

Further genetic analysis of a major quantitative trait locus controlling root length and related traits in common wheat

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Abstract Roots are essential for normal growth, development, and reproduction of higher plants. Consequently, improvement of root system architecture and functionality is of fundamental importance in crop improvement. However, the genetic mechanisms controlling root morphology and function are still not well understood, especially in common wheat, which possesses a complex and unsequenced hexaploid genome. Here we report a more detailed genetic analysis of *qTaLRO-B1*, a major quantitative trait locus (QTL) previously detected to affect root length and related traits in common wheat. A pair of QTL isolines with different *qTaLRO-B1* alleles was developed. Line 178B, carrying the *longer root* allele, was significantly more efficient in taking up phosphate nutrient and biomass accumulation

than line 178A, with the *shorter root* allele. We mapped *qTaLRO-B1* to a 0.9-cM interval on common wheat chromosome 2BS with seven sequence-tagged-site (STS) markers developed from the genes conserved between wheat and *Brachypodium distachyon*. The seven STS markers were collinearly conserved in tetraploid wheat, but they covered a much larger genetic distance (22.8 cM) in the latter species. In conclusion, we have converted *qTaLRO-B1* into a major gene that affects common wheat root length in a qualitative manner, and improved understanding of the genetic location of *qTaLRO-B1* and the chromosomal segment carrying this important locus. The implications of our data for further study of *qTaLRO-B1* are discussed.

Keywords Root · Major QTL · Genetic mapping · Wheat · *Brachypodium distachyon*

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Introduction

Wheat is one of the most important food crops in the world. However, the current annual rate of increase in

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wheat productivity (about 1.1 %) is considerably below that (≥ 1.7 %) demanded by global wheat consumption (Dixon 2009; Rosegrant and Agcaoili 2010; Reynolds et al. 2012). Furthermore, wheat production in many regions of the world still relies on the use of excessive water and chemical fertilizers, which is not sustainable either ecologically or economically (Rosegrant et al. 2009; Good and Beatty 2011). Consequently, there is an urgent need to raise the yield potential of wheat and its efficiencies of using water and fertilizer resources. To fulfill this task, the morphology and function of the wheat root system must be improved.

In the past, crop varieties have been bred generally by selection of above-ground organs, whereas the below-ground roots were largely neglected (Dunbabin et al. 2003; Waines and Ehdai 2007; Den Herder et al. 2010). However, more and more studies show that root development and function, as well as the genes involved, can differ substantially among different genotypes, which can be harnessed for raising water and nutrient use efficiencies and yield potential of crop plants (Coudert et al. 2010; Den Herder et al. 2010). For example, Wasson et al. (2012) recently proposed that, by using appropriate germplasm and combining with suitable laboratory and field screens, it is possible to develop wheat varieties with a deeper and more active root system for achieving higher yield levels in water limited environments. White et al. (2013) suggested a series of morphological and physiological traits for enhancing root uptake of various types of mineral nutrients through breeding. Despite these suggestions, the selection for root traits is difficult at present. In general, root traits are controlled by polygenes with quantitative effects, highly prone to environmental influences, and difficult to observe and quantify under field conditions (Tuberosa et al. 2002a, b; Malamy 2005; Wasson et al. 2012; Hamada et al. 2012). To overcome these problems, large efforts have been devoted to studying the genes and quantitative trait loci (QTLs) controlling root traits in the hope that the resultant resources can facilitate the improvement of root morphology and function through molecular marker-assisted selection (Coudert et al. 2010; Den Herder et al. 2010; Jung and McCouch 2013).

In common wheat, molecular mapping of root trait QTLs have been reported by a number of studies (Sanguineti et al. 2007; Landjeva et al. 2008; Sharma

