypic variation in population in two experiments, respectively.

Conclusions

By two separate field trials for investigating mineral concentration in seeds and paper culture experiments for investigating morphological traits, a great number of QTL for the tested traits were detected. Some of them were overlapped and found in different P supplies or experiments, and some exist solely. These would be important bases for us to further understand the molecular mechanism that *Brassica napus* root system or mineral nutrient accumulation in seeds responds to low P stress.

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