Genome-wide association study on root traits under different cultivation patterns in wheat

Fengdan Xu (✉ fengdanxu1989@163.com)

Henan Agricultural University

Shulin Chen

Henan Agricultural University

Xiwen Yang

Henan Agricultural University

Sumei Zhou

Henan Agricultural University

Xu Chen

Henan Agricultural University

Junsen Wang

Henan Agricultural University

Ziliang Zhang

Henan Agricultural University

Yuan Huang

Henan Agricultural University

Miao Song

Henan Agricultural University

Shaoyu Han

Henan Agricultural University

Kehui Zhan

Henan Agricultural University

Dexian He

Henan Agricultural University

Research article

Keywords: wheat, Yellow and Huai Winter Wheat Region (YHW), root traits, yield, Genome-wide association study (GWAS), co-localization region

Posted Date: June 12th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-27846/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Background

Roots are critical for water and nutrient acquisition, environmental adaptation and yield formation.

Results

Here, 196 accessions from Yellow and Huai Winter Wheat Region (YHW) of China were collected for investigating the performance of six root traits under three cultivation patterns—Indoor Hydroponic Culture (IHC), Outdoor Hydroponic Culture (OHC) and Outdoor Pot Culture (OPC) at different growth stages—for three consecutive years. In the same growth period, OPC root traits always varied greatly, followed by OHC and IHC. The correlation coefficients between IHC and OPC at stooling stage (SS) were lower (0.016 ~ 0.278) than those between OHC and OPC (0.29 ~ 0.378). Root traits were negatively correlated grain yield (GY), the canonical correlation coefficient between root traits and yield was the highest (0.232) at SS. Genome-wide association study (GWAS) was furtherly conducted by a wheat 660K SNP array. It was revealed that 1105 SNP loci were significantly associated with root traits. A co-localized chromosomal segment regulating total root length (TRL) was detected on chromosome 4A, spanning from 737.85 to 742.00 Mb, under different cultivation patterns at stooling stage. Another co-localization region regulating total root area (TRA) was detected on chromosome 5A, an approximately 6.17 ~ 18.76 Mb region at SS, wintering stage (WT) and jointing stage (JS) under OPC. LD analysis and blast comparison revealed 27 and 31 genes related to root development were found from these two segments, respectively. Among them, *TraesCS4A02G493900*, *TraesCS4A02G494200*, *TraesCS5A02G021700*, *TraesCS5A02G021800* and *TraesCS5A02G011600* were predicted to be highly expressed in root.

Conclusion

This work could deepen our understanding of the molecular mechanisms of root development under different cultivation patterns in common wheat.

Background

Wheat (*Triticum aestivum* L.) is a vital food crops for human beings, with many varieties grown in arid and semiarid regions [1]. As the population increases, the demand for food doubles approximately every 20 years, therefore, the continuous improvement of yield in wheat production is an urgent issue. Root systems facilitate aerial growth and are critical to yield [2]. Morphological characteristics of the root system include root length, surface area, volume and diameter and the number of root tips. Root length, surface area and volume affect the spatial arrangement of roots underground and are significantly associated with nutrient and water uptake. Larger-diameter roots have larger xylem vessels and thus enhance a plant's drought tolerance [3]. Branching roots (root tips) constitute the major components of the root system. A greater number of root tips leads to a higher absorption efficiency of water and nutrients by increasing root surface area and volume, thus enhancing yield [4].

However, extracting complete roots from the soil is time consuming and labor intensive and often yields low-accuracy measurements of root traits. To overcome these challenges, researchers have adopted indoor cultivation methods combined with digital imaging to dissect root traits from wheat seedlings. These methods include the use of hydroponic culture [5], gel-filled chambers [6], germination bags [7], sand-filled pots [8], soil-filled pots [9], a germination paper-based 'pouch and wick' system [10, 11] and soil-filled columns scanned by X-rays [12]. Researchers are increasingly aiming to elucidate the mechanisms regulating root growth and development through forward genetic strategies, although polygenic traits present a challenge. For instance, Liu et al. [13] identified 46 QTLs of root morphological traits located on 17 of 21 chromosomes, i.e., all except 1D, 4D, 6B and 6D, which explained 4.98 to 24.31% of the phenotypic variation in these traits. Ren et al. [14] studied a recombinant inbred line (RIL) population of Xiaoyan 54 × Beijing 411 wheat and detected 35 QTLs, which were mainly distributed on chromosomes 2B, 2D, 4B, 6A, 6B and 7B. Furthermore, researchers identified a root length QTL (*qTaLRO-B1*) between *Xgwm210* and *Xbarc1138.2* on chromosome 2BS. Cao et al. [9] mapped *qTaLRO-B1* to a 0.9-cM interval by crossing near-isogenic lines (NILs) 178A and 178B. Furthermore, a few genes regulating wheat root morphology have been found by reverse genetics methods, including *TaZFP34* [15], the lateral organ boundaries (LOB) family member *TaMOR* [16] and the family of zinc finger proteins *KRAB-ZFPs* [17].

Due to the polyploidy of wheat and the lack of a reliable reference sequence, research on quantitative traits in hexaploid wheat has lagged behind that in other crops, especially research on root traits. Genome-wide association study (GWAS) is a powerful tool for identifying loci that are significantly associated with target traits based on linkage disequilibrium (LD) in natural populations. In recent years, with the increasing availability of genomic sequences and genotyping by sequence data, GWAS has become a rapid and cost-effective way to detect and transform markers for marker-assisted breeding [18]. Many types of SNP arrays from functional genes have been successfully developed for the identification of QTLs for various traits in natural populations; such arrays include 9K [19], 55K [20], 90K [21, 22] and 660K arrays [23]. Yield traits have been reported to be closely related to root traits, and some QTLs for root traits have been found to co-localize with yield traits [11, 24].

The root system is underground and difficult to identify in situ. Previous research on wheat roots has mainly involved QTL mapping of seedlings from specific populations (RIL, NIL and DH populations) by using a small number of markers under hydroponic indoor culture; thus, the molecular basis of many traits remain unclear. In view of this, in the present study, 196 wheat accessions from YHW of China were used as representatives of natural populations, and root traits were investigated under three cultivation patterns at four growth stages (SS, WT, JS and maturation stage (MS)). In addition to investigating yield in the field, GWAS of the panel was conducted with the wheat 660K SNP array. The aim of this study was to elucidate the relationships between root and yield and explore associated SNPs or candidate genes for root traits. This work not only provides valuable genetic information for genetic improvements to the wheat root system but can also be used to screen favorable genotypes for breeding new lines with better root systems.

Results

Phenotypic variation of root traits in wheat

The variations in root traits among the three cultivation patterns at the four growth stages of the 196 wheat accessions are provided in Table S3. The frequency distributions of the six studied root traits were continuous and distributed normally (Fig. S1). The joint variance analysis of root traits indicated that the effects of genotype (variety) and genotype $\times$ environment were highly significant ($p < 0.01$) (Table 1). These results indicated that the root traits were quantitative traits and controlled by minor polygenes.

Table 1
Phenotype variation and heritability of 6 root traits of the 196 wheat accessions at different growth stages and patterns

IHC							OHC					
Trait	Range	mean	CV (%)	F (G)	F (G*E)	h_B^2 (%)	Range	mean	CV (%)	F (G)	F (G*E)	h_B^2 (%)
TRL (cm)	310.3 ~ 806.4	524.8	22.6	-	-	-	564.7 ~ 1271.3	833.3	14.2	11.4**	4.6**	63.2
TRA (cm ²)	40.2 ~ 125.9	77.4	23.3	-	-	-	77.5 ~ 191.1	112.0	14.4	9.2**	4.9**	59.2
ARD (mm)	0.39 ~ 0.62	0.47	8.3	-	-	-	0.32 ~ 0.52	0.43	5.8	7.1**	6.1**	45.9
TRV (cm ³)	0.4 ~ 1.6	0.9	26.8	-	-	-	0.6 ~ 2.6	1.6	17.0	6.1**	4.7**	50.8
NRT (count)	196.3 ~ 847.0	471.1	31.2	-	-	-	335.8 ~ 923.7	606.5	17.2	9.1**	5.0**	53.9
RDW (mg)	9.8 ~ 37.5	21.7	27.6	-	-	-	25.2 ~ 53.8	37.8	12.3	81.7**	72.7**	54.0
SS							WT					
Trait	Range	mean	CV (%)	F (G)	F (G*E)	h_B^2 (%)	Range	mean	CV (%)	F (G)	F (G*E)	h_B^2 (%)
TRL (cm)	224.0 ~ 1008.0	524.6	27.0	7.1**	6.8**	48.0	637.9 ~ 1556.6	1021.0	35.9	6.0**	5.4**	54.2
TRA (cm ²)	44.1 ~ 168.8	95.3	24.4	6.7**	5.8**	49.9	93.3 ~ 244.2	155.2	23.7	5.9**	5.4**	51.5
ARD (mm)	0.42 ~ 0.80	0.58	8.1	5.9**	6.4**	43.0	0.28 ~ 0.38	0.33	27.1	2.7**	2.4**	49.7
TRV (cm ³)	0.6 ~ 3.0	1.5	28.3	6.6**	5.1**	51.8	0.9 ~ 3.7	2.1	27.3	4.9**	4.7**	48.5
NRT (count)	214.3 ~ 1198.8	589.2	38.2	8.3**	8.1**	47.1	944.0 ~ 2826.7	1832.1	37.5	5.4**	5.7**	50.7
RDW (mg)	22.6 ~ 71.1	44.0	24.4	131.2**	115.7**	51.0	72.5 ~ 185.0	120.0	19.1	243.6**	200.5**	55.9
JS							MS					
Trait	Range	mean	CV (%)	F (G)	F (G*E)	h_B^2 (%)	Range	mean	CV (%)	F (G)	F (G*E)	h_B^2 (%)
TRL (cm)	1241.0 ~ 3651.4	2268.3	22.1	3.2**	3.1**	39.9	1595.3 ~ 5310.9	3139	24.5	3.7**	3.2**	46.3
TRA (cm ²)	170.4 ~ 578.0	363.2	23.7	2.5**	2.5**	36.7	173.4 ~ 911.4	455.1	30.8	3.9**	3.7**	43.1
ARD (mm)	0.22 ~ 0.51	0.39	15.0	4.3**	4.4**	43.8	0.25 ~ 0.52	0.36	15.2	4.3**	4.1**	44.6

Note: G: Genotype; E : Environment; h_B^2 : broad sense heritability; - : indicated that data was missing; **Significant at $p < 0.01$. The same below

	IHC						OHC					
TRV (cm ³)	2.0 ~ 8.2	4.8	28.8	2.2**	2.3**	33.8	2.1 ~ 13.7	5.90	41.9	3.9**	3.8**	43.4
NRT (count)	1535.3 ~ 5187.4	3256.3	24.3	2.4**	2.1**	40.0	2999.4 ~ 9369.3	5040.0	23.8	3.4**	2.5**	49.5
RDW (mg)	200.1 ~ 572.8	383.0	22.7	7.4**	5.1**	57.3	150.2 ~ 922.9	448.5	34.8	72.3**	57.8**	55.0
Note: G: Genotype; E : Environment; h_B^2 : broad sense heritability; - : indicated that data was missing; **Significant at $p < 0.01$. The same below												

Within the same developmental period, root growth outdoors was more rapid than that indoors. The root traits of OPC plants (22.6 ~ 71.1 mg) varied the most among the culture systems, followed by those of OHC (25.2 ~ 53.8 mg) and IHC (9.8 ~ 37.5 mg). The root heritabilities under OHC were 45.9 ~ 63.2%, which were slightly higher than those under OPC (43.0 ~ 51.8%) (Table S4). The average u value for each accession was used to evaluate the root development. Luomai 23, Anmai 8, Pu 2056, Yumai 54, Pingan 9 and Bonong 6 had similar u values under the three cultivation patterns. Root system development of the Luomai 23 cultivar was poor, with low u values under IHC (-1.32), OHC (-1.29) and OPC (-1.23). Yumai 54 developed roots quickly, as evidenced by its high u values, under IHC (1.33), OHC (1.18) and OPC (1.15).

The growth of the wheat root systems was not uniform across the growth period. The average u value of each variety was used to evaluate the root morphology response during different growth stages. Zhengpinmai 8, Luohan 6 and Yanke 028 had high u values at SS; Zhoumai 13, Zhengyumai 043 and Chinese Spring had high u values at WT; Zhengmai 379, Yunong 9901 and Jinmai 47 had high u values at JS; Luohan 3, Zhengyumai 043 and Yumai 51 had high u values at MS; Kaimai 18, Zhongchuang 805 and San 160 had low u values at SS; Pu 2056, San 160 and Jinan 17 had low u values at WT; Luomai 24, Yumai 52 and Fanmai 11 had low u values at JS; and Yunong 949, Luomai 24 and Yujiao 5 had low u values at MS. Some varieties developed rapidly in the early growth stage, while others developed rapidly after being stimulated by the environment (e.g., low temperature).

Correlations Among Root Traits

Canonical correlation analysis showed that among the three cultivation patterns at stooling stage in wheat, the strongest correlation of root traits was between OHC and OPC (0.515), followed by OHC and IHC (0.421) and then IHC and OPC (0.385) (Table S5). The correlation coefficients of all root traits except average root diameter (ARD) were highest for IHC and OHC ($r > 0.3$). The correlations between IHC and OHC were moderately high, with correlation coefficients of approximately 0.2. However, the correlations between IHC and OPC were low, with correlation coefficients close to zero (Table 2). The canonical correlation analysis showed that the correlation between IHC and OPC was low at every stage. The correlation between OHC and OPC was higher than that between IHC and OPC and highly significant at SS (0.515) and WT (0.409). Under OPC, the correlation between SS and WT was stronger than the correlations involving JS and MS. The results indicated that root traits showed stronger correlations between OHC and OPC and weaker correlations between IHC and OPC. However, the significant correlations among root traits revealed strong coordination under the three cultivation patterns and four growth stages (Table S6). The coefficients of correlation between TRL and TRA and between TRA and total root volume (TRV) were the highest among the traits ($r > 0.85$); the correlation coefficients among TRL, TRA, TRV and number of root tips (NRT) were moderately high ($r > 0.60$), while the correlation between ARD and TRV was weak ($r = 0.5$). In contrast, ARD was not correlated with TRL, TRA, NRT or root dry weight (RDW), with correlation coefficients close to zero or negative (except for MS).

Table 2
Correlation coefficients of wheat root traits at different growing stages and growing pattern

Traits	TRL	TRA	ARD	TRV	NRT	RDW
IHC-OHC	0.335**	0.256**	0.189*	0.162*	0.271**	0.124
IHC-SS	0.059	0.066	0.163*	0.103	0.278**	0.016
IHC-WT	0.048	0.046	0.079	0.015	0.058	-0.016
IHC-JS	0.057	0.034	-0.010	0.002	0.168**	-0.066
IHC-MS	0.061	0.104	0.027	0.133	0.080	0.183*
OHC-SS	0.292**	0.377***	0.093	0.337***	0.323***	0.378***
OHC-WT	0.163*	0.172*	0.174*	0.162*	0.131*	0.161*
OHC-JS	0.067	0.109	0.161*	0.122*	-0.002	-0.030
OHC-MS	0.086	0.119*	0.010	0.101	0.085	0.116*
SS-WT	0.376***	0.337***	0.060	0.218**	0.218**	0.347***
SS-JS	0.101	0.138	-0.057	0.096	0.121*	0.135*
SS-MS	0.163*	0.255**	-0.007	0.236**	-0.037	0.255**

Correlations Of Root Traits With Yield

Canonical correlation analysis indicated that root traits and yield had the strongest relationship during SS (0.232), followed by JS (0.183), WT (0.167) and MS (0.143) (Table 3). Grain yield (GY) was significantly negatively correlated with TRL (-0.146), TRA (-0.157) and NRT (-0.150) at SS and negatively correlated with TRA (-0.122) and TRV (-0.131) at WT. The coefficients of correlation between root traits and GY were low at both MS and JS. The results showed that root traits were negatively correlated with GY and that yield was most strongly correlated with root traits at stooling stage.

Table 3
Correlation coefficients between root traits and grain yield traits of 196 wheat accessions

Traits	SS	WT	JS	MS
TRL	-0.146*	-0.098	0.002	-0.055
TRA	-0.157*	-0.122	-0.003	-0.048
ARD	-0.052	-0.001	0.014	0.045
TRV	-0.123	-0.131	-0.015	-0.031
NRT	-0.150*	-0.011	-0.036	-0.015
RDW	-0.138	-0.009	-0.037	-0.066
V	0.232	0.167	0.183	0.143
Note: V was the Canonical correlation coefficient				

GWAS of root traits in wheat

A total of 2361 SNP loci associated with root traits were detected. The loci were distributed across all 21 chromosomes, with an R^2 range of 1.2 ~ 22.50% (Table S7). Among the three cultivation patterns, OHC was associated with the largest number of SNP loci (824), followed by OPC (402), and the fewest root-related SNP loci were detected under IHC (298). Among the four root developmental stages, JS was associated with the largest number of SNP loci (726), followed by MS (490), SS (402) and WT (292). Root traits had an h_B^2

range of 40 ~ 60% and were strongly affected by the environment. Using best linear unbiased estimation (BLUE) values of wheat root traits, a total of 1105 SNPs were detected, with an R^2 range of 6.38 ~ 19.43% (Table S7, S8; Fig. S2). The largest number of SNP loci were detected for ARD (391), followed by TRA (267), TRL (244), NRT (233), TRV (215) and RDW (194).

Some SNP loci for the same root trait from two or more stages or cultivation patterns were detected in similar physical locations. Taking TRL as an example (Table 4), in the 737.85 ~ 742.00 Mb region on chromosome 4A, 1, 1 and 1 SNP was significantly associated with TRL under IHC, OHC and OPC, respectively, at stooling stage. Similarly, in nearby intervals of chromosomes 1B, 1D, 2B, 4B, 5B and 6B, TRL-associated SNP loci were detected for two stages or cultivation patterns. Significant associations between chromosomal segments and other root traits are presented in Table S9. 1, 1 and 5 SNP loci associated with TRA were detected within a 6.17 ~ 18.76 Mb segment in chromosome 5A at SS, WT and JS, respectively.

Table 4
The genetic positions of SNPs associated with TRL at different growing stages

SNP marker	genetic position (Mb)	Growing Stages
1B	641.24 ~ 642.27	OHC,MS
1D	464.37 ~ 465.44	OHC,SS
1D	480.52 ~ 484.26	OHC,WT
2B	3.41 ~ 13.07	OHC,JS
2B	781.58 ~ 785.89	IHC,JS
4A	737.85 ~ 742.00	IHC,OHC,SS
4B	665.59 ~ 657.45	OHC,MS
5B	696.34 ~ 701.69	OHC,SS
6B	658.86 ~ 668.71	SS,JS

Because the six root traits were closely associated with one another, some SNP loci were associated with multiple traits. For instance, under IHC, OHC and OPC, 9, 29 and 21 SNP loci associated with two or more root morphology traits, respectively, were detected, which were distributed on all chromosomes except 1A and 4D. At WT and JS, 5 and 39 SNP loci associated with two or more root traits, respectively, were detected, which were from chromosomes 1D, 2A, 2B, 3B, 4A, 5A, 5B, 6A, 6B, 6D and 7B. However, at MS, no pleiotropic-effect SNP loci were found. *AX-94437665* and *AX-111613906*, detected at JS, were associated with four root traits (TRL, TRA, TRV and NRT). Moreover, 5, 1 and 11 SNP loci detected at SS under OHC, SS under OPC and JS under OPC, respectively, were simultaneously associated with three root traits (Table S10).

Haploblock analysis of TRL and TRA on associated chromosomal regions

Haploblock analysis was performed for the interval 737.85 ~ 742.00 Mb on chromosome 4A and the interval 6.17 ~ 18.76 Mb on chromosome 5A. At $r^2 = 0.1$, two and three blocks were identified at the chromosomal segments on 4A and 5A, respectively. Further analysis with the LD heatmap (Fig. 1; Fig. S3) revealed that the two blocks on chromosome 4A were located in the ranges of 738.86 ~ 739.51 Mb and 742.17 ~ 742.33 Mb, respectively, containing 18 and 9 genes, respectively. The three blocks on chromosome 5A were located in the ranges of 7.72 ~ 8.23 Mb, 8.28 ~ 8.80 Mb and 17.69 ~ 18.63 Mb, respectively, containing 16, 6 and 9 genes, respectively. Blast search against the Chinese Spring sequence (IWGSC V1.1) on the NCBI website revealed that 55 of the 58 genes were annotated. Functional prediction of orthologous genes in other species was performed; 46 and 18 genes were found to be homologous to those in rice and *Arabidopsis*, respectively (Table S11).

The gene expression data for the 58 genes in different wheat tissues were downloaded (Table S12) from the WheatExp website. Among them, 29 genes were expressed in all of the tissues root, stem, leaf, ear and grain, and eight genes were expressed in only some tissues; the remaining 21 genes were not expressed. In particular, *TraesCS4A02G493900*, *TraesCS4A02G494200*, *TraesCS5A02G021700* and *TraesCS5A02G021800* showed high expression levels in roots at three plant growth stages, and *TraesCS5A02G011600* was specifically expressed in the roots (Fig. 2). These five genes may participate in regulating root development.

Discussion

Effects of different cultivation patterns on wheat root development

Methods to acquire root system data for crops have always been a focus of root studies. Currently, approaches are mainly divided into two categories: field detection and indoor detection [25]. Traditional trench digging, soil core sampling [26] and soil column testing [27] in the field are labor-intensive and time-consuming methods for root phenotyping. Furthermore, traditional trench digging is not suitable for studies with large-population root QTLs or high-throughput analysis. Indoor detection involves growing plants in an incubator, an artificial climate chamber or a greenhouse to identify root traits. This method is convenient for obtaining the complete root system, although the root system may not be reflective of the root system in a complex field environment [28]. In this study, comparative analysis of root morphology under three cultivation patterns revealed that root development was least similar between IHC and OPC among the combinations of culture methods. Similarly, in rice, the ability of roots to penetrate a solid wax layer was found to be limited, and therefore, deep root lines were not consistently observed in the field [29]. Bai et al. [30] found that the correlation between laboratory-based root length screening data and field-based root depth data was weak and that there was no consistency in root depth across two years. However, in the present study, root development was comparable between OHC and OPC, and the root traits under OHC had a higher broad heritability. Under IHC, the root phenotypes showed little variation; however, the environment of root development in this type of system cannot reflect the complex root environment in the field. Under OHC, root development is affected by not only genotype but also the environment and the interaction of environment and genotype. Furthermore, under OPC, the soil environment is complex, reducing the effect of genotype on the root system relative to that in hydroponic culture. Considering the integrity and feasibility of root trait collection, OHC can be used to perform multiyear and multisite GWASs in large populations, and it can incorporate genomics information to elucidate the genetic basis of root development. Hence, OHC is a rapid root identification method worthy of promotion.

Although the root system is very sensitive to the external environment, correlations between root traits were observed under different cultivation methods. As indicated by their u values, a few wheat varieties had more uniform root development; Luomai 23 had a poor root system, and Yumai 54 had a large root system under all three cultivation patterns. These varieties may contain the main genes responsible for root development and provide breeding materials for wheat root research.

Genetic Basis Of Wheat Root Traits

A total of 1105 SNP loci associated with root system traits were detected. Alignment against the reference wheat genome (Chinese Spring) revealed that seven SNP loci detected in this study were at the same or similar positions as loci related to root system traits detected in previous studies. For example, under OHC, TRL-associated *AX-94990677* (2.49 Mb), detected on chromosome 2A, was in the same interval as *Xbarc1138_2A* detected by previous researchers [31]. Furthermore, RDW-associated *XPsr1327_1A* on chromosome 1A was close to the locus *AX-111044604* (583.36 Mb) detected at SS under OPC in the present study [32]. The GWAS results also showed that some SNP loci that control multiple root traits were detected under all three cultivation patterns or at all stages except the MS. These results are consistent with previous studies on roots in the seedling stage [14, 33]. A similar phenomenon for other wheat traits has been detected in previous studies. For example, disease resistance genes *Lr26*, *Yr9* and *Pm8* [34] and insect resistance genes are located on the 1B/1R chromosome translocation; these genes enhance yield and help maintain greenness [35].

In the present study, although the correlations of root traits among the four developmental stages were not high, some shared SNPs or segments among different cultivation patterns or among multiple stages were found. Candidate segments regulating TRL were detected within 737.85 ~ 742.00 Mb on chromosome 4A under the different cultivation patterns at stooling stage. Furthermore, candidate segments regulating TRA were detected within 6.17 ~ 18.76 Mb on chromosome 5A at three developmental stages.

Relationships Between Root Traits And Yield

Previous studies have identified some yield QTLs on chromosomes 2B, 2D, 4A, 4B, 5A, 7A, 7B and 7D [19–23]. Furthermore, QTLs of yield traits have been found to overlap with QTLs of root number and TRL in tetraploid wheat [3]. A QTL for TRL and seminal root number was mapped to chromosome 7DS and found to be associated with GY [11, 24, 36]. In addition, studies of the relationship between the root traits and yield of indoor seedlings revealed that the seminal root number and TRL of wheat were positively correlated with GY [3, 36] but negatively correlated or uncorrelated with thousand-kernel weight [25]. Atta et al. [37] found that wheat root traits

had weak positive correlations with grain number per spike, spikelet number, water use efficiency and yield. In the present study, root traits were negatively correlated with GY, inconsistent with previous studies. It is possible that in previous studies, the focus was on the indoor seedling stage, where the light is lower than natural light and the cultivation period is short. Therefore, only the seminal roots but no nodal roots were produced. In wheat production, the root system comprises both seminal and nodal roots [3]. During the middle and late growth stages, nodal roots are the main component of the root system, accounting for more than 70% of the root system [21]. Large roots can extend the grain-filling period. Developed roots lead to strong competition for effective assimilation within the plant (limited sources), and the formation of spikes may be restricted [38]. Accordingly, root traits were negatively correlated with GY, and the correlations were significant at stooling stage. An excessive root mass has been shown to cause inefficient consumption and reductions in yield [39]. Ma et al. [40] showed that reducing the root mass can improve water use efficiency in the late flowering stage of wheat and thus increase the harvest index. What's more, root traits were negatively correlated with GY and that yield was most strongly correlated with root traits at stooling stage. A robust root system in seedlings is conducive to the uptake of soil resources (such as N, P and others) during the early growth stage of plants [9], thereby largely relieving internal competition within the plant. However, some wheat grows in arid regions that lack water resources, and deep root systems are conducive to the use of deep water. Therefore, the root system at stooling stage may play a key role in wheat yield.

Prediction Of Candidate Genes Related To Wheat Root Development

Through GWAS and haplotype analysis, five out of 59 candidate genes regulating root development were found on chromosomes 4A and 5A. Functional annotation identified several similar genes that might affect root development. For example, two candidate genes on chromosome 5A, *TraesCS5A02G022100* and *TraesCS5A02G022300*, encode zinc finger domain proteins, these genes potentially play important roles in plant growth regulation and development, signaling and responses to abiotic stress [17, 41]. In wheat roots, the overexpression of *TaZFP34*, a gene encoding a zinc finger protein, can inhibit aboveground growth and increase the root-to-shoot ratio of plants [15]. Functional annotation showed that a root development-related candidate gene, *TraesCS4A02G484800*, is homologous to *OSINV3* and *AT1G12240*. The glycoside hydrolase family 32 encoded by the *AT1G12240* gene in *Arabidopsis* is involved in the decomposition and transformation of sucrose, which can promote hypocotyl elongation [42]. Similarly, *OSINV3* in rice is a gene encoding glycoside hydrolase family proteins. Electron microscopy revealed that the panicles of *OsINV3* mutant plants had small and few cells on the inner and outer membranes, indicating that *OsINV3* plays an important role in cell expansion [43]. However, the function of *TraesCS4A02G484800* in wheat has not been reported. This gene might regulate the elongation and formation of root cells through glycoside hydrolase, thereby affecting the development of the root system.

Expression data of five genes, *TraesCS4A02G493900*, *TraesCS4A02G494200*, *TraesCS5A02G011600*, *TraesCS5A02G021700* and *TraesCS5A02G021800*, revealed high expression levels in roots at three plant growth stages. Gene annotation revealed that the threonine dehydratase encoded by *TraesCS5A02G021800* is the first enzyme in the isoleucine biosynthetic pathway, and this gene's homologous gene, *OMR1*, similarly encodes a threonine dehydratase. Yu et al. [44] studied an *Arabidopsis* mutant with low isoleucine biosynthesis that has defects in cell proliferation and cell expansion during root development and found that the mutant root phenotype can be completely restored when externally supplementing isoleucine or introducing the *OMR1* gene into the mutant. Thus, *OMR1* might be involved in cell differentiation and formation during root development.

Conclusions

Within a given growth period, among the cultivation systems, OPC yielded the greatest RDW, OHC yielded rapid root elongation, and IHC yielded minimal variation in root morphological traits. The correlation coefficients of root traits were high between OHC and OPC, and root traits under OHC had higher broad heritability than those under the other systems; thus, OHC can be considered a rapid root identification method worthy of promotion. Root traits were negatively correlated with GY; the correlations were significant at SS. Because of the close relationships among root traits, the SNP loci detected by GWAS were found to be pleiotropic. Five candidate genes regulating root development were found on chromosomes 4A and 5A and might participate in regulating root development. Among these candidate genes, *TraesCS4A02G493900*, *TraesCS4A02G494200*, *TraesCS5A02G021700* and *TraesCS5A02G021800* showed high expression levels in roots at three plant growth stages, and *TraesCS5A02G011600* was exclusively expressed in roots. The proteins of these genes were identified as mainly involved in carbon metabolism, nitrogen metabolism, signal transduction, stress responses and DNA synthesis. Among the 196 wheat accessions, Luomai 23 had a poor root system, and Yumai 54 had a large root system under all three cultivation patterns.

Methods

Plant materials

The 196 wheat accessions used in this study included newly bred varieties, elite varieties and historical varieties from YHW. The accessions comprised 158 accessions from Henan Province, nine from Shaanxi, eight from Jiangsu, 10 from Shandong, four from Hebei, four from Beijing, one from Anhui, one from Shanxi and one land variety from Sichuan (Chinese Spring) (Table S1). Seeds were provided by Collaborative Innovation Center of Henan Grain Crops, Henan Agricultural University.

Experimental design

Hydroponic culture

Individuals were grown in an IHC system in a greenhouse, and the experiment was repeated three times. The greenhouse conditions included a 16 h/8 h light/dark photoperiod at 20 °C/16 °C and a light intensity of 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Fig. 3a). Plants grown in an OHC system were cultivated for three consecutive years from 20th October to 20th November in 2016, 2017 and 2018. The seeds were germinated in Petri dishes and grown on germination substrate for seven days with sterile water. Then, the germinated seeds with residual endosperm removed were transferred to plastic pots containing 20 L nutrient solution (Fig. 3b). The composition of the nutrient solution was as described by Ren et al. [14]. The nutrient solution was refreshed every three days, with the pH maintained at 6.0. Eight plants of each wheat accession were measured in each hydroponic culture experiment. The root experiments were conducted at Zhengzhou (34.7°N, 113.6°E), Henan, China.