et al. 2011; Hamada et al. 2012; Ren et al. 2012; Bai et al. 2013; Christopher et al. 2013; Liu et al. 2013). Collectively, these studies have demonstrated the presence of multiple QTLs for several major root traits, such as seminal root angle (SRA), number (SRN), and elongation rate (SRER), primary root length (PRL), maximum root length (MRL), lateral root length (LRL) and number (LRN), total root length (TRL), and root surface area (RSA). Furthermore, it has been generally realized that the development and morphological characteristics of seminal roots at seedling stage have important influences on the function of the root system in mature wheat plants. For example, the number of seminal roots has been found to correlate strongly with seed size and grain yield (MacKey 1979; Liu et al. 2013) and to contribute significantly to water uptake (Manschadi et al. 2008) in common wheat. Thus, identifying and analyzing QTLs controlling seminal root traits will likely provide important information and resources for improving root function and its contribution to wheat yield increase. In accordance with this realization, we have previously identified a number of QTLs for seminal root traits using recombinant inbred lines (RILs) derived from two elite common wheat varieties (Xiaoyan 54 and Jing 411; Ren et al. 2012). One QTL uncovered by us, *qTaLRO-B1*, resided on the short arm of chromosome 2B and explained a large proportion of the phenotypic variation of MRL (68 %) and PRL (59 %). Moreover, through marker allele-assisted genotyping in parental lines and F₂ populations, this QTL was detected, and found active, in two other common wheat varieties. Together, these results suggest that *qTaLRO-B1* is a major genetic determinant of root length, and may be useful for understanding and improving the genetic basis of root size and function in common wheat. Therefore, it is worthwhile conducting further genetic analysis of *qTaLRO-B1*.

From the information above, the main objective of this work was to map the chromosomal location of *qTaLRO-B1* in more detail and to shed new light on the chromosomal region hosting *qTaLRO-B1*. Towards this end, a pair of QTL isolines (178A and 178B) segregating for *qTaLRO-B1* alleles was developed, and new sequence-tagged-site (STS) markers were developed from the genes conserved between *Brachypodium distachyon* and wheat. The common wheat chromosomal interval hosting *qTaLRO-B1* was

compared to its orthologous regions in tetraploid wheat and *B. distachyon*. The new understanding obtained for *qTaLRO-B1* and its implications for further research of *qTaLRO-B1* are discussed.

Materials and methods

Development of QTL isolines and comparison of their growth performance

178A and 178B were developed using the heterogeneous inbred family analysis method (Tuinstra et al. 1997). Briefly, immediately after identifying *qTaLRO-B1* in the RIL population of Xiaoyan 54 and Jing 411 (Ren et al. 2012), the DNA markers flanking this QTL (i.e., *Xgwm210* and *Xbarc1138.2*) were used to screen the F5 progenies that were accumulated while developing the RILs. Several F5 plants were found to be heterozygous at the *qTaLRO-B1* locus. After selfing one such F5 plant, two F6 lines, homozygous for the *qTaLRO-B1* alleles from Jing 411 or Xiaoyan 54, were identified through marker-assisted selection (MAS). After three rounds of selfing and MAS, the two lines were designated 178A and 178B, respectively, and used in the experiments described in this study.

Pot culture trail was carried out to compare the root growth and function of 178A and 178B. The soil used for the trail was calcareous, and its available phosphorus (Olsen P) content was 1.6 mg kg⁻¹. The Olsen P content of the soil was adjusted to two different levels, 26 mg kg⁻¹ (high P treatment) and 8 mg kg⁻¹ (low P treatment), by adding the required amounts of KH₂PO₄. The soil was also supplied with 75 mg kg⁻¹ nitrogen (as urea), 1.0 mg kg⁻¹ zinc (as ZnSO₄), 1.0 mg kg⁻¹ boron (as H₃BO₃), and appropriate quantities of KCl to balance the potassium level. Two uniformly germinated seedlings were planted in each pot, with four replicates arranged per P level per genotype. At 4 weeks after sowing, shoot dry weight, root dry weight, and shoot P content were investigated for every pot. The above- and below-ground parts of the plants in each pot were collected and dried at 80 °C for determining shoot and root dry weight. Shoot P content was measured using the molybdate-blue colorimetric method (Murphy and Riley 1962). Two independent pot culture trails were conducted, with identical results obtained.

Preparation of genetic populations and evaluation of root phenotypes

For investigating the genetic control of the long root phenotype in 178B as well as the mapping of *qTaLRO-B1*, 57 F1 seeds were created by crossing 178A with 178B, and a segregating F2 population was developed by selfing 20 F1 plants. To aid comparative genomic analysis of *qTaLRO-B1*, a tetraploid wheat F2 population was constructed using the durum wheat variety Langdon and a wild emmer wheat accession (WEW270) as parents. The seedling root length of Langdon was substantially longer than that of WEW270.

Previous studies have reported that the root trait QTLs detected in hydroponic culture coincided with those for nutrient uptake and grain yield in common wheat (An et al. 2006; Su et al. 2009; Liu et al. 2013). Thus, in the present study, hydroponic culture was used to investigate root morphological parameters of wheat seedlings in the growth chamber (set at 18 °C, with 60 % relative humidity and 16-h light/8-h dark photoperiod). Seeds were germinated in water for 18 h at 20 °C. The germinated seeds were placed onto a nylon mesh to grow for 6 days in water. After removing residual endosperm materials, the seedlings (supported by the nylon mesh) were transferred to plastic boxes (35 × 25 × 10 cm, each containing 5 L of nutrient solution) to grow for another 12 days. The nutrient solution was refreshed every 3 days. The oxygen content in the nutrient solution was enriched by adding 125 µl of 30 % H₂O₂. At the end of hydroponic culture, MRL was measured for 178A, 178B, F1, and F2 progenies.

