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QTLs and epistasis for seminal root length under a different water supply in rice (*Oryza sativa* L.)

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Abstract To identify the genetic background of seminal root length under different water-supply conditions, a recombinant inbred (RI) population consisting of 150 lines, derived from a cross between an *indica* lowland rice, IR1552, and a tropical *japonica* upland rice, Azucena, was used in both solution culture (lowland condition) and paper culture (upland condition). Quantitative trait loci (QTLs) and epistatic loci for seminal root length were analyzed using 103 restriction fragment length polymorphism (RFLP) markers and 104 amplified fragment length polymorphism (AFLP) markers mapped on 12 chromosomes based on the RI population. One QTL for seminal root length in solution culture (SRLS) and one for seminal root length in paper culture (SRLP) were detected on chromosomes 8 and 1, and about 11% and 10% of total phenotypic variation were explained, respectively. The QTL for SRLP on chromosome 1 was very similar with the QTL for the longest nodal root referred to in a previous report; this QTL may be phenotypically selectable in a breeding program using paper culture. Five pairs of epistatic loci for SRLS were detected, but only one for SRLP, which accounted for about 60% and 20% of the total variation in SRLS and SRLP, respectively. The results indicate that epistasis is a major genetic basis for seminal root length, and there is a different genetic system responsible for seminal root growth under different water supply conditions.

Keywords Rice (*Oryza sativa* L.) · Seminal root length · Different water supply · Quantitative trait loci (QTLs) · Epistasis

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Introduction

Rice (*Oryza sativa* L.) is one of the world's most important food crops. One-half of the 148 million hectares of rice planted annually is in a rainfed environment (IRRI 1993). In these areas rainfall is the major source of water supply, and yields may be seriously restricted by water deficit.

The root system directly affects the amount of water available to a crop. In the dry season crops largely rely on water stored in the soil profile. It is essential that roots are able to grow sufficiently deep to access soil-stored water (Monteith 1986), especially at the seedling stage. So a deep root system is beneficial in avoiding water stress by absorbing water from deep soil layers (Chang et al. 1982; Yoshida and Hasegawa 1982).

The root system of rice consist of seminal and nodal roots with first- and higher- order lateral roots. Lowland rice has a shallow and compact root system, most of which is distributed in the top 15-cm of soil (Kang et al. 1994; Morita et al. 1995). Upland rice often has a deeper root system compared with lowland rice, and some roots of upland rice can reach a 70-cm to 80-cm soil depth (Morimoto 1940). Upland rice is usually directly planted under rainfed conditions. Therefore, the growth of seminal roots is important for the emergence and establishment of seedlings under an upland condition. It is necessary to understand the genetic background of the seminal root of upland rice in order to allow breeders to improve the productivity of rice under a rainfed system.

Recent advances in molecular-marker technology have led to well-developed molecular genetic maps of rice (Causse et al. 1994; Kurata et al. 1994;), which make it possible to identify and locate genes or quantitative trait loci (QTLs). Several studies have previously reported QTLs for maximum root length (MRL) and other root traits in rice under different conditions (Champoux et al. 1995; Redoña and Mackill 1996; Price and Tomos 1997; Yadav et al. 1997). However, we do not have sufficient knowledge of the genetic background responsible for seminal root length in rice under different water-supply conditions.

From previous screening experiments, it was found that elongation of the seminal root was accelerated under upland conditions at the seedling stage in different solid media. The difference in seminal root length between Azucena and IR1552 was greater under paper culture than under solution culture. A recombinant inbred (RI) population (F_{10}), derived from Azucena and IR1552, was used to identify the genetic basis of seminal root length under different growth conditions.

Materials and methods

Plant material

A recombinant inbred (RI) population consisting of 150 lines, derived from a cross between IR1552, an *indica* variety of lowland adaptation, and Azucena, an upland tropical *japonica* variety, was used in this study. The RI population was developed by single-seed descent to the F_{10} generation, using bagged panicles.