Soil-filled pot experiments in the field

Pots were placed in the field in 2017~2018 and 2018~2019. Each pot (height, 10 cm; diameter, 12 cm) was filled with 2 kg of sieved tillage soil, and the top edge of each pot was on the same plane as the ground surface (Fig. 3c). The plants were regularly watered to 70~80% of field capacity at 15-day intervals after sowing. Other field management followed local agronomic practices. Uniform germinating seeds were placed in each pot. Ten days after sowing, each plot was thinned to two seedlings. Due to labor limitations, each wheat accession was planted in eight pots, with two plants per pot, and two pots with four plants were measured in each stage. To facilitate management, the total 1568 pots, representing all accessions, were distributed among 20 quadrats (length, 150 cm; width, 160 cm), with each quadrat containing 80 pots. A border was planted to protect the plants around the quadrats from marginal effects. The root experiments were conducted at Zhengzhou (34.7°N, 113.6°E), Henan, China.

Phenotyping of the 196 wheat accessions

For the seedlings in the hydroponic culture system, 30 days after sowing, the roots were rinsed with sterile water, and root traits were recorded. For the plants in the soil-filled pots, 30 days (SS), 60 days (WT), 150 days (JS) and 220 days (MS) after sowing, roots were removed from the soil and rinsed with fresh water before the root traits were measured.

The WinRHIZO system (Canada Regent Instruments, LA6400XL) was used to scan and analyze root morphology and measure ARD, TRL, TRV, TRA and NRT. After scanning, the roots were oven-dried at 80 °C to determine RDW using an analytical balance (Germany SARTORIUS, QUINTIX224-ICN) (Table S2).

We harvested plants to measure grain yield in Zhengzhou (34.7°N, 113.7°E) during 2014~2015 and 2015~2016, Shangqiu (33.4°N, 115.4°E) during 2014~2015 and Zhumadian (33.0°N, 114.1°E) during 2014~2015. The wheat had been planted in a north-south orientation. Each accession had been planted with a 23-cm row spacing in four rows, and the two middle rows (0.5 m) were harvested to measure GY·667 m². The 196 wheat accessions had been planted in a randomized block design with two replicates.

Statistical analysis

Joint variance analysis, estimation of broad-sense heritability and BLUE were performed for the phenotypic values obtained in each environment using IciMapping v4.0 software [63, 64]. Other analyses, including descriptive statistics and correlation and canonical correlation analyses, were conducted by using SPSS 19 software.

To facilitate a comprehensive evaluation, each root trait was converted into u values by the standardized normal distribution method using the equation where, for a given trait, x is the average value for the different environments and $\bar{x}$ and s are the arithmetic mean and standard deviation, respectively, of the 196 accessions. Then, the average u value was calculated for each accession at each stage. For each trait, a higher average u value suggested a larger root system.

GWAS and prediction of candidate genes

Genetic diversity, population structure and LD analyses of panel data were conducted according to Chen et al. [23]. Previous reports indicated that the 196 accessions could be divided into two subpopulations, which were largely consistent with the pedigrees and geographic origins. The optimal model for traits was taken into account when the actual $-\log_{10}(p)$ value was found to be closest to the expected value of $-\log_{10}(p)$. A mixed linear model correcting for both the Q-matrix and K-matrix (PCA + Q) was used to reduce population structure and relative kinship errors [46]. The best model was used to analyze associations among phenotypic data and BLUE values for each trait in Tassel v5.0. Here, the threshold for the p -value of $-\log_{10}(p\text{-value})$ was determined according to a uniform suggestive genome-wide significance threshold ($-\log_{10}(p) \geq 3.5$) [47]. Manhattan and Q-Q plots were generated in R using the CMplot package. Haploview 4.2 software was used to analyze local LD decay and haplotype block structure. The identification of candidate genes for root system traits and the analysis of expression patterns were performed according to Zheng et al. [36].

Abbreviations

TRL: Total root length; TRV: Total root volume; NRT: Number of root tips; TRA: Total root area; ARD: Average root diameter; RDW: Root dry weight; GY: Grain yield per unit area; SS: Stooling stage; WT: Wintering stage; JS: Jointing stage; MS: Maturing stage; OHC: Outdoor Hydroponic culture; IHC: Indoor Hydroponic culture; OPC: Outdoor Pot culture

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable. The datasets used and/or analyzed during the current study are available from the corresponding author on request.

Competing interests

The authors declare that they have no competing interest.

Funding

The work was supported National Key Research and Development Program "Science and Technology Innovation of High Grain Production Efficiency" of China (2018YFD0300701). The supporters did not play any role in the design analysis, or interpretation of this study and the relevant data. Shulin Chen was supported with funds from China Scholarship Council (CSC).

Author's contributions

FX, KZ and DH conceived the topic. SC and KZ provided gene chip, YH, LZ, MS and YH collected the phenotype regulation data. SZ, XY and XC performed the data analysis. FX wrote the manuscript with edits from other co-authors. All authors read and approved the

Acknowledgements

We thank Xuehai Zhang, Jingjing Mei, Chaonan Shi and Congwei Sun (Henan Agriculture University) for their technical assistance in the field, glasshouse and laboratory work. We also thank American Journal Experts for language editing of this manuscript.

Author details

¹ College of Agronomy Henan Agricultural University/ National Engineering Research Center for Wheat/ Co-construction State Key Laboratory of Wheat and Maize Crop Science /Collaborative Innovation Center of Henan Grain Crops, Henan Agricultural University, 15 Longzihu College District, Zhengzhou 450046, China.

² Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, OT Gatersleben, 06466 Seeland, Germany.