DNA isolation and PCR conditions

Genomic DNA was extracted from seedling leaves using the cetyltrimethylammonium bromide method (Allen et al. 2006). PCR was conducted in a total volume of 20 µl containing 10 mM Tris-HCl, pH 7.5, 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM of each of the four dNTPs, 50 ng of each of the two primers, 50–100 ng genomic DNA template, and 0.5 U *Taq* DNA polymerase. Amplification was performed at 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 50–60 °C (adjusted for specific primer set) for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 10 min. PCR products were separated on

Table 1 The seven STS markers developed in this work for mapping *qTaLRO-B1*

Marker	Primer sequence (5'–3')	T_m (°C)	Wheat EST	Ortholog in <i>B. distachyon</i>	Marker type	Polymorphism detection method
<i>STS1^{TaLRO-B1}</i>	F: CATACCACAAGAGTCAGTTGC R: GGCTCTACCATACATACCAGG	56	CA600621	<i>Bradi5g00700</i>	Co-dominant	Length polymorphism
<i>STS2^{TaLRO-B1}</i>	F: AGCAGCAGCGAGAAGAGAAG R: GTTCGACGCTGATCCTGATT	60	BE471132	<i>Bradi5g01880</i>	Co-dominant	Single strand conformation polymorphism(SSCP)
<i>STS3^{TaLRO-B1}</i>	F: GATGGTGATGATGCCATACG R: AGGGTCTTGGTGGGCTTAGT	60	BE586168	<i>Bradi5g01430</i>	Dominant	Length polymorphism
<i>STS4^{TaLRO-B1}</i>	F: GCTGCTGCGGTA CTG R: GGACGCACATGATGGA	50	CJ717809	<i>Bradi5g01410</i>	Co-dominant	Length polymorphism
<i>STS5^{TaLRO-B1}</i>	F: ATGTCCGTGCATATGGAACA R: GTTGCTGGAAGGATGGATA	60	CJ726624	<i>Bradi5g01280</i>	Co-dominant	SSCP
<i>STS6^{TaLRO-B1}</i>	F: AACTGCTTCGAGAGCTCCAC R: CATGCAGCAAGTCTTGTCGT	60	CD882067	<i>Bradi5g01010</i>	Co-dominant	SSCP
<i>STS7^{TaLRO-B1}</i>	F: CGGCAATGTCTTGAGTGCTA R: GCGATGTATTTCCGGAGCATT	60	CJ685262	<i>Bradi5g02170</i>	Co-dominant	SSCP

F forward, R reverse

6 % denaturing polyacrylamide gels with a 19:1 ratio of acrylamide:bisacrylamide. Gels were silver-stained and photographed.

Development of new STS markers and linkage analysis

Previous work indicated that *qTaLRO-B1* was flanked by the microsatellite markers *Xgwm210* and *Xbarc1138.2* (Ren et al. 2012). Since *Xgwm210* had been located to the distal bin of chromosome 2BS (2BS4-0.84-1.00, <http://wheat.pw.usda.gov/GG2/index.shtml>), and the counterpart of 2BS4-0.84-1.00 in *B. distachyon* was on the short arm of chromosome 5 (Bd5S, Liu et al. 2012), the genes annotated for a region of Bd5S (from 0.5 to 2.5 Mb) were used to search wheat expressed sequence tag (EST) databases (http://www.cerealsdb.uk.net/CerealsDB/Documents/DOC_database.php, <http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The positive hits were then employed for designing gene-specific conserved primers (<http://probes.pw.usda.gov/cgi-bin/ConservedPrimers/ConservedPrimers.cgi>, You et al. 2009). The designed primer sets were each tested by PCR with the genomic DNA samples of 178A or 178B as template. For the primer sets that yielded PCR polymorphic fragments between 178A and 178B, they were further analyzed by PCR with the genomic DNA samples of Chinese Spring and the

nulli-tetrasomic lines of wheat group 2 chromosomes (i.e., N2AT2B, N2BT2D, and N2DT2A; Sears 1954) as template. In this way, the primer sets capable of amplifying polymorphic PCR fragments originating from chromosome 2B were identified, and STS markers specific for chromosome 2B were developed. The newly developed STS markers were all labeled by the superscript “*TaLRO-B1*” to indicate that they were located in the chromosomal region hosting *qTaLRO-B1* (Table 1). The alleles of the STS markers were differentiated using 6 % denaturing PAGE optimized as described previously (Creste et al. 2001). In this gel system, the alleles could be distinguished by either length polymorphism or single strand conformation polymorphism (SSCP).

