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Identification of QTLs for new formed root architectural traits in rice (*Oryza sativa* L.) after transplantation

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Abstract

Transplanting has been the most widespread planting technique for rice production in Asia. It is estimated that more than 85% of rice production in china, and almost 100% in Northeast China adopted transplanting. The healthy and vigorous seeding can reduce thetransplanting shock' and is the most important for final yield formation. The large root diameter, long root length, large surface area and more root number of new formed root are the major parameters for healthy and vigorous seeding. Nipponbare, Kasalath and ninety-eight backcross recombinant inbred lines (BC_1F_5) derived from a cross between them were used to detect QTL controlling new formed root traits after transplanting. The healthy seeding was transplanted at fourth leaf stage. And the average root diameter, total root length, average surface area, root length and root number of new formed root were measured at 7 days after transplanting. The *qARD3* associated with average root diameter was detected on chromosome 3.Two putative QTLs controlling total root length were detected on chromosomes 5 (*qRL5*) and 8 (*qRL8*). One QTL *qASA5* affecting average surface area was detected on chromosomes 5. The *qARL5* affecting average root length was identified at the same region with *qRL5* and *qASA5*.Three QTLs associated with root number were detected on chromosomes 1 (*qRN1*), 3 (*qRN3*) and 5 (*qRN5*). The phenotypic variations (VE) explained by individual QTL were ranged from 8.6% to 24.2%. A QTLs cluster formed on chromosome 5. The *qRN3* and *qRL8* were new locus for new formed root traits after transplanting. The QTLs cluster on chromosome 5 was also a new region. These QTLs provided "elements" and basic-information for transplanting root architecture and elucidation of root architecture molecular mechanism after transplanting of rice.

Keywords: rice (*Oryza sativa* L.), transplanting, new formed root traits, QTL mapping.

Introduction

Transplanting is the most widespread planting technique for rice production in Asia (IRRI, 2002). It started at Han dynasty (206 BC -AD 220), spread at Song dynasty (960 - 1279) and became universal at Ming-Qing dynasty (1368 - 1911) (Xu and Xu, 2006). It is estimated that more than 85% of rice production in china adopted transplanting; particularly, almost 100% in Northeast China, the largest *japanoca* rice planting region of China (5.53 million hectares) (Database of ministry of agriculture of CHINA: http://www.zzys.moa.gov.cn/). To obtain high yield by the transplanting method, nursery culture is of prime importance to provide healthy and vigorous seeding. Mechanical transplanting becomes more and more popular recently because of the short supplying of transplanting laborers. Damage to rice seedlings is inevitable in the process of pulling and transplanting of seedlings especially in mechanical transplanting, and it leads to growth stagnation for several days or even a week after transplanting, being known as 'transplanting shock' (Matsuo et al., 1995). Transplanting shock becomes worse than before as wide spreading of seedling transplanter (Liu, 2011). Gradually, the original old root loss its function and the new formed roots becomes the main absorption unit of water and nutrients after transplanting.

Rice has a fibrous root system, which consists of one primary root originating from the seed and a mass of adventitious roots formed from the stem during post-embryonic development. A growing number of studies have focused on the characterization of root development in rice using mutational analysis. Lateral roots are important to plants for the uptake of nutrients and water. Several members of the *Aux*/*IAA* gene family have been shown to play crucial roles in lateral root development (Nakamura et al., 2006; Zhu et al., 2012; Kitomi et al., 2012; Ni et al., 2011). Several gene controlling crown root included *crl1*, *crl4*, *Crl5* and *CAND1* were cloned and characterized (Inukai et al., 2005; Kitomi et al., 2008; Kitomi et al., 2011; Wang et al., 2011). In plants, root hairs are important organs for the uptake of nutrients and water from the rhizosphere and serve as sites of interaction with soil microorganisms. A novel basic helix-loop-helix (bHLH) transcription factor and *OsAPY* had been reported regulating root hair development in rice (Ding et al., 2009; Yuo et al., 2009). In recently, *OsARF12* controlling root length were cloned (Qi et al., 2012). Although, so many genes related rice root had been cloned, but they all characterized by rice mutants. As well known, the rice root traits were controlled by quantitative trait loci (QTLs) derived from natural variations. Horii et al (2006) identified nine QTLs controlled root axis length, root dry weight and branching index on chromosome 1, 6, 9 and 11. Price et al (2002) detected 6 root weight QTLs，11 root to shoot ratio QTLs，12 root number QTLs and 1 root thickness QTLs. Uga et al (2008) identified 10 root traits QTLs including 2 for stele transversal area, 4 for total area of late metaxylem vessels, 2 for total number of late metaxylem vessels and 2 for root thickness. Topp et al (2013) used 3D