Growth conditions and measurement of root length

The difference in seminal root length between the parents, and the segregation for root length among the RI population, were measured in solution-culture and paper-culture experiments conducted in a greenhouse at Zhejiang University Hua-jia-chi Campus in 1999 by using a completely randomized design. Seeds of each line and the parents were sterilized in water bath at 55°C for 10 min, washed with distilled water and allowed to germinate at 35°C for 2 days. Germinated seeds were vertically planted on filter paper for 2 days. Seedlings with a 2.5-cm-long root were transplanted to solution culture using two treatments: solution culture (lowland condition) and paper culture (upland condition). All the lines were planted with five replications, but with 20 replications for the two parents. For solution culture, ½-strength Yoshida's nutrient solution (Yoshida et al. 1976) at pH 5.0 was used. For paper culture, anchor papers (Anchor Paper Ltd. USA) were covered with holed plastic bags; seedlings were transplanted to papers after the root length reached 2.5 cm. The anchor paper was hung vertically with 2 cm of the lower part soaked in $^1/_2$-strength Yoshida's nutrient solution (Yoshida et al. 1976) at pH 5.0. During the experiment, the day/night temperature was 28/22°C with 60% relative humidity and a 12-h photoperiod of approximately 158 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided by a 1000 W dysprosium lamp every day. The pH of the solution was adjusted daily and the nutrient was replaced every week. Fifteen days after sowing, when the difference in seminal root length between the parents in paper culture was pronounced, the root length was determined after staining with basic fuchsin, using a scanner connected to an image-analysis system. All 750 plants were analyzed at one run.

Molecular-map construction

A molecular map with 104 amplified fragment length polymorphism (AFLP) markers and 103 restriction fragment length polymorphism (RFLP) markers was constructed for the RI population (Wu et al. 2000). The total map length was 2,419.5 cM and encompassed the 12 rice chromosomes. The average distance between markers was 11.7 cM. RFLP probes were from Cornell University and the Japanese Rice Genome Project. The RFLP markers were determined by hybridizing radioactive DNA (Feinberg and Vogelstein 1984) to fragments of plant DNA separated on agarose gels after digestion with six restriction enzymes, *DraI, EcoRI, EcoRV, HindIII, ScaI* and *XbaI*, respectively. The 103 informative RFLP markers distributed throughout the 12 chromosome were selected and scored on 150 RI lines. AFLP analysis was conducted following the method of Vos et al. (1995), with minor modifications employed by Maheswaran et al (1997). *EcoRI/MseI* systems (Life Technologies, 10544–013) were used to generate polymorphic AFLP markers, and a total of 16 primer-pair combinations were employed. One hundred and four AFLP markers were assigned to the 12 linkage groups at LOD>3 based on their linkage to the anchor RFLP markers using the Mapmaker/EXE 1.0 program (Lander 1993).

Statistical analysis

One-way ANOVA (SAS/6.11, GLM) and interval-mapping analysis (Mapmaker/QTL) (Lander 1993) were performed in order to detect QTLs for the variations in seminal root length in solution culture (SRLS) and seminal root length in paper culture (SRLP) of the RI population. $P<0.005$ for Type-I errors and a $\log_{10}$ likelihood ratio (LOD) value of 2.4 were used as criteria to indicate the putative QTL position. The additive effect and the percentage of variation explained by individual QTLs were estimated. Epistasis in the parameters was analyzed using the program QTL mapper (Wang et al. 1999).

Results

Water content of growth medium

The water content of papers at different heights from the solution level is shown in Fig. 1. From the upper part of papers to the lower part, the water content increased gradually. It ranged from about 66% to 71%. It was acceptable to consider paper culture as an upland condition because moderate soil moisture was approximately 62% of the maximum water-holding capacity.

Phenotypic variation

The phenotypic values of the parents and of the RI population are presented in Fig. 2. A significant difference ($P<0.05$) in the seminal root length in solution culture (SRLS) between the two parents was observed in this case, but the most-significant difference ($P<0.01$) was in paper culture. Transgressive segregation in the RI popu-

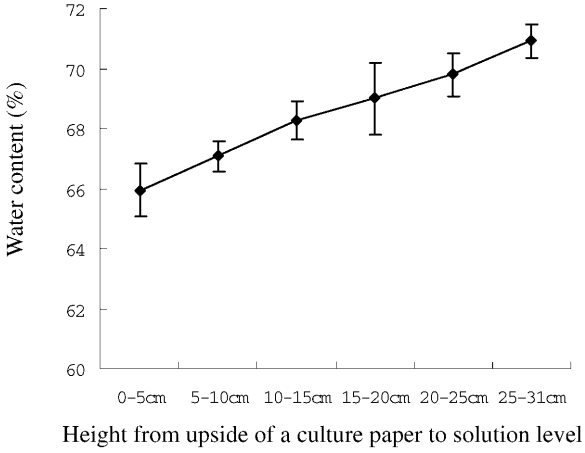

Fig. 1 Water content of different heights from the upside of a culture paper to the solution level. *Bar*=standard error