To accomplish linkage analysis of *qTaLRO-B1*, 5087 F2 individuals were subject to root phenotyping by hydroponic culture and DNA genotyping by PCR as described above. Apart from the newly developed STS markers, three simple sequence repeat (SSR) markers *Xgwm210*, *Xbarc1138.2*, and *Xgwm429*, previously reported in the vicinity of *qTaLRO-B1* (Ren et al. 2012), were also used during genotyping. The phenotype and genotype data obtained were deployed to construct a linkage map of *qTaLRO-B1* using the software Joinmap 4.0 (Van Ooijen and Voorrips 2006). Using the same software, the linkage relationships of the newly developed STS markers in

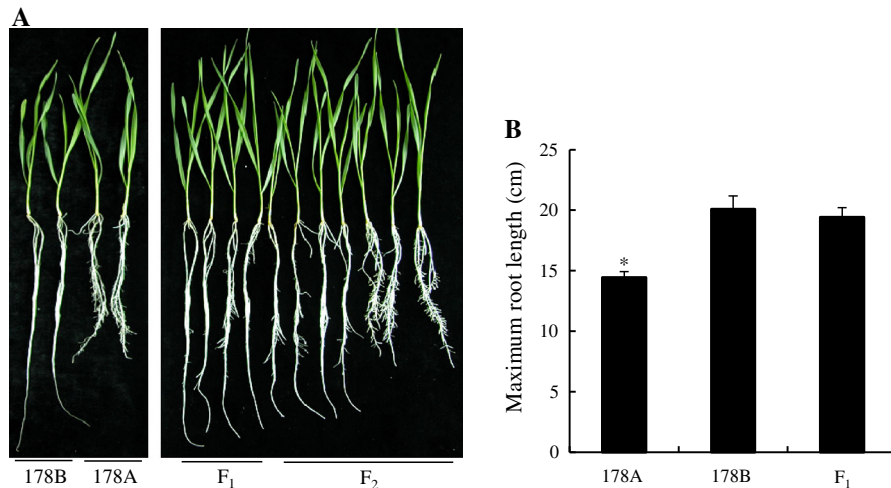


Fig. 1 Comparison of the seedling roots of 178A, 178B, and the F₁ and F₂ individuals derived from 178A × 178B. **a** Morphological differences between the root systems of typical 178A, 178B, and F₁ and F₂ plants. The seedlings shown were photographed under the same magnification. **b** Quantitative

differences in maximum root length (MRL) among 178A, 178B, and F₁ plants. The data set displayed is representative of three separate measurements. Asterisk indicates statistically significant difference ($P < 0.01$) from the mean MRL values of 178B and F₁ plants

tetraploid wheat were investigated with the genotyping data collected from 127 F₂ individuals descended from the cross Langdon × WEW270 (see above).

Statistical analysis

All data were calculated using Excel 2010, and the results were presented as mean ± standard error. Statistical comparison of the data was performed with the software IBM SPSS Statistics 19. Chi squared tests for goodness-of-fit were used to compare the observed and theoretically expected segregation ratios in the F₂ population as described previously (Chernoff and Lehmann 1954).

Results

Genetic analysis of root system difference between 178A and 178B

When hydroponically cultured seedlings of 178A, 178B, and their F₁ hybrid were inspected, the root system of 178B was substantially longer than that of 178A, with all F₁ samples exhibiting a longer root phenotype (Fig. 1a). Quantitative comparison of MRL confirmed the above observation (Fig. 1b). In contrast, F₂ seedlings exhibited clear segregation of longer and

shorter root phenotypes (Fig. 1a). The segregation ratio between the two root phenotypes was examined in a subsample of 1,933 F₂ seedlings derived from 178A × 178B. The numbers of F₂ individuals with longer or shorter roots were 1,458 and 475, respectively, which fitted a 3:1 segregation ratio according to the Chi squared test ($\chi^2 = 0.021$, $\chi^2_{0.05, 1} = 3.84$). This segregation ratio, plus the root phenotype of the F₁ seedlings (Fig. 1a), indicated that the longer root phenotype displayed by 178B was dominant over the shorter root phenotype exhibited by 178A.

Comparison of growth performance between 178A and 178B

Two independent pot cultivation experiments were carried out to compare the growth of 178A and 178B under the conditions of high and low levels of P supply. In both experiments, considerable differences were found between 178A and 178B in terms of shoot and root dry weight accumulation and shoot P content. Generally, the values determined for 178B were significantly higher than those for 178A (Fig. 2).

Further genetic mapping of *qTaLRO-B1*

A prerequisite for further mapping of *qTaLRO-B1* was the finding of DNA markers that were polymorphic

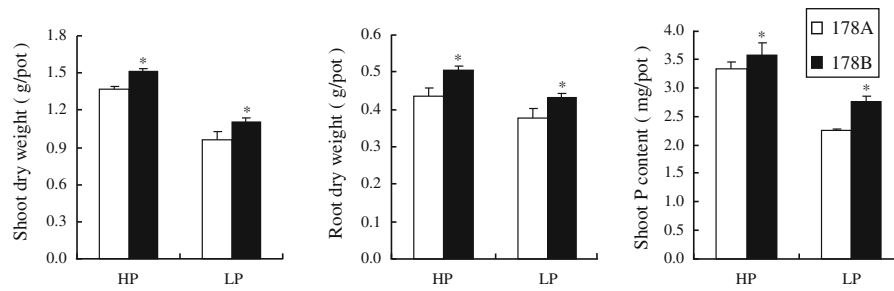


Fig. 2 Comparison of shoot dry weight, root dry weight, and shoot phosphorus (P) content between 178A and 178B under high P (HP) or low P (LP) growth conditions. The data set shown

is representative of two separate pot culture experiments. Asterisk indicates statistically significant difference ($P < 0.05$) between the measurements of 178A and 178B

between 178A and 178B and yet located in the chromosomal bin 2BS4-0.84-1.00 where *qTaLRO-B1* resided (see “Materials and methods”). We tested a wide range of SSR and EST markers previously found in 2BS4-0.84-1.00, but none of them showed polymorphism between 178A and 178B. Consequently, efforts were made to develop new STS markers from the genes syntenically conserved between the distal part of wheat 2BS chromosome and its orthologous region on the short arm of chromosome 5 of *B. distachyon* (Bd5S, Liu et al. 2012). Although more than 120 genes from Bd5S were used to search conserved wheat ESTs, and nearly 100 pairs of conserved primers were designed, only seven primer sets amplified polymorphic PCR products that were confirmed to be located on the distal region of 2BS by analysis with Chinese Spring, N2AT2B, N2BT2D, and N2DT2A. The seven primer sets represented STS markers newly developed by this work, and were designated *STS1^{TaLRO-B1}* to *STS7^{TaLRO-B1}* (Table 1). Except for *STS3^{TaLRO-B1}*, which was a dominant marker, the remaining six were all co-dominant for 178A and 178B (Table 1). The alleles of *STS1^{TaLRO-B1}*, *STS3^{TaLRO-B1}*, and *STS4^{TaLRO-B1}* were differentiated by length polymorphism, whereas those of the remaining four markers were distinguished by SSCP (Table 1; Supplementary Fig. 1).

With the genotyping and MRL data from 5087 F2 individuals, a linkage map consisting of 10 DNA markers and *qTaLRO-B1* was constructed (Fig. 3). The total genetic distance covered by this regional map was 58.7 cM. *STS5^{TaLRO-B1}* and *STS6^{TaLRO-B1}* were the closest flanking markers of *qTaLRO-B1*, and their genetic distances to *qTaLRO-B1* were 0.1 and 0.3 cM, respectively (Fig. 3). The distances of *qTaLRO-B1* to *Xgwm210* and *Xbarc1138.2* were 0.4

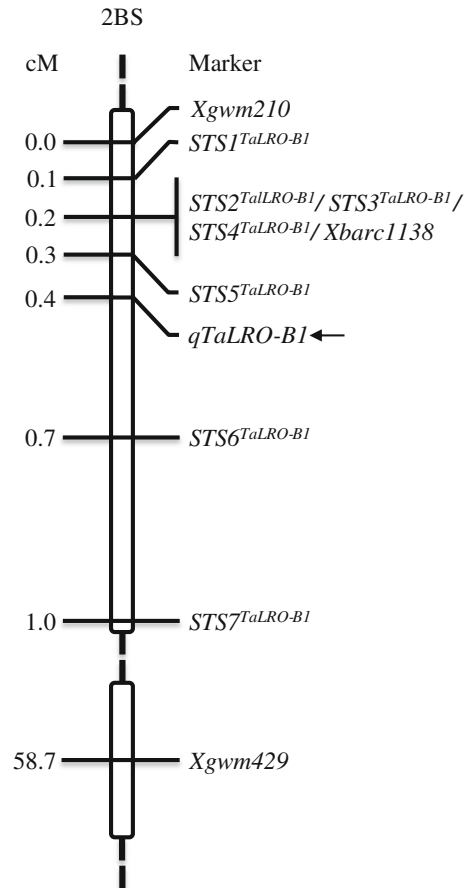


Fig. 3 Genetic linkage map of the region carrying *qTaLRO-B1* on the short arm of chromosome 2B (2BS) of common wheat, constructed with seven STS (*STS1^{TaLRO-B1}*–*STS7^{TaLRO-B1}*) and three microsatellite (*Xgwm210*, *Xbarc1138*, and *Xgwm429*) markers. The genetic distance value (cM) is shown in the left panel. Arrow indicates the position of *qTaLRO-B1*

and 0.2 cM, respectively (Fig. 3). Finally, it was observed that *STS2^{TaLRO-B1}*, *STS3^{TaLRO-B1}*, *STS4^{TaLRO-B1}*, and *Xbarc1138.2* were genetically linked together

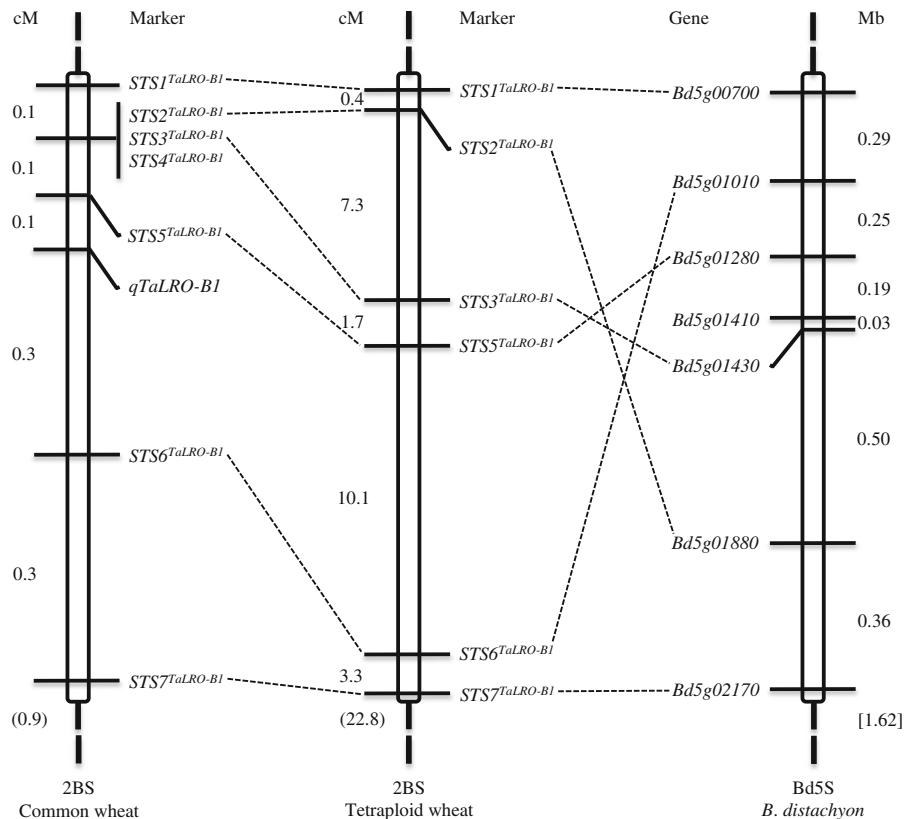


Fig. 4 Comparative genomic analysis of the 2BS chromosomal region hosting *qTaLRO-B1* and its orthologous counterparts located on the 2BS chromosome of tetraploid wheat or the short arm of chromosome 5 of *B. distachyon* (Bd5S). Orthologous loci are connected by *dashed lines*. *STS4^{TaLRO-B1}* could not be mapped in the tetraploid wheat population developed by this work owing to lack of polymorphism

(Fig. 3), despite the analysis of more than 5000 F2 plants.

Comparison of *qTaLRO-B1* region and its orthologous counterparts in tetraploid wheat and *B. distachyon*

For this set of analyses, we concentrated on the newly developed STS markers as they were more likely to be collinearly conserved among the three species. In tetraploid wheat, the loci corresponding to *STS1^{TaLRO-B1}* and *STS7^{TaLRO-B1}* were all conserved. However, *STS4^{TaLRO-B1}* could not be mapped because it was not polymorphic between the parents of the tetraploid wheat F2 population developed in this work. For the six markers that were mapped in tetraploid wheat, their genetic

between the parental lines. The total genetic distance value from *STS1^{TaLRO-B1}* to *STS7^{TaLRO-B1}*, as shown in the *parentheses*, was substantially larger in tetraploid wheat (22.8 cM) than in common wheat (0.9 cM). The total physical distance covered by the seven *B. distachyon* gene loci, as indicated in the *square brackets*, was approximately 1.62 Mb

positions were collinear to those found in common wheat (Fig. 4). Interestingly, the genetic distance value from *STS1^{TaLRO-B1}* to *STS7^{TaLRO-B1}* was 22.8 cM, which was approximately 25.3 times larger than that covered by the two markers in common wheat (Fig. 4). Moreover, *STS2^{TaLRO-B1}* and *STS3^{TaLRO-B1}* were separated in tetraploid wheat, in contrast to their tight linkage in common wheat (Fig. 4). Although MRL also segregated among the F2 progenies in the tetraploid population, the gene involved was not genetically linked to any of the seven STS markers (data not shown).

In *B. distachyon*, the loci corresponding to *STS1^{TaLRO-B1}* and *STS7^{TaLRO-B1}*, as represented by seven annotated genes, were also conserved, but the chromosomal segment corresponding to that covered from *STS2^{TaLRO-B1}* and *STS6^{TaLRO-B1}* in wheat was

inverted (Fig. 4). The physical distance spanned by the seven *B. distachyon* gene loci was approximately 1.62 Mb, whereas that between *Bd5g01280* and *Bd5g01010*, which were orthologous to *STS5^{TaLRO-B1}* and *STS6^{TaLRO-B1}*, respectively, was about 0.25 Mb (Fig. 4).

Discussion

Conversion of *qTaLRO-B1* into a major gene controlling root growth and function in common wheat

Prior to this work, many QTLs controlling root traits have been identified in common wheat (see “Introduction”) and other crop species (e.g., Zhang et al. 2001; Tuberosa et al. 2002a, b; Fita et al. 2008; Liang et al. 2010; Obara et al. 2010; Ron et al. 2013; Topp et al. 2013), indicating that genetic and functional dissection of QTLs is becoming an essential avenue for understanding the genes involved in root growth and function of crop plants. However, for more advanced characterization of an important QTL, it is often necessary to convert it into a major gene with qualitative effect on its target trait (Shen et al. 2001; Tuberosa and Salvi 2007). Therefore, we developed the QTL isolines 178A and 178B, and investigated whether *qTaLRO-B1* might behave as a major gene to control root length in a Mendelian fashion in the genetic population derived from 178A × 178B. From the data shown in Figs. 1 and 2, it is clear that the phenotypic difference between the root systems of 178A and 178B seedlings was caused by allelic variation of a single major gene, and that the root system of 178B was not only longer (in length) and larger (in biomass), but also more active (in inorganic P uptake), than that of 178A. Under our hydroponic culture conditions, the total root surface area (RSA) of 178B ($69.7 \pm 7.1 \text{ cm}^2$) was also significantly higher than that of 178A ($36.7 \pm 2.7 \text{ cm}^2$). The longer length, larger biomass, and higher RSA of 178B’s root system agree well with the enhanced P accumulation found in this genotype relative to its isolate 178A (Fig. 2). Because 178A and 178B were isolines derived from a single F5 plant and differed mainly at the *qTaLRO-B1* locus, we suggest that the difference between the root systems of 178A and 178B is controlled by allelic variation of *qTaLRO-B1*, and that

the long root (*LR*) allele in 178B (originally from Xiaoyan 54) is dominant over the short root (*SR*) allele in 178A (originally from Jing 411).

The contrasting effects conferred by the *LR* or *SR* alleles of *qTaLRO-B1* are consistent with previous observations on the possession of larger root biomass and higher phosphorus uptake capacity by Xiaoyan 54 but not Jing 411 (Yan et al. 2010). Since Xiaoyan 54 has also been found to be efficient in acquiring nitrogen (N) from soil (Zhang et al. 2005), we are now in the process of investigating whether 178B has a higher N uptake rate than 178A. As several major root trait QTLs have been found to affect grain production (Landi et al. 2007, 2010; Ehdai et al. 2010; Suji et al. 2012), it will also be important in further research to test whether 178A and 178B differ in yield level.

Improved understanding of *qTaLRO-B1* and the chromosomal region carrying *qTaLRO-B1*

Although our earlier work detected *qTaLRO-B1* in common wheat, knowledge of the genetic location of this locus and the chromosomal segment carrying it was limited (Ren et al. 2012). Based on the linkage mapping and comparative genomic analysis data collected in this work, we have improved understanding of *qTaLRO-B1* in three respects. First, with the development and mapping of seven STS markers, the genetic location of *qTaLRO-B1* in 2BS is now better defined than before. Second, judging from the collinearity between the seven STS markers and their orthologs in tetraploid wheat, there is a high level of synteny between the *qTaLRO-B1* region in common wheat and its orthologous counterpart in tetraploid wheat, although no MRL controlling gene was detected in the latter by this work. The loci corresponding to the seven STS markers are also conserved in *B. distachyon*. However, the order of these loci in *B. distachyon* shows some differences from that in common wheat because of a local inversion occurring in the relevant chromosomal region in the former species. Finally, the recombination frequency of the chromosomal region carrying *qTaLRO-B1* is substantially lower than that of its syntenic counterpart in tetraploid wheat. This is supported by (1) a large difference between the genetic distances covered by the same set of markers in common wheat and tetraploid wheat, and (2) a clear separation of *STS2^{TaLRO-B1}* from *STS3^{TaLRO-B1}* in tetraploid wheat but not common wheat.

The low recombination frequency in the *qTaLRO-B1* region, as compared to that of its syntenic segment in tetraploid wheat, is very likely caused by the suppression of meiotic recombination. This phenomenon has been found for a number of chromosomal regions harboring important genes in plants. For example, recombination frequency is low in the region carrying *Pi-ta*, a gene conferring strong resistance to *Magnaporthe oryzae* in rice (Jia 2009; Jia et al. 2012). In wheat, suppressed recombination has been found in the genomic region hosting *Lr20-Pm1* conferring resistance to leaf rust and powdery mildew diseases (Neu et al. 2002), and the chromosomal region carrying *Ppd-B1* regulating photoperiod response (Mohler et al. 2004). Although the molecular basis of recombination suppression in plants is still not completely resolved (Jia et al. 2012), two possible mechanisms are frequently discussed. The first is that the presence of complex chromosomal structure or rearrangements tends to lead to recombination suppression (Gill et al. 1996a, b; Neu et al. 2002; Kim et al. 2005). The second mechanism is that strong natural or artificial selections may result in certain linkage blocks that have greatly suppressed recombination (Yonemaru et al. 2012). Since *qTaLRO-B1* confers positive benefits on the growth of both root and above-ground organs, and is detected in different elite common wheat varieties (Ren et al. 2012; this work), it is possible that the *qTaLRO-B1* region has experienced enhanced artificial selection, and thus exhibits substantially lowered recombination frequency. As an initial test for this possibility, we investigated the allelic variation of *STS2^{TaLRO-B1}*, *STS3^{TaLRO-B1}*, and *STS4^{TaLRO-B1}* in 94 elite common wheat varieties released mainly from the 1980s to 2000s (Zhang et al. 2013). The number of varieties having 178B-type alleles in the three loci was 60, whereas that with 178A-type alleles across these loci was only nine, with the remaining varieties carrying alternative allele(s) in one or more of the three loci (data not shown). This provides a preliminary support for the existence of strong artificial selection for the *qTaLRO-B1* region. Molecular cloning of *qTaLRO-B1* will enable a more detailed examination of this possibility in the future.

Implications for further research of *qTaLRO-B1*

From the discussions above, it is apparent that substantial efforts are still needed to fine-map, and to

clone, *qTaLRO-B1*. Due to recombination suppression, the populations derived from 178A × 178B are not suitable for fine mapping of *qTaLRO-B1*. Consequently, more genetic populations involving 178B are being prepared and evaluated. Concomitantly, additional STS markers are being developed by comparing *B. distachyon* genes located between *Bd5g01280* and *Bd5g01010* (which corresponded to *STS5^{TaLRO-B1}* and *STS6^{TaLRO-B1}*, respectively; Fig. 4) with wheat ESTs. The application of these new resources should enable further improvement in understanding the *qTaLRO-B1* region, and may help to identify more DNA markers and donor genotypes suitable for transferring *qTaLRO-B1* in MAS breeding aimed at improving common wheat root size and function.

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