techonlogy to identify quantitative trait locus controlling 25 root architecture traits. 89 QTLs were detected on chromosome 1, 2, 6, 7, 9 and 11. Wang et al (2013) fine mapped a root length QTL, qRL7, on chromosome 7. Obara et al (2010) Fine mapped qRL6.1, a major QTL for root length of rice seedlings grown under a wide range of NH_4 (+) concentrations. Uga et al (2012) fine mapped qSOR1, a major rice QTL involved in soil-surface rooting on chromosome 7. Uga et al (2010, 2011) Fine mapped two linked QTLs for root traits on rice chromosome 9, one is *Sta1* controlled determining stele transversal area, and the other is *Dro1* controlled ratio of deep rooting. The *Dro1* was cloned and reported negatively regulated by auxin and is involved in cell elongation in the root tip, and causes asymmetric root growth and downward bending of the root in response to gravity (Uga et al., 2013). Although so many root genes have been functionally characterized, information about new formed root after transplanting is still limited (Ikeda et al., 2007). In this study, we report the identification and confirmation of useful QTLs controlling new formed root traits after rice transplanting. These results will be useful for map-based cloning of useful QTL and marker-assisted selection for high transplanting efficiency varieties.

Results

The performance and correlation analysis of new formed root traits after transplanting

Before transplanting, seedlings of Nipponbare and Kasalath have no significant difference for the five target traits. The average root diameter, total root length, average surface area, root length and root number of Nipponbare were significantly higher than Kasalath at 7 days after transplanting (Table 1). The average root diameter, total root length, average surface area, root length and root number of Nipponbare were 1.62, 2.85, 2.35, 1.09 and 2.62 times of Kasalath. All the five traits showed a continuous normal distribution in the BILs population according to Skewness and Kurtosis value. Transgressive segregation was also identified. These results suggested that this population was adapted to QTL analysis.

The correlation analysis of new formed root traits after transplanting

The average root diameter showed positive correlation with both total root length (0.4142^{*}) and average surface area (0.4657^{*}). The total root length showed positive correlations with average surface area (0.9596^{**}), root length (0.6659^{**}) and root number (0.74556^{**}). Highly positive correlations were detected between average surface area and root length (0.6279^{**}) as well as root number (0.7273^{**}). No significant correlation was identified between root length and root number.

QTL controlling new formed root traits after transplanting in rice

A total of eight significant QTLs were identified for the five traits using CIM as summarized in Figure 1 and Table 3. The number of QTLs detected ranged from one to three per trait. These eight QTLs were located in five intervals distributed along the chromosome 1, 3, 5 and 8 (Fig. 1).

One QTL on chromosome 3 (*qARD3*) was associated with average root diameter. The Nipponbare allele conferred a positive effect at this locus, increasing the root diameter. The phenotypic variation explained by this QTL was 22.6 % in the BC_1F_5 lines. Two putative QTLs controlling total root length were detected on chromosomes 5 (*qRL5*) and 8 (*qRL8*). Phenotypic variation explained by *qRL5* and *qRL8* were 10.9 % to 24.2 %, respectively, and these two QTLs explained 35.1 % of the total phenotypic variation in the BC_1F_5 population. At *qRL5*, the Nipponbare allele increased the total root length whereas the Nipponbare allele decreased the total root length at *qRL8*. One putative QTLs affecting average surface area was detected on chromosomes 5. The Nipponbare allele increased the average surface area at this locus. The *qASA5* accounted for 18.0 % of the phenotypic variation. One putative QTL on chromosome 5 affecting average root length was identified at the same region with *qRL5* and *qASA5*. The Nipponbare allele increased the average root length at this locus. The phenotypic variation explained by *qARL5* was 14.2 %. Three QTLs associated with root number were detected on chromosomes 1, 3 and 5. The Nipponbare allele decreased the root number at *qRN1* and *qRN3* but increased the root number at *qRN5*. The *qRN5* was also located at the *qRL5* and *qASA5* region. The phenotypic variation explained by the three QTLs ranged from 8.6 % and 10.1 %.

Discussion

The finally yield of rice is formed from synergistic effect of roots and shoots. In recently, research focus gradually shifted to the underground part (root) of rice, and proposing the "Root Breeding". Although many root mutant genes and QTLs for root trait have been functionally characterized or mapped, limited information is available on new formed root after transplanting (Nakamura et al., 2006; Zhu et al., 2012; Kitomi et al., 2012; Ni et al., 2011; Topp et al., 2013; Li et al., 2005; Kamoshita et al., 2002; Qu et al., 2004). In this study, we identified eight QTLs controlling average diameter, total root length, average surface area, root length and root number. And they distributed on chromosome 1, 3, 5 and 12 and formed a QTLs clusters on chromosome 5. These QTLs provided "breeding elements" and could help us to clone rice new formed root genes after transplanting. To compare the QTLs identified in this paper with the other research team report by QTL Annotation Rice Online (http://qtaro.abr.affrc.go.jp/index.html), we founded that *qRN1* was located in the same region with the QTLs controlling penetrated root thickness (Zheng et al., 2000), deep root ratio (Kamoshita et al., 2002) and adventitious root number (Zheng et al., 2006). The *qARD3* was located in the same region with the QTLs for Root value (Fang et al., 1999). The *qRN3* and *qRL8* were new locus for new formed root traits after transplanting. The QTL cluster on chromosome 5 was also a new region with high research and application value controlling total root length, average surface area, root length and root number of new formed root after transplanting. As the rapid development of agricultural machinery and large-scale transfer of rural labor, Mechanical transplanting has become more and more widespread in recent years instead of traditional manual transplanting. The mechanical transplanting area has occupied 80% of the total rice area in Heilongjiang province (the rice planting area has been more than 4 million hectares). Damage to rice seedlings is inevitable in the process of pulling and transplanting of seedlings especially in mechanical transplanting. The 'transplanting shock' by mechanical transplanting is always more than manual transplanting with 3-5 days or even more than a week. The new breeding target of rice was proposed as the new variety must have higher root re-architecture ability. We identified stable positive alleles for new formed root traits, Nipponbare positive allele was locked between C624 and C1018

Table 1. Performance of transplanting rice root traits in Nipponbare, Kasalath and BILs population.

Trait	Kasalath	Nipponbare	Difference	Range	Mean±SD	Skweness	Kurtosis
Root average diameter	0.39	0.63	0.24**	0.385-0.767	0.529±0.066	0.28	1.00
Total root length	9.54	27.20	17.66**	5.112-33.079	16.135±6.172	0.56	0.18
Average surface area	1.99	4.67	2.68**	0.817-6.033	2.710±1.136	0.54	0.08
Average root length	2.21	2.40	0.20*	1.166-3.958	2.305±0.594	0.43	-0.02
Root number	4.32	11.32	7.00**	3.333-14.000	6.985±2.058	0.90	1.15

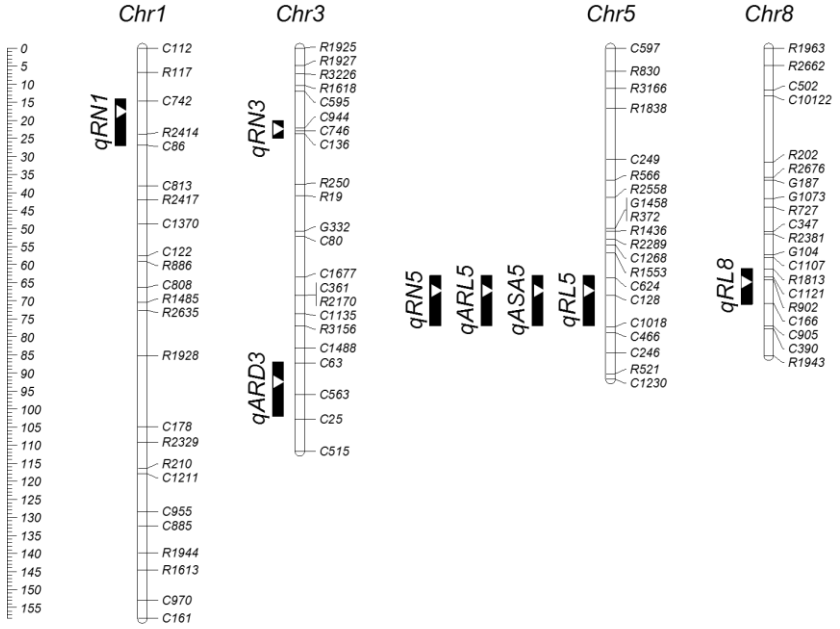

Fig 1. Putative QTLs for root traits identified at 7 days after transplanting. The legends on the chromosomes represent putative regions of QTLs for each trait.

Table 2. Correlation indexes of transplanting rice root traits in Kasalath, Nipponbare and BILs population.