Table 1 QTLs for seminal root length in solution culture and paper culture (SRLS and SRLP)

Traits [a]	Chrom.	Marker interval	LOD [b]	%Variation	Additive [c]
SRLS	8	RG1	2.53	11.2	–0.74
SRLP	1	RG109B/RG690	3.37	10.0	–1.86

[a] SRLS: seminal roo t length in solution culture; SRLP: seminal root length in paper culture
[b] LOD: Log_{10}-likelihood
[c] The additive effect is the effect associated with the substitution of an IR1552 allele by its corresponding Azucena allele

Table 2 Epistatic loci for seminal root length in solution culture and paper culture (SRLS and SRLP)

Trait[a]	Chrom.	Locus	Chrom.	Locus	LOD[b]	Effect	%Var
SRLS	1	RG222/RZ801	6	RG64/AAC-CTT5	5.17	–0.8579	10.16
	1	AGG-CAA6/AGC-CTA6	4	AGG-CAA2/AGC-CAG6	4.43	0.7564	7.9
	5	RZ390/ACA-CTG9	5	CDO105/RG13	4.99	–0.9871	13.45
	9	ACA-CTA4/AAC-CAG3	11	AAG-CAT13/AAG-CTC4	5.27	–1.1279	17.56
	10	G2155/AGC-CAG7	12	RG341/ACA-CTT1	3.13	0.8909	10.96
SRLP	6	RZ508/AGG-CAG3	9	AGC-CAG3/RZ698	4.33	2.117	19.88

[a] SRLS: seminal root length in solution culture; SRLP: seminal root length in paper culture
[b] LOD: Log_{10}-likelihood

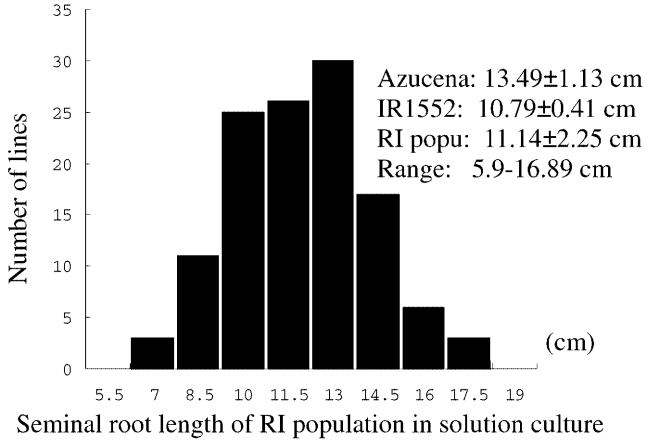

Fig. 2 Distribution of SRLS and SRLP among the RI population

lation was observed and SRLS values ranged from 5.90 to 16.89 cm, while SRLP values ranged from 5.98 to 25.22 cm. Both SRLS and SRLP among the RI population were normally distributed.

QTLs analysis

Two QTLs were detected for the two traits, respectively. One QTL linked with RG1 on chromosome 8 explained about 11% of the total variation in SRLS (Table 1 and Fig. 3). The other QTL for SRLP was detected on chromosome 1 flanked by RZ730 and RG109B (Table 1 and Fig. 3); the contribution of the QTL to the total variation in this case was 10%. The positive alleles of the two QTLs were from Azucena.

It was shown that the positive allele in Azucena at the locus linked with RZ730 and RG109B on chromosome 1 was especially expressed under the upland condition. While the positive allele at the locus linked with RG1 on chromosome 8 was especially expressed under the lowland condition.

Epistatic effect

Significant epistatic loci (P<0.005) for target traits were detected by the QTLmapper (Wang et al. 1999). Five pairs of epistatic loci for SRLS were identified on chromosomes 1, 4, 5, 6, 9, 10, 11 and 12, which together accounted for 60% of the total variation in SRLS (Table 2 and Fig. 3). Among the five epistatic loci, the loci between chromosomes 9 and 11 explained as high as about 18% of the total variation in SRLS. Only one pair of epistatic loci was identified from paper culture between chromosomes 6 and 9, which explained about 20% of the total phenotypic variation (Table 2 and Fig. 3). This indicated that the interaction of different loci can explain a portion of the genetