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Research article

Genome-wide association study and protein network analysis for understanding candidate genes involved in root development at the rapeseed seedling stage

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ABSTRACT

Root system is essential for plants to absorb water and nutrients. The root related traits are complex quantitative traits and regulated by genetic control. Here, we used two association mapping panels to perform a genome-wide association study (GWAS) on seven root related traits in *Brassica napus* at the seedling stage and obtained 27 SNP loci significantly associated with the phenotypes. We further conducted a genome-wide LD block analysis of the candidate peak regions and obtained 295 candidate genes with high association peaks across seven phenotypes in LD region. In addition, a protein interaction network using the candidate genes identified here was constructed, and 113 genes were associated. Seven genes, BnaA03g47330D, BnaC09g16810D, BnaA06g22840D, BnaA03g28390D, BnaA08g19920D, BnaA03g28930D and BnaA03g11440D were in a large cluster, and may play important roles in interacting with other related genes. Our data may provide resources for molecular breeding and functional analysis of root growth and development in rapeseed.

1. Introduction

Roots are essential for plants. As the main interface between the plants and various biotic and abiotic factors in the soil environment (Smith and De, 2012), they absorb water and nutrient from the soil and supply a variety of growth substances for the shoots. In addition, roots provide anchor and mechanical support to make the plants more firmly in the soil (Smith and De, 2012). Based on their multiple roles in plant development and adaptation, root parameters are considered to be important breeding traits for higher yield in crop plants.

Brassica napus have a complex root system architecture, which is mainly composed of the primary root and the lateral roots (Santosh et al., 2015). In the process of plant growth and development, root morphology traits can be directly evaluated from primary root length, root weight, lateral root number, root volume. Moreover, plant weight, shoot weight and root-shoot ratio can also indirectly reflect the root morphology traits since plant growth is a biological process highly affected by the coordination of above-ground shoots and below-ground roots (Naz et al., 2012, 2014; Robinson et al., 2016).

In the progress of root growth and development, the phytohormone plays an essential role in root growth and development, especially for auxin (Sabatini et al., 1999; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4455775/, Mockaitis and Estelle, 2008; Hernández-Barrera et al., 2011). In addition to phytohormone, intercellular signaling molecules, such as ion, protein kinases, and phosphatases, and their respective receptors, as well as specific transcription factors (TFs), are essential for root development (Drisch and Stahl, 2015). To date, many genes have been reported that involved in the development of the root system in plants. In *Arabidopsis*, The *PIN* genes mediate polar auxin transport and regulate cell division and cell expansion in the primary root (Blilou et al., 2005). *HDG11* gene was found that can up-regulates cell-wall-loosening protein genes to promote root elongation (Xu et al., 2014). The P*LT1* and *PLT2* genes, which determine root stem cell positioning and differentiation based on their differential expression in the stem cell niche (Aida et al., 2004; Galinha et al., 2007). The *SHR* and *SCR* were found to be required for the maintenance of the stem cell niche and control cell division in the root apical meristem (Sozzani et al., 2010; Cruz-Ramírez et al., 2012; Wachsman et al., 2015). In maize, several genes have been described that affect the development of the root system, such as *Rtcs, Rth1* and *Rth3* (Taramino et al., 2007; Hochholdinger et al., 2008). *Rtcs* controls crown root and seminal root formation (Taramino et al., 2007). *Rth1* and *Rth3* control root hair elongation with *Rth3* being shown to affect grain yield in maize (Hochholdinger et al., 2008). In rice, *RRL1, RRL2, CRL2* and *WOX11*

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genes have been reported that affect the formation of root crown (Inukai et al., 2001a; Inukai et al., 2001b; Zhao et al., 2009). *OsCKH1*, *ARL1* and *FPF1* genes have been reported that affect the formation of adventitious root (Liu et a1., 2003; Liu et a1., 2005; Xu et al., 2005). *Srt5* and *rrl3*genes have been reported that affect the root length. *Dro1* gene was reported that is negatively regulated by auxin and involved in cell elongation in the root tip that causes asymmetric root growth and control root growth angle in rice (Uga et al., 2013).

Root related traits are complex quantitative traits and believed to be controlled by many genes, each with a small genetic effect. Researches have been undertaken to map root QTL in *Arabidopsis* (Gerald et al., 2006), rice (Xu et al., 2016), wheat (Iannucci et al., 2017), common bean (Ochoa et al., 2006), maize (Pace et al., 2015), soybean (Zhou et al., 2011) and barley (Naz et al., 2012, 2014; Robinson et al., 2016; Sayed et al., 2017a,b). Recently, in *Brassica napus*, Genetic variation and QTLs for root related traits have been studied in a recombinant inbred line population and 23 stable QTL for root related traits were identified (Wang et al., 2017). However, the genetic control of root characteristics is poorly understood among the rapeseed diverse genetic resources.

Genome-wide association studies (GWAS) are an efficient method to detect marker-trait associations and can be applied to mapping and hunting for candidate genes related to certain traits (Huang et al., 2010; Zhao et al., 2011). Instead of previous commonly used SSRs (Zhao et al., 2009, 2013; Li et al., 2014), SNPs have become more popular for GWASs (Huang et al., 2010, 2012). Compared with linkage mapping, association mapping does not require constructing special mapping populations and it uses high recombination in natural populations. In rapeseed, many QTL have been identified for above-ground traits of agronomic importance by GWASs (F. Li et al., 2014; Cai et al., 2014; Luo et al., 2015; He et al., 2017), however, there is less genes or QTLs has been reported on below-ground root related trait.

The objectives of the present study were to: (1) detect SNP markers associated with seven root related traits in two rapeseed association panels by genome-wide association studies, and (2) identify the candidate genes having an effect on root development and investigate a protein interaction network using the candidate genes. Our study may provide resources for molecular breeding and functional analysis of root growth and development in rapeseed.

2. Materials and methods

2.1. Plant materials and phenotypic evaluation

Two association mapping panels were used for genome-wide association study in the current study. Panel 1 was composed of 296 diverse inbred lines, including 48 winter ecotype lines, 197 Chinese semi-winter accessions and 51 spring ecotype lines. Panel 2 was composed of 126 diverse inbred lines, including 41 winter ecotype lines, 37 Chinese semi-winter accessions and 48 spring ecotype lines. These lines were grown in the green house under the condition of 70% relative humidity with 16 h of light at 24 °C followed by 8 h of dark at 20 °C, and two replications were planted for each line.

After growth for 35 days, 10 plants from each accession (5 plants per replicate) were sampled to detect the root related traits. Seven root related trait including whole plant weight (PW), root weight (RW), primary root length (RL), total root number (RN), total root volume (RV), shoot weight (SW) and Root-shoot ratio (RSR) were detected. After they had been sampled, the total roots were separated from the shoot and root part and measured respectively. The Root-shoot ratio (RSR) was calculated. The primary root length (RL) was investigated by manually using a ruler and the total root number (RN) was determined by manually counting. The root were scanned by a scanner (EPSON V700, Japan), and then the total root volume (RV) were analyzed by WinRHIZO software.

Table 1
Phenotypic variation of root related traits.

Population	Traits	MIN	MAX	MEANtst	CV%
Panel 1	PW	0.36	10.81	2.59 ± 1.18	45.68%
	RW	0.02	0.98	0.11 ± 0.08	73.53%
	RL	2.30	15.83	4.99 ± 1.31	26.24%
	RN	12.44	104.82	44.63 ± 16.66	37.33%
	RV	0.01	0.35	0.11 ± 0.06	53.73%
	SW	0.34	10.36	2.48 ± 1.14	46.14%
	RSR	0.01	0.57	0.05 ± 0.04	89.49%
Panel 2	PW	0.56	10.81	2.73 ± 1.38	50.55%
	RW	0.02	0.46	0.10 ± 0.06	60.33%
	RL	2.79	15.83	5.15 ± 1.58	30.71%
	RN	12.00	98.00	44.18 ± 17.73	40.13%
	RV	0.01	0.28	0.08 ± 0.05	54.60%
	SW	0.53	10.36	2.63 ± 1.34	51.05%
	RSR	0.01	0.16	0.04 ± 0.02	44.75%

PW, Plant weight; RW, Root weight; RL, Root length; RN, Root number; RV, Root volume; SW, Shoot weight; RSR, Root-shoot ration.

2.2. Genotyping

The panel 1 comprised of 296 inbred lines was genotyped by the *Brassica* 60 K Illumina® Infinium SNP array by Emei Tongde Co. (Beijing) in accordance with the manufacturer's protocol (http://www.illumina.com/technology/beadarray-technology/infinium-hd-assay.html).We excluded SNPs with either an AA or a BB frequency equal to zero, call frequency < 0.9, or minor frequency < 0.05.

The panel 2 comprised of 126 inbred lines was sequenced using an Illumina Hiseq TM 4000 (Illumina Co, Ltd.) with a 5X sequencing depth and 125-bp paired-end sequencing length. The reads were mapped to the reference genome of *Brassica napus*. annotation_v5 (http://www.genoscope.cns.fr/brassicanapus/data/) with BWA software (http://bio-bwa.sourceforge.net/). SNPs were detected among accessions by GATK software (https://www.broadinstitute.org/gatk/guide/best-practices.php). The missing SNPs with the missing rate below 0.6 were filled using the software beagle v4.1 (https://faculty.washington.edu/browning/beagle/beagle.html#download). The SNP loci with heterozygous rate over 25% and minor allele frequency (MAF) less than 0.05 were removed.

2.3. Genome-wide association analysis

The software package STRUCTURE v2.3.4 was employed to analyze the Population structure (Pritchard et al., 2000). The program was run with the following parameters: Five independent runs were performed with a K-value (the putative number of genetic groups) from 1 to 10, with 10,000 MCMC (Markov chain Monte Carlo) replications and 10,000 burn-ins. The optimal K-value was determined by the log probability of data [LnP(D)] and an ad hoc statistic Δk based on the rate of change of LnP(D) between successive K (Evanno et al., 2005). SPAGeDi v1.4 was used to calculate the relative kinship matrix (Hardy andVekemans, 2002). Negative values between two individuals were set to 0 (Yu et al., 2006). For the mapping panel 1, the genotype data was from 60K SNP array and totally 33186 SNPs were obtained after filtering. We selected 5700 SNPs to analyze population structure. On each chromosome, we select 300 SNPs according to the SNPs distributions distance on the chromosome. We used the whole 33186 SNPs to performed PCA analysis and kinship estimation. For the mapping panel 2, the genotype data was from whole genome resequencing and the total SNP number is 690,953 after filtering. Since the too large data produced by whole genome resequencing and the software limited, we selected 10000 SNPs according to the SNPs distributions distance on the whole genome to analyze population structure, PCA, and estimate kinship.

For mapping panel 1, TASSEL 5.0 (Yu et al., 2006; Yang et al., 2010)

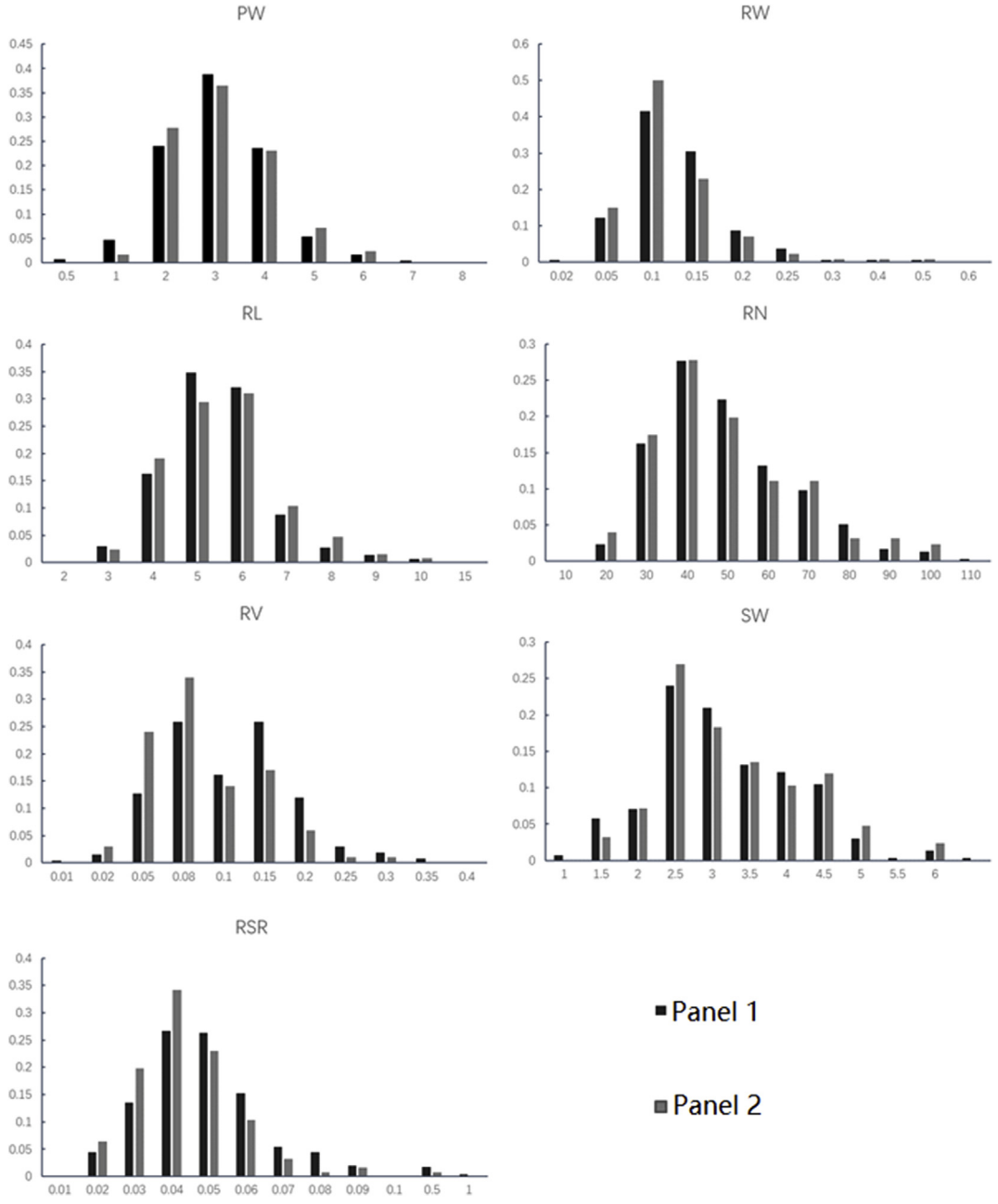

Fig. 1. Phenotype frequency distribution of seven root related traits in the two mapping populations.