References

1. Monneveux P, Jing R, Misra SC. Phenotyping for drought adaptation in wheat using physiological traits. *Front Physiol.* 2012;3:429.
2. Fang Y, Du Y, Wang J, Wu A, Qiao S, Xu B, Zhang S, Siddique KHM, Chen Y. Moderate drought stress affected root growth and grain yield in old, modern and newly released cultivars of winter wheat. *Front Plant Sci.* 2017;8:672.
3. Maccaferri M, El-Feki W, Nazemi G, Salvi S, Canè MA, Colalongo MC, Stefanelli S, Tuberosa R. Prioritizing quantitative trait loci for root system architecture in tetraploid wheat. *J Exp Bot.* 2016;67(4):1161–78.
4. Bishopp A, Lynch JP. The hidden half of crop yields. *Nat Plants.* 2015;1(8):15117.
5. Ayalew H, Desalegn T, Hui L, Yan G. Performance of ethiopian bread wheat (*Triticum aestivum* L.) genotypes under contrasting water regimes: Potential sources of variability for drought resistance breeding. *Aust J Crop Sci.* 2016;10(3):370–6.
6. Christopher J, Christopher M, Jennings R, Jones S, Fletcher S, Borrell A, Manschadi AM, Jordan D, Mace E, Hammer G. QTL for root angle and number in a population developed from bread wheats (*Triticum aestivum* L.) with contrasting adaptation to water-limited environments. *Theor Appl Genet.* 2013;126(6):1563–74.
7. Robertson BM, Waines JG, Gill BS. Genetic variability for seedling root numbers in wild and domesticated wheats1. *Crop Sci.* 1979;19(6):843–7.
8. Hamada A, Nitta M, Nasuda S, Kato K, Fujita M, Matsunaka H, Okumoto Y. Novel QTLs for growth angle of seminal roots in wheat (*Triticum aestivum* L.). *Plant Soil.* 2012;354(1–2):395–405.
9. Cao P, Ren Y, Zhang K, Teng W, Zhao X, Dong Z, Liu X, Qin H, Li Z, Wang D. Further genetic analysis of a major quantitative trait locus controlling root length and related traits in common wheat. *Mol Breeding.* 2014;33(4):975–85.
10. Bai C, Liang Y, Hawkesford MJ. Identification of QTLs associated with seedling root traits and their correlation with plant height in wheat. *J Exp Bot.* 2013;64(6):1745–53.
11. Atkinson JA, Wingen LU, Marcus G, Pound MP, Oorbessy G, John FM, Le GJ, Simon G, Bennett MJ, Julie K. Phenotyping pipeline reveals major seedling root growth QTL in hexaploid wheat. *J Exp Bot.* 2015;66:2283–92.
12. Gregory PJ, Bengough AG, Grinev D, Schmidt S, Thomas WTB, Wojciechowski T, Young IM. Root phenomics of crops: opportunities and challenges. *Funct Plant Biol.* 2009;36(11):922–9.
13. Liu XL, Li R, Chang XP, Jing R. Mapping QTLs for seedling root traits in a doubled haploid wheat population under different water regimes. *Euphytica.* 2013;189(1):51–66.
14. Ren YZ, He X, Liu DC, Li JJ, Zhao XQ, Li B, Tong YP, Zhang AM, Li ZS. Major quantitative trait loci for seminal root morphology of wheat seedlings. *Mol Breeding.* 2012;30(1):139–48.
15. Chang H, Chen D, Kam J, Richardson T, Drenth J, Guo X, McIntyre CL, Chai S, Rae AL, Xue GP. Abiotic stress upregulated TaZFP34 represses the expression of type-B response regulator and *SHY2* genes and enhances root to shoot ratio in wheat. *Plant Sci.* 2016;252:88–102.
16. Li B, Liu D, Li Q, Mao X, Li A, Wang J, Chang X, Jing R. Overexpression of wheat gene *TaMOR* improves root system architecture and grain yield in *Oryza sativa*. *J Exp Bot.* 2016;67:4155–67.
17. Ecco G, Imbeault M, Trono D. KRAB zinc finger proteins. *Development.* 2017;144(15):2719–29.

18. Neumann K, Kobiljski B, Denčić S, Varshney RK, Börner A. Genome-wide association mapping: a case study in bread wheat (*Triticum aestivum* L.). *Mol Breeding*. 2011;27:37–58.
19. Suraj G, Sujana M, Michael BJ, Mai X, Gina BG, Adhikari TB, Xu M. Genome-wide association study reveals novel quantitative trait loci associated with resistance to multiple leaf spot diseases of spring wheat. *Plos One*. 2014;9(9):e108179.
20. Ye X, Li J, Cheng Y, Yao F, Chen G. Genome-wide association study reveals new loci for yield-related traits in Sichuan wheat germplasm under stripe rust stress. *BMC Genomics*. 2019;20:640.
21. Sukumaran S, Dreisigacker S, Lopes M, Chavez P, Reynolds MP. Genome-wide association study for grain yield and related traits in an elite spring wheat population grown in temperate irrigated environments. *Theor Appl Genet*. 2015;128(2):353–63.
22. Wang SX, Zhu YL, Zhang DX, Shao H, Ma CX. Genome-wide association study for grain yield and related traits in elite wheat varieties and advanced lines using SNP markers. *Plos One*. 2017;12(11):e0188662.
23. Chen SL, Cheng XY, Yu K, Chang XN, Bi HH, Xu HX, Wang JS, Pei XX, Zhang ZL, Zhan KH. Genome-wide association study of differences in 14 agronomic traits under low and high density planting models based on the 660 k SNP array for common wheat. *Plant Breeding*. 2019;139(2):272–83.
24. Xie Q, Fernando KMC, Mayes S, Sparkes DL. Identifying seedling root architectural traits associated with yield and yield components in wheat. *Ann Bot*. 2017;119(7):1115–29.
25. Neumann G, George TS, Plassard C. Strategies and methods for studying the rhizosphere—the plant science toolbox. *Plant Soil*. 2009;321(1–2):431–56.
26. Azevedo MCB, Chopart JL, Sugarcane CCM. Sugarcane root length density and distribution from root intersection counting on a trench-profile. *Sci Agricola*. 2017;68:94–101.
27. Jose S, Gillespie AR, Seifert JR, Pope PE. Comparison of minirhizotron and soil core methods for quantifying root biomass in a temperate alley cropping system. *Agroforest Syst*. 2001;2:161–8.
28. Fang S, Yan X, Liao H. 3D reconstruction and dynamic modeling of root architecture in situ and its application to crop phosphorus research. *Plant J*. 2009;60(6):1096–108.
29. Clark LJ, Cope RE, Whalley WR, Barraclough PB, Wade LJ. Root penetration of strong soil in rainfed lowland rice: comparison of laboratory screens with field performance. *Field Crop Res*. 2002;76(2–3):189–98.
30. Bai C, Ge Y, Ashton RW, Evans J, Bartsch M. The relationships between seedling root screens, root growth in the field and grain yield for wheat. *Plant Soil*. 2019;440:311–26.
31. Ren YZ, Qian YY, Xu YH, Zou CQ, Liu DC, Zhao XQ, Zhang A, Tong YP. Characterization of QTLs for root traits of wheat grown under different nitrogen and phosphorus supply levels. *Front Plant Sci*. 2017;8:2096.
32. Petrarulo M, Marone D, Ferragionio P, Cattivelli L, Rubiales D, De Vita P, Mastrangelo AM. Genetic analysis of root morphological traits in wheat. *Mol Genet Genomics*. 2015;290(3):785–806.
33. Li L, Peng Z, Mao XG, Wang JY, Chang XP, Reynolds M, Jing RL. Genome-wide association study reveals genomic regions controlling root and shoot traits at late growth stages in wheat. *Ann Bot*. 2019;124(6).
34. Qi W, Tang Y, Zhu W, Li DY, Diao CD, Xu LL, Jian Zeng J, Wang Y, Fan X, Sha L, Zhang HQ, Zheng YL, Zhou YH, Kang HY. Molecular cytogenetic characterization of a new wheat-rye 1BL·1RS translocation line expressing superior stripe rust resistance and enhanced grain yield. *Planta*. 2016;244(2):405–16.
35. Howell T, Hale I, Jankuloski L, Bonafede M, Gilbert M, Dubcovsky J. Mapping a region within the 1RS·1BL translocation in common wheat affecting grain yield and canopy water status. *Theor Appl Genet*. 2014;127:2695–709.
36. Zheng XW, Wen XJ, Qiao L, Zhao JJ, Zhang XJ, Li X, Zhang SW, Yang ZJ, Chang ZJ, Chen JL, Zheng J. A novel QTL *QTrl.saw-2D.2* associated with the total root length identified by linkage and association analyses in wheat (*Triticum aestivum* L.). *Planta*. 2019;250(1):129–43.
37. Atta BM, Mahmood T, Trethowan RM. Relationship between root morphology and grain yield of wheat in north-western NSW, Australia. *Aust J Crop Sci*. 2013;7:2108–15.
38. González FG, Miralles DJ, Slafer GA. Wheat floret survival as related to pre-anthesis spike growth. *J Exp Bot*. 2011;62(14):4889–901.
39. Passioura JB. Roots and drought resistance. *Water Manage*. 2012;7:265–80.
40. Ma SC, Xu BC, Li FM, Liu WZ, Huang ZB. Effects of root pruning on competitive ability and water use efficiency in winter wheat. *Field Crop Res*. 2008;105(1–2):56–63.