Trait	Root average diameter	Total root length	Average surface area	Average root length	Root number
Root average diameter	1.000				
Total root length	0.4142*	1.000			
Average surface area	0.4657*	0.9596**	1.000		
Average root length	0.163	0.6659**	0.6279**	1.000	
Root number	0.167	0.74556**	0.7273**	0.030	1.000

Table 3. QTLs controlling root ability related trait in transplanting rice.

QTL	Chromosome	Marker interval	Physical location	Peak LOD	PEV(%) [a]	Additive effect [b]
qARD3	3	C63-C25	3,965,273-8,410,886	5.74	22.6	-0.0354
qRL5	5	C624-C1018	21,372,388-24,477,046	5.59	24.2	-3.4974
qRL8	8	R1813-C166	3,174,171-7,595,028	3.37	10.9	2.3817
qASA5	5	C624-C1018	21,372,388-24,477,046	4.76	18.0	-0.5569
qARL5	5	C624-C1018	21,372,388-24,477,046	3.41	14.2	-0.2578
qRN1	1	C742-C86	37,713,253-40,919,341	2.52	8.6	0.6980
qRN3	3	C944-C136	26,729,079-28,528,927	2.93	10.1	0.7973
qRN5	5	C624-C1018	21,372,388-24,477,046	2.80	9.8	-0.7660

[a]Percentage of explained phenotypic variation, [b] Positive and negative values indicate that alleles of Kasalath and Nipponbare, respectively.

region on chromosome 5. The molecular markers needed for breeding will be developed by sequence these regions. In rice production, the *japonica* rice always showed more suitable for dry land nursery and transplanting than *indica* rice. But we still found the allele from *indica* variety Kasalath had the positive function. It may be because of the "hidden diversity". Using diverse *indica* germplasm as donors in rice breeding could effectively improve transplanting new formed root traits of *japonica* rice in Northeast China with increasing genetic diversity and without losing their high yield potential.

Materials and Methods

Plant materials

A total of 98 backcross recombinant inbred lines (BC_1F_5) were used. They were constructed by Lin et al. (1998) from an intersubspecific backcross, Nipponbare / Kasalath // Nipponbare, by the single seed descent method. The chromosome map adopted for this study was developed earlier using 245 RFLP markers (Lin et al., 1998). Nipponbare, a Japan *japonica* rice, is more suitable for transplanting than *indica* rice Kasalath.

Cultivation and morphological analysis

The field experiments were conducted in the rice growing seasons on the experimental farm of Cultivation and Farming Research Institute of Heilongjiang Academy of Agricultural Sciences, Harbin, China (Longitude 126°83′E; Latitude 45°85′N).

The dry land nursery was used to obtain high quality seedling. The sowing date was April 18 and transplanting date was May 21. Field and seeding bed management essentially followed standard agricultural practice. Fertilizers applied were 60, 90, and 90 kg/ha for N, P_2O_5, and K_2O, respectively.

One healthy seeding with one tiller at fourth leaf stage was transplanted in each hill. Four-row plots with 12 plants per row were planted in a 30 cm × 15 cm distance in a completely randomized block design with three replications. Ten plants in each plots were sampled at 7 days after transplanting and then another plant was transplanted again. The average root diameter, total root length, average surface area, root length and root number of new white root were measured by software WinRHIZO Reg 2009c (Regent Instruments Inc.) at 7. The mean value of three replications were used to QTL analysis.

QTL analysis

QTL detection was carried out by Composite Interval Mapping (CIM) method in Qgene 4.3.2 software (Joehanes and Nelson, 2008). Composite interval analysis was undertaken using automatic parameter setting and controlling marker forward stepwise. The threshold of LOD score was determined by the method of permutation (1,000 times). A putative QTL was detected between the markers when the LOD score was larger than the threshold.

Conclusion

The QTLs controlling new formed root traits after transplanting were detected by using Nipponbare, Kasalath and ninety-eight backcross recombinant inbred lines (BC_1F_5) at 7 days after transplanting. The *qARD3* associated with average root diameter was detected on chromosome 3.Two putative QTLs controlling total root length were detected on chromosomes 5 (*qRL5*) and 8 (*qRL8*).One QTL *qASA5* affecting average surface area was detected on chromosomes 5. The *qARL5* affecting average root length was identified at the same region with *qRL5* and *qASA5*.Three QTLs associated with root number were detected on chromosomes 1 (*qRN1*), 3 (*qRN3*) and 5 (*qRN5*). The *qRN3* and *qRL8* were new locus for new formed root traits after transplanting. The QTLs cluster on chromosome 5 was also a new region.

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