Table 2
Correlation coefficients among root related traits in the two mapping Panels.

	PW	RW	RL	RN	RV	SW	RSR
PW		0.72671**	0.39243**	0.34045**	0.49699**	0.99954**	−0.14812**
RW	0.50285**		0.48812**	0.39330**	0.56849**	0.70565**	0.52205**
RL	0.38631**	0.38347**		0.40233**	0.48547**	0.38335**	00.21431**
RN	0.40934**	0.24493**	0.30348**		0.39431**	0.33389**	0.06521
RV	0.0.4381*	0.50341**	0.30820**	0.25861**		0.48792**	0.13844
SW	0.99816**	0.44953**	0.37233**	0.40585**	0.41711**		−0.17570*
RSR	−0.10789	0.69726**	0.22028**	−0.05923	0.13806*	−0.16039**	

Below diagonal: Panel 1; Above diagonal: Panel 2.
**Significantly correlated at $P < 0.001$ level.
*Significantly correlated at $P < 0.05$ level.
Abbreviations are the same as those given in Table 1.

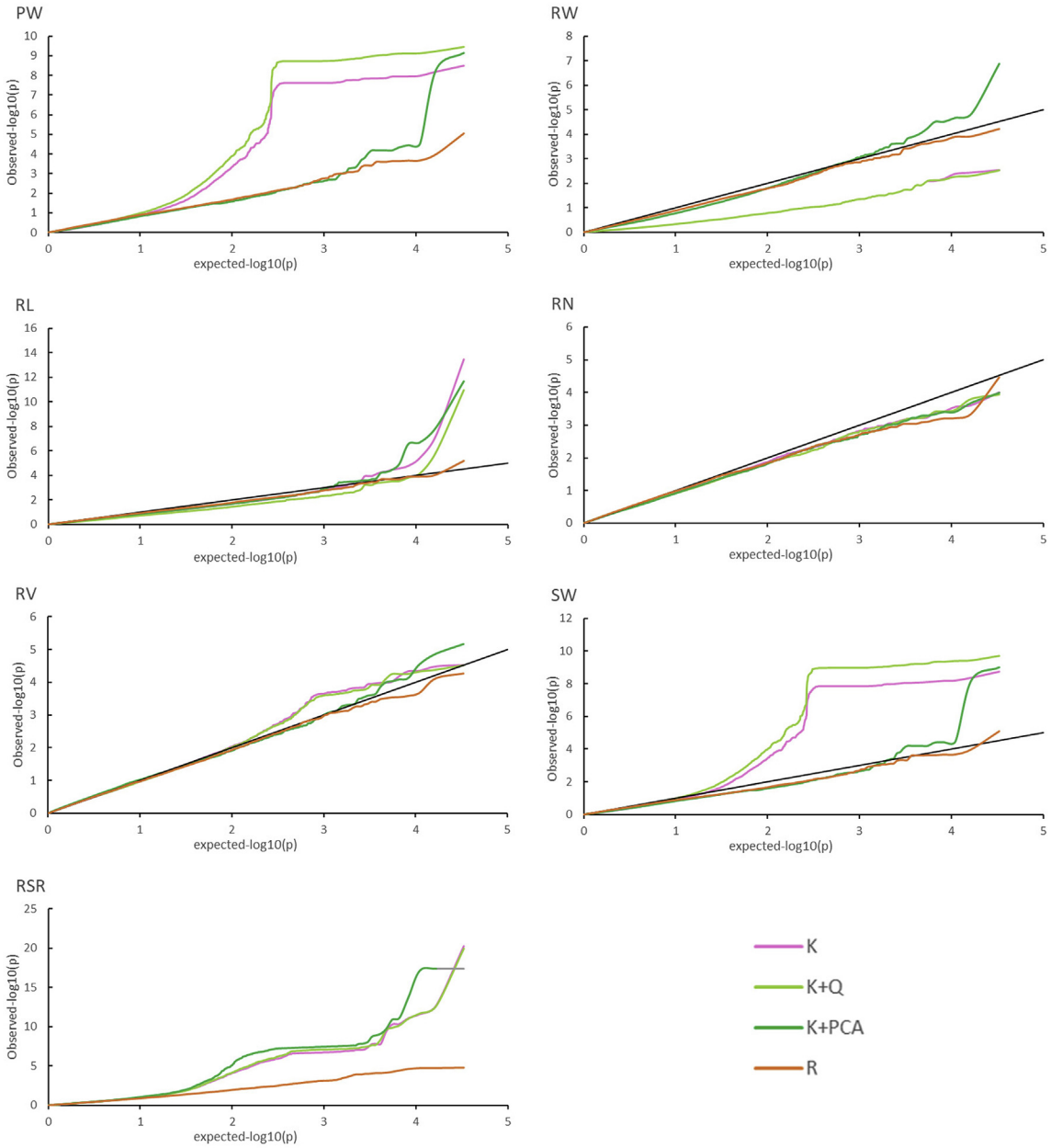

Fig. 2. Quantile-quantile plots of R, K model, Q + K model and PCA + K model. The black line is the expected line under the null distribution.

and R package (Team, 2014) were both employed to perform the genome-wide analysis. By TASSEL 5.0, three mixed models controlling relative kinship, K model, Q + K model, and PCA + K model were chosen to determine the statistical associations between phenotypes and genotypes to evaluate the effects of population structure (Q, PC) and relative kinship (K) on root related traits. These three models were performed with optimum compression and population parameters previously determined (P3D) by variance component estimation in TASSEL 5.0. For the mapping panel 2, because of the too large data produced by whole genome resequencing, only the R package (Team, 2014) was employed to perform the genome-wide analysis. The genome-wide threshold was set at p = 1/number of total SNPs (Luo et al., 2015).

2.4. Candidate gene identification and protein network interaction analysis

Gene loci containing the SNPs in highly associated peaks were considered as candidate genes related to root related traits. All the identified candidate genes associated with root related traits at seedling stage were used for network analysis. Based on integrating with the Biological General Repository for Interaction Datasets (BioGRID, http://thebiogrid.org/) that provides genetic and protein interaction data from model organisms (Chatr-Aryamontri et al., 2015), Network analysis was performed for the candidate genes related to root related trait. Cytoscape v3.6.0 was employed to visualize the protein–protein interaction network (Shannon et al., 2003).

3. Results

3.1. Phenotypic variation of root related traits

Seven root related traits were investigated in both of the two association mapping populations. Minimum, maximum, mean values and coefficient of variations (CVs) for all the investigated traits in each population were presented in Table 1. The CVs ranging from 26.24 to 89.49% within the population suggested the investigated root related

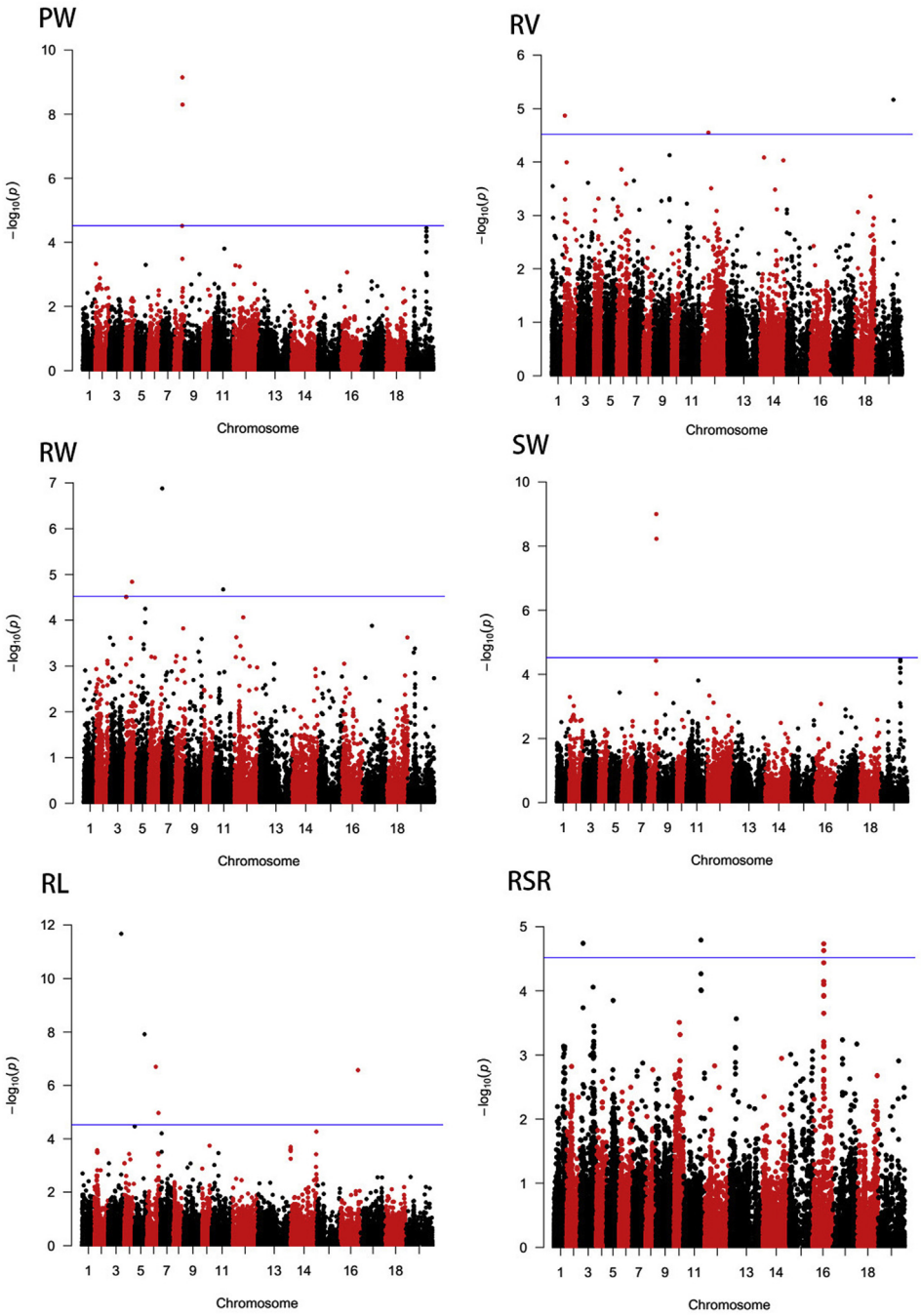

Fig. 3. Manhattanplots of association analysis for root related traits in panel 1. Each dot represents a SNP. The significance threshold -$\log_{10}$(p) = 4.52.

traits had extensive phenotypic variations (Table 1). The distribution of the seven traits were approximately normal in both of the two populations (Fig. 1) indicating that these traits were quantitative inheritance pattern and suitable for GWAS analysis. Except for RSR trait, significant pairwise correlations were detected among these traits in both populations (Table 2). RSR was positively correlated with RW and RL and negatively correlated with SW in both populations. However, RSR was positively correlated with RV only in Panel 1 and negatively correlated with PW only in Panel 2.

3.2. Genome-wide association analysis

For panel 1, According to the Q-Q plots of the R and three models in tassel 5.0, the observed P values of the PCA + K model were closer to the expected P values than other models for the six traits including the PW, RW, RL, RN, RV and SW (Fig. 2), this indicated that PCA + K model could effectively control false positive associations and avoid false negative associations for these six traits. Thus, we choose PCA + K model to perform the association analysis for these six traits. For the RSR trait, the observed P values of the R were closer to the expected P values, thus, we choose R to perform the association analysis for Root shoot ration (Fig. 2). For panel 2, only R package were used to genome wide association analysis because of the large genotyped data produced by whole genome resequencing.

In panel 1, totally 22 SNP loci were identified significantly associated with the seven root related traits at -lg(P) > 4.52, including 3 SNPs associated with PW on chromosome A08 and C09, 3 SNPs associated with RW on A04, A07 and C01, 6 SNPs associated with RL on

Table 3
Genome-wide significant association signals detected in the two mapping populaions

Traits	SNP ID	Chr.	Position	P value	R^2	$-\log_{10}(P)$	Population
PW	39852*	C09	13737563	8.97E-06	0.1078	5.0470	Panel 1
PW	18225*	A08	14842805	7.09E-10	0.2286	9.1496	Panel 1
PW	18282*	A08	15148135	5.02E-09	0.2497	8.2994	Panel 1
PW	14149995*	A03	14149995	5.24E-07	0.3128	6.2808	Panel 2
RW	16449	A07	243867	1.32E-07	0.1546	6.8784	Panel 1
RW	8306	A04	11231342	1.45E-05	0.0937	4.8386	Panel 1
RW	52132	C01	18269465	2.13E-05	0.1093	4.6711	Panel 1
RL	42482	C04	47289490	6.53E-06	0.1235	5.1851	Panel 1
RL	6832	A03	24364511	2.11E-12	0.2380	11.6750	Panel 1
RL	11422	A05	18326481	1.21E-08	7.9169	0.1569	Panel 1
RL	13047	A06	16000043	2.00E-07	6.6994	0.1348	Panel 1
RL	35372	C06	32036971	2.68E-07	6.5716	0.1243	Panel 1
RL	13726	A06	20842625	1.08E-05	4.9672	0.0932	Panel 1
RV	37132	C09	32713842	6.81E-06	5.1671	0.1359	Panel 1
RV	4318	A02	1242248	1.35E-05	4.8690	0.1404	Panel 1
RV	42321	C02	11293971	2.81E-05	4.5518	0.1283	Panel 1
SW	39852*	C09	13737563	8.05E-06	0.1132	5.0943	Panel 1
SW	18225*	A08	14842805	9.96E-10	0.2248	9.0018	Panel 1
SW	18282*	A08	15148135	5.89E-09	0.2493	8.2300	Panel 1
SW	14149995*	A03	14149995	5.14E-07	0.3178	6.2895	Panel 2
RSR	1123	C01	31588804	1.61E-05	0.1062	4.7944	Panel 1
RSR	40599	A03	5245888	1.81E-05	0.1054	4.7416	Panel 1
RSR	25799	C06	20178116	1.84E-05	0.1052	4.7346	Panel 1
RSR	25808	C06	20189683	2.35E-05	0.1036	4.6296	Panel 1
RSR	13924962	A03	13924962	3.17E-07	0.4237	6.4991	Panel 2
RSR	13924995	A03	13924995	3.17E-07	0.4239	6.4991	Panel 2
RSR	13924969	A03	13924969	3.44E-07	0.4224	6.4630	Panel 2

* Common SNP loci detected from different traits.
Abbreviations are the same as those given in Table 1.

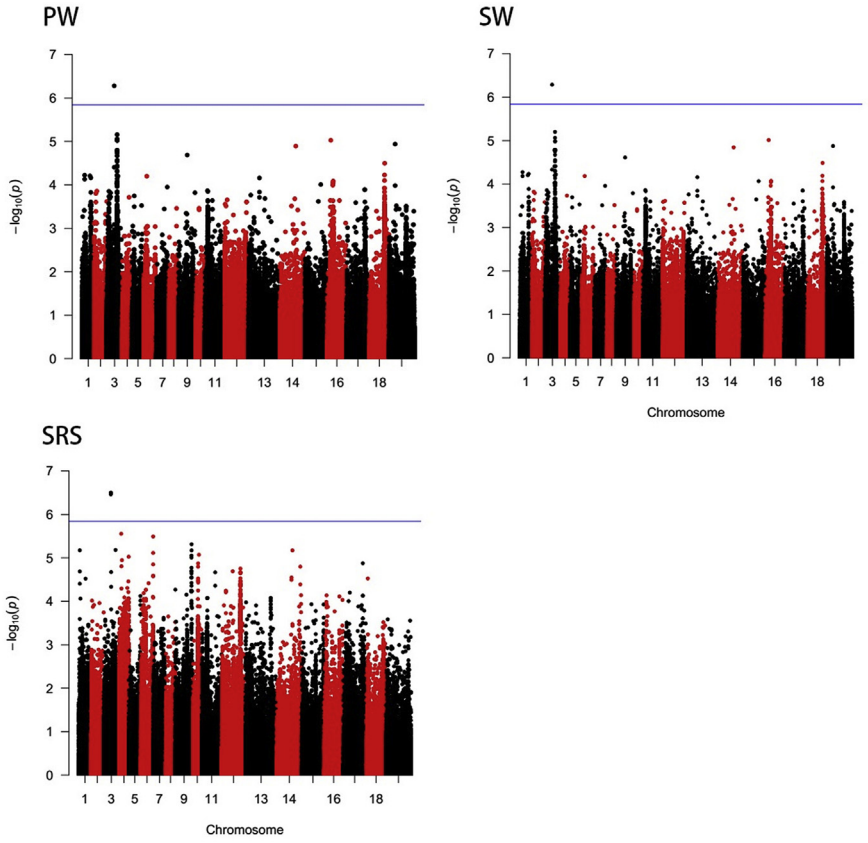

Fig. 4. Manhattanplots of association analysis for root related traits in panel 2. Each dot represents a SNP. The significance threshold $-\log_{10}(p) = 5.84$.

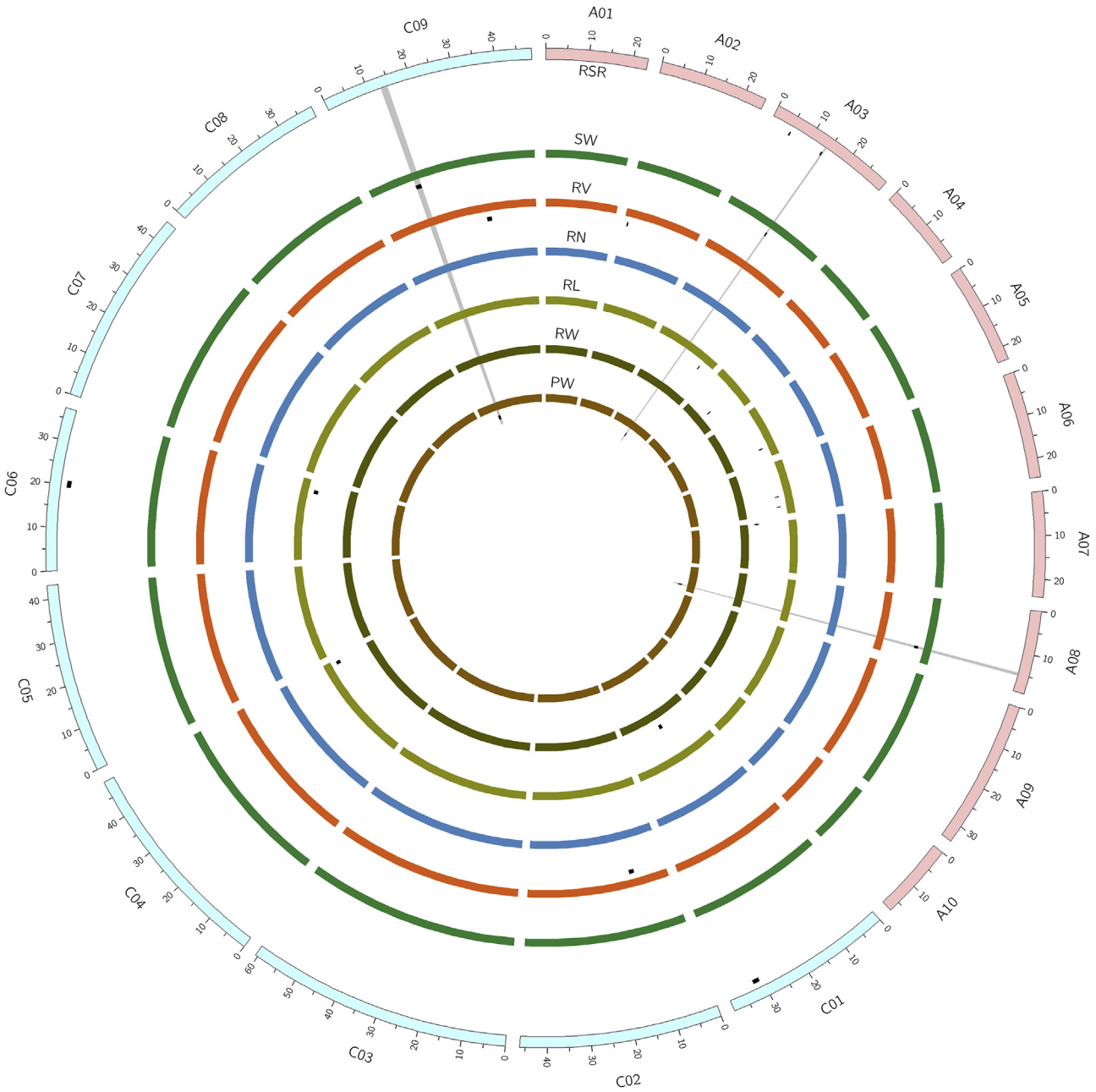

Fig. 5. GWAS result for seven root related traits in the two mapping populations.

A03, A05, A06, C04 and C05, 3 SNPs associated with RV on A02, C02 and C09, 3 SNPs associated with SW on A08 and C09, and 4 SNPs associated with RSR on A03, C01 and C06 (Fig. 3, Table 3).

In panel 2, totally 5 SNP loci were identified significantly associated with the root related trait at -lg(P) > 5.84, including one SNP associated with PW on A03, one SNP associated with SW on A03, and 3 SNPs associated with RSR on A03 (Fig. 4, Table 3).

In all these significant SNPs, 4 common SNPs on A03, A08 and C09 were found associated with the trait of PW and SW. (Fig. 5, Table 3).

3.3. Candidate gene identification and protein network interaction analysis

We further conducted a genome-wide LD (linkage disequilibrium) block analysis of the candidate peak regions and only significant SNPs that were harbored by LD blocks were supposed to be the regions containing putative candidate genes. According to our previous studies, the extent of LD is about 150 kb in A subgenome and 750 kb in C subgenome in *Brassica napus* (Wei et al., 2017). Thus, we selected 150 kb upstream and downstream of the highest point of SNP in A subgenome and 750 kb in C subgenome, respectively. Gene loci containing the SNPs in highly associated peaks were considered as candidate genes related to root development. We successfully identified 295 candidate genes in the peak SNP sites (or adjacent to those sites) of associated loci for seedling root development (Supplementary Table 1). Among the 295 genes, 12, 113, 4, 10, 17, 7, 28, 22, 16, 11, 37 and 18 genes were in A02, A03, A04, A05, A06, A07, A08, C01, C02, C04, C06 and C09, respectively. Among all the genes, 96, 21, 68, 35, 96, and 95 genes were identified to be associated with the traits of PW, RW, RL, RV, SW and RSR, respectively. Twenty common genes were found associated with the traits of PW, SW and RSR.

To further explore the gene's functional interactions, we constructed a protein interaction network using the GWAS candidate genes (Supplementary Table 1) as nodes and protein–protein interaction data from BioGRID as edges (http://thebiogrid.org/). The network contains

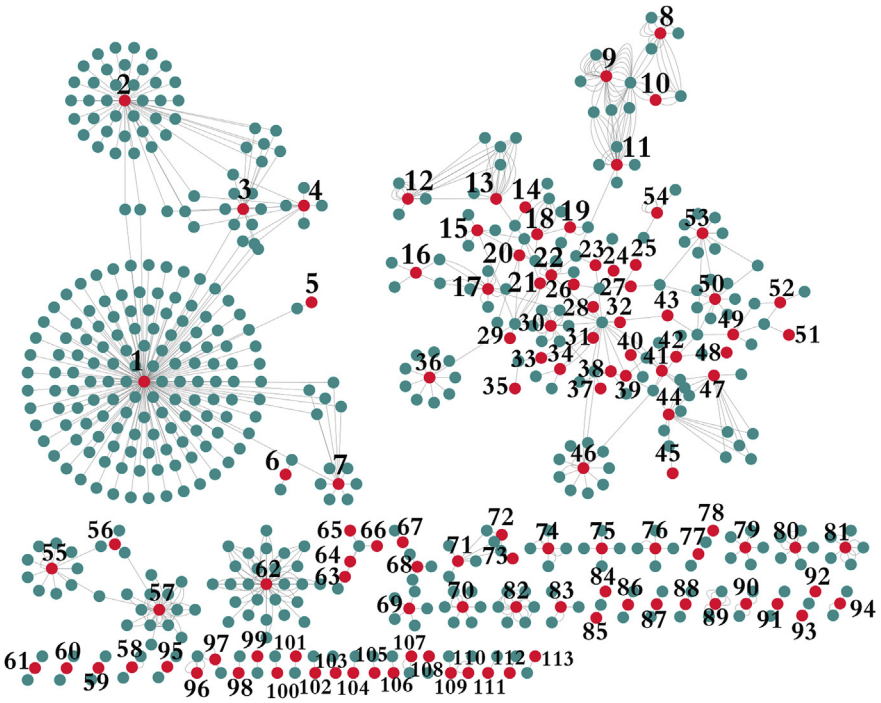

Fig. 6. Protein interaction Cytoscape network. The network suggested the interactions between the genes. Red node is the candidate genes which -log10(p) > 5 with FDR < 0.05. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

599 nodes and 821 edges, as visualized by Cytoscape in Fig. 6. A total of 113 GWAS candidate genes with FDR < 0.05 were marked in the network with a red node (Fig. 6, Table 4). As shown in the network, the gene numbers 1 (*BnaA03g47330D*), 2 (*BnaC09g16810D*), 3 (*BnaA06g22840D*), 4 (*BnaA03g28390D*) 5 (*BnaA08g19920D*), 6 (*BnaA03g28930D*) and 7 (*BnaA03g11440D*) were in a large cluster, and may play important roles by interacting with other related genes.

4. Discussion

As below-ground part of plants, root is very important for plant and has numerous functions in plant development including water and nutrient uptake (MacMillan et al., 2006), so root highly affected the plant productivity by the coordination of above-ground part and below-ground part. However, fewer researchers focus on the below-ground plant parts than above-ground plant parts since it is more difficult to observe and measure the root related trait (Shen et al., 2001). In the present study, we measured seven root related traits in two rapeseed association panels and performed a genome-wide association study (GWAS). Moreover, the possible candidate genes involved in root development are predicted and a protein interaction network was constructed using the candidate genes.