41. Eom KS, Jin SC, Lee SJ. Structural analyses of zinc finger domains for specific interactions with DNA. *J Microbiol Biotechnol*. 2016;26:2019–29.
42. Sergeeva LI, Keurentjes JJ, Bentsink L, Vonk J, van der Plas LH, Koornneef M, Vreugdenhil D. Vacuolar invertase regulates elongation of *Arabidopsis thaliana* roots as revealed by QTL and mutant analysis. *P Natl Acad Sci Usa*. 2006;103(8):2994–9.
43. Morey SR, Hirose T, Hashida Y, Miyao A, Hirochika H, Ohsugi R, Yamagishi J, Aoki N. Genetic evidence for the role of a rice vacuolar invertase as a molecular sink strength determinant. *Rice*. 2018;11(1):6.
44. Yu HL, Zhang FX, Wang GD, Liu YL, Liu D. Partial deficiency of isoleucine impairs root development and alters transcript levels of the genes involved in branched-chain amino acid and glucosinolate metabolism in *Arabidopsis*. *J Exp Bot*. 2013;2:599–612.
45. Lei M, Li HH, Zhang LY, Wang JK. QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. *Crop J*. 2015;3(3):269–83.
46. Liu YX, Wang L, Mao SS, Liu K, Zheng YL. Genome-wide association study of 29 morphological traits in *Aegilops tauschii*. *Sci Rep*. 2015;5:15562.
47. Beyer S, Daba S, Tyagi P, Bockelman H, Brown-Guedira G, IWGSC, Mohammadi M. Loci and candidate genes controlling root traits in wheat seedlings-a wheat root GWAS. *Funct Integr Genomic*. 2019;19:91–107.

Additional Files

Additional files 1:Table S1 Information of the 196 wheat cultivars (lines) assessed in the study. (XLS 353 kb)

Additional files 1:Table S2 Plant growth traits in the study. (XLS 353 kb)

Additional files 1:Table S3 Phenotypic data of 7 agronomical traits (average). (XLS 353 kb)

Additional files 1:Table S4 Normalized standard transformation of u average coefficients for root traits. (XLS 353 kb)

Additional files 1:Table S5 Canonical correlation of wheat root. (XLS 353 kb)

Additional files 1:Table S6 Correlation coefficients of the 6 root traits among the 196 accessions. (XLS 353 kb)

Additional files 1:Table S7 GWAS results of wheat root traits among the 196 wheat accessions by the mixed linear model (MLM Q+K). (XLS 353 kb)

Additional files 2:Table S8 List of significant ($-\log_{10}(P) > 3.5$) SNP associations of root traits. (XLS 214 kb)

Additional files 1:Table S9 The genetic positions of SNPs that are associated with root traits ($-\log_{10}(P) > 3.5$). (XLS 353 kb)

Additional files 1:Table S10 List of significant ($-\log_{10}(P) > 3.5$) marker trait associations of the SNP markers for pleiotropic. (XLS 353 kb)

Additional files 1:Table S11 Gene annotation of candidate region on the Chinese Spring chromosome 4A and 5A. (XLS 353 kb)

Additional files 1:Table S12 The expression data in different stages of the organs. (XLS 353 kb)

Additional files 2:Fig. S1 Plots of frequency distributions of differences among 6 root traits. (pdf 125 kb)

Additional files 3:Fig. S2 Manhattan and quantile–quantile (Q-Q) plots for 6 root traits. (pdf 1885 kb)

Additional files 4:Fig. S3 Haploview plot for LD decay within the candidate region on chromosome 4A and 5A. (pdf 568 kb)

Figures

Figure 1

Haploview plot for LD decay within the 6.17~18.76 Mb region of chromosome

Figure 2

Expression patterns of genes in different wheat organs at three growing stages. (a)TraesCS4A02G493900; (b)TraesCS4A02G494200; (c)TraesCS5A02G021700; (d)TraesCS5A02G021800; (e) TraesCS5A02G011600

Figure 3

Root experiment of 196 accessions under three culture patterns. (a) Indoor hydroponic culture (IHC); (b) Outdoor hydroponic culture (OHC); (c) Outdoor Pot culture in the field (OPC)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [supplement1.pdf](#)
- [supplement2.pdf](#)
- [supplement3.xls](#)
- [supplement4.xls](#)