In this study, we totally identified 27 SNP loci that significantly associated with the root related traits. These SNPs could possibly be regarded as candidate loci for root development and be used for development of molecular markers for rapeseed breeding.

Among the 295 candidate genes identified in the current study, 8 of them have been reported that their homologous genes in *Arabidopsis* were associated with root growth and development (Table 5) (Lee et al., 2010; Prasad et al., 2010; Jin et al., 2012; Sundaravelpandian et al., 2013; Zhou et al., 2013; Velasquez et al., 2015; Li et al., 2017; Crawford et al., 2015). *Cand2* gene is a G-protein-coupled-receptor candidate, which involved in the regulation of Arabidopsis root growth (Lee et al., 2010). *Lrs1* gene was reported that regulated the lateral root development in *Arabidopsis* seedlings (Prasad et al., 2010). *P4H2* gene was reported that impact on root hair growth in *Arabidopsis* (Jin et al., 2012). *PFT1* is critical for multiple stages of root hair development in *Arabidopsis* by controlling the ROS balance (Sundaravelpandian et al., 2013). *AHL4* was reported that regulated the vascular tissue boundaries in roots of *Arabidopsis* (Zhou et al., 2013). *ACS4* is an ethylene biosynthesis enzyme, which interacts with *XBAT32*, an *Arabidopsis* RING E3 Ligase, regulates the lateral root production through its role in ethylene biosynthesis (Velasquez et al., 2015). *HDT1* gene regulates the GIBBERELLIN 2-OXIDASE2 expression to control *Arabidopsis* root meristem cell number (Li et al., 2017). *WIP4* was identified to be required for the initiation of the *Arabidopsis* root meristem (Crawford et al., 2015).

According to the protein–protein interaction analysis, 113 candidate genes were associated in the interaction networks. Seven genes, the gene numbers 1 (*BnaA03g47330D*), 2 (*BnaC09g16810D*), 3 (*BnaA06g22840D*), 4 (*BnaA03g28390D*) 5 (*BnaA08g19920D*), 6 (*BnaA03g28930D*) and 7 (*BnaA03g11440D*) were in a large cluster, and may play important roles by interacting with other related genes. Especially for the gene number 1, *BnaA03g47330D*, it may play a key role in the networks. Besides the largest cluster, we found that gene number 62 (*BnaA08g19480D*) was also in a key position in the networks. As mentioned above, *PFT1*, the homologous gene of *BnaA08g19480D* in *Arabidopsis* was reported that is critical for root hair development (Sundaravelpandian et al., 2013). This result suggests that the key genes can be investigated to explore its possible role in the network. These key candidate genes generated here may provide additional resources for molecular breeding and functional analysis of root growth and development in rapeseed seedlings.

Author contributions

Yajun He and Dingxue Hu wrote the manuscript. Wei Qian designed the study. Yajun He, Dingxue Hu, Jingcan You, Daoming Wu and Hongli Dong conducted the experiment. Yajun He, Dingxue Hu and Yixin Cui analyzed the data. Wei Qian and Jiana Li provided resources. All authors read and approved the final manuscript.

Table 4
Protein interaction cytoscape network of Candidate genes for root related traits.

Number	Gene ID	Annotation
1	BnaA03g47330D	TRICHOME BIREFRINGENCE-LIKE 18
2	BnaC09g16810D	Calcineurin-like metallo-phosphoesterase superfamily protein
3	BnaA06g22840D	Leucine-rich repeat protein kinase family protein
4	BnaA03g28390D	Leucine-rich repeat protein kinase family protein
5	BnaA08g19920D	RING/U-box superfamily protein
6	BnaA03g28930D	CONTAINS InterPro DOMAIN/s: mRNA splicing factor, Cwf18 (InterPro:IPR013169)
7	BnaA03g11440D	MBOAT (membrane bound O-acyl transferase) family protein
8	BnaA03g47510D	myb domain protein 18 (MYB18)
9	BnaA06g22980D	UVB-RESISTANCE 8 (UVR8)
10	BnaC06g31050D	SHORT HYPOCOTYL IN WHITE LIGHT1 (SHW1)
11	BnaA05g24250D	SPA1-related 3 (SPA3)
12	BnaA03g28730D	CLP-similar protein 3 (CLPS3)
13	BnaC02g15600D	cleavage and polyadenylation specificity factor 160 (CPSF160)
14	BnaA05g24330D	unknown protein
15	BnaA08g19740D	general regulatory factor 12 (GRF12)
16	BnaA06g22810D	transducin family protein/WD-40 repeat family protein
17	BnaC09g29790D	ARM repeat superfamily protein
18	BnaA03g28550D	SUVR4
19	BnaC06g18000D	BECLIN1 (BECLIN1)
20	BnaA03g11400D	SHV3-like 1 (SVL1)
21	BnaA05g24610D	phosphoenolpyruvate carboxylase 3 (PPC3)
22	BnaA03g47480D	FLOWERING WAGENINGEN (FWA)
23	BnaC06g31060D	BED zinc finger
24	BnaA03g29350D	Protein phosphatase 2C family protein
25	BnaA03g29320D	long-chain acyl-CoA synthetase 6 (LACS6)
26	BnaA03g29220D	neurofilament protein-related
27	BnaA03g28400D	methionine synthase 2 (MS2)
28	BnaA03g28630D	UV-B LIGHT INSENSITIVE 3 (ULI3)
29	BnaA03g28280D	RING/U-box superfamily protein
30	BnaA03g28480D	glyceraldehyde-3-phosphate dehydrogenase C subunit 1 (GAPC1)
31	BnaC01g32800D	ubiquitin-conjugating enzyme19 (UBC19)
32	BnaA02g02850D	prenylcysteine methylesterase (PCME)
33	BnaA06g22720D	glutamate-1-semialdehyde-2,1-aminomutase (GSA1)
34	BnaC01g32880D	RING/U-box superfamily protein
35	BnaA03g28760D	pathogenesis-related 4 (PR4)
36	BnaC01g24030D	putative mitochondrial RNA helicase 1 (PMH1)
37	BnaC04g48640D	polyamine oxidase 2 (PAO2)
38	BnaA02g02620D	casein lytic proteinase B3 (CLPB3)
39	BnaA08g19980D	Ribosomal protein L34e superfamily protein
40	BnaA03g29070D	Ribosomal L22e protein family
41	BnaA03g28820D	Ribosomal protein S3Ae
42	BnaA03g29090D	ribosomal protein L18 (RPL18)
43	BnaC09g16990D	Sugar isomerase (SIS) family protein
44	BnaA03g28860D	Ribosomal protein S24e family protein
45	BnaA08g19090D	unknown protein
46	BnaA03g29050D	regulatory particle triple-A ATPase 5A (RPT5A)
47	BnaA08g19260D	Ribosomal protein L10 family protein
48	BnaA03g11520D	O-Glycosyl hydrolases family 17 protein
49	BnaA03g29120D	RING/U-box protein
50	BnaA03g29270D	TCP-1/cpn60 chaperonin family protein
51	BnaA03g47600D	FtsJ-like methyltransferase family protein
52	BnaC06g30880D	alpha-amylase-like 3 (AMY3)
53	BnaA03g28450D	TCP-1/cpn60 chaperonin family protein
54	BnaA03g28270D	peroxin 19-1 (PEX19-1)
55	BnaC01g32210D	ZIM-like 1 (ZML1)
56	BnaA03g29110D	Tetratricopeptide repeat (TPR)-like superfamily protein
57	BnaA05g24550D	cobalt ion binding
58	BnaA04g13310D	1-aminocyclopropane-1-carboxylate synthase 4 (ACS4)
59	BnaA03g28960D	ROP binding protein kinases 2 (RBK2)
60	BnaA02g02590D	U-box domain-containing protein
61	BnaC09g29480D	SPEECHLESS (SPCH)
62	BnaA08g19480D	PHYTOCHROME AND FLOWERING TIME 1 (PFT1)
63	BnaA08g19200D	THO2
64	BnaC04g48330D	TRAF-like family protein
65	BnaC01g23930D	histone deacetylase 3 (HDA3)
66	BnaA03g28620D	Nucleotidylyl transferase superfamily protein
67	BnaA03g28750D	ankyrin repeat family protein
68	BnaA04g13210D	basic helix-loop-helix (bHLH) DNA-binding superfamily protein
69	BnaC01g32850D	translocase of the outer mitochondrial membrane 40 (TOM40)
70	BnaA08g19220D	microtubule-associated proteins 70–2 (MAP70-2)
71	BnaC06g17120D	F-box/RNI-like superfamily protein
72	BnaC06g17160D	F-box/RNI-like superfamily protein
73	BnaC04g49450D	Ankyrin repeat family protein
74	BnaA03g29360D	SAC3/GANP/Nin1/mts3/eIF-3 p25 family
75	BnaC02g15530D	unknown protein
76	BnaA03g29000D	acyl-CoA binding protein 4 (ACBP4)
77	BnaA06g22890D	shoot apical meristem arrest 1 (SHA1)
78	BnaA03g11650D	Uncharacterised protein family (UPF0497)
79	BnaA08g19490D	TEMPRANILLO 1 (TEM1)
80	BnaA03g29200D	anaphase-promoting complex/cyclosome 11 (APC11)
81	BnaA04g13390D	methyltransferase 1 (MET1)
82	BnaC02g15550D	PPHB SUSCEPTIBLE 2 (PBS2)
83	BnaA03g29080D	Calcineurin-like metallo-phosphoesterase superfamily protein
84	BnaA08g19760D	FKBP-like peptidyl-prolyl cis-trans isomerase family protein
85	BnaA02g02740D	ARM repeat superfamily protein
86	BnaA03g28870D	cysteine synthase D1 (CYSD1)
87	BnaA05g24640D	phosphoesterase
88	BnaA02g02690D	SPIRAL1-like4 (SP1L4)
89	BnaA03g28850D	with no lysine (K) kinase 1 (WNK1)
90	BnaA03g28670D	flowering locus KH domain (FLK)
91	BnaA03g28570D	peroxin-12 (PEX12)
92	BnaA08g19690D	Phosphatidylinositol 3- and 4-kinase family protein
93	BnaC02g15200D	INVOLVED IN: biological_process unknown
94	BnaA02g03100D	ROP-interactive CRIB motif-containing protein 4 (RIC4)
95	BnaA03g28320D	ribosomal protein S2 (RPS2)
96	BnaC02g15620D	AT hook motif DNA-binding family protein
97	BnaA03g11360D	beta-1,2-xylosyltransferase (XYLT)
98	BnaA03g47280D	Expressed protein
99	BnaA08g19060D	Acid phosphatase/vanadium-dependent haloperoxidase-related protein
100	BnaC04g49820D	DROUGHT TOLERANCE REPRESSOR (DOR)
101	BnaC04g49070D	FKBP-like peptidyl-prolyl cis-trans isomerase family protein
102	BnaC01g32410D	NIMA-related kinase 5 (NEK5)
103	BnaA06g22750D	flavonol synthase 3 (FLS3)
104	BnaA06g30770D	methyl esterase 11 (MES11)
105	BnaC02g16510D	mitochondrial acyl carrier protein 2 (mtACP2)
106	BnaC06g17240D	Pentatricopeptide repeat (PPR) superfamily protein
107	BnaA03g11640D	FIP2
108	BnaA03g47490D	homolog of DNA mismatch repair protein MSH3 (MSH3)
109	BnaA03g47680D	vacuolar ATP synthase G3 (VATG3)
110	BnaA03g29180D	ATSK12
111	BnaA03g28720D	WRKY DNA-binding protein 39 (WRKY39)
112	BnaA03g28950D	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
113	BnaA02g02750D	DNA/RNA polymerases superfamily protein

Notes

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Table 5
The reported candidate genes for root growth and development.

Trait	SNP position	-lg(P)	Bna. genes	ATH. genes	Distance(kb)	Gene function	Reference
PW	A03:14149995	6.28	BnaA03g28920D*	AT3G05010	89.01	Cand2	Jin et al., (2012)
PW	A03:14149995	6.28	BnaA03g28940D*	AT3G05090	83.14	LRS1	Lee et al., (2010)
PW	A03:14149995	6.28	BnaA03g29370D*	AT3G06300	139.18	P4H2	Velasquez et al., (2015)
PW	A08:14842805	9.15	BnaA08g19480D*	AT1G25540	132.74	PFT1	Sundaravelpandian et al., (2013)
RV	C02:11293971	4.55	BnaC02g15620D	AT5G51590	65.80	AHL4	Zhou et al., (2013)
RW	A04:11231342	4.84	BnaA04g13310D	AT2G22810	16.66	ACS4	Prasad et al., (2010)
RW	C01:18269465	4.67	BnaC01g23930D	AT3G44750	136.01	HDT1	Li et al., (2017)
RSR	A03:13924962	6.5	BnaA03g28940D*	AT3G05090	141.89	LRS1	Lee et al., (2010)
RSR	C01:31588804	4.79	BnaC01g32390D	AT3G20880	6.56	WIP4	Crawford et al., (2015)
SW	A03:14149995	6.29	BnaA03g28920D*	AT3G05010	89.01	Cand2	Jin et al., (2012)
SW	A03:14149995	6.29	BnaA03g28940D*	AT3G05090	83.14	LRS1	Lee et al., 2010
SW	A03:14149995	6.29	BnaA03g29370D*	AT3G06300	139.18	P4H2	Velasquez et al., (2015)
SW	A08:14842805	9.00	BnaA08g19480D*	AT1G25540	132.74	PFT1	Sundaravelpandian et al., (2013)

* Common candidate genes screened from different traits.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.01.028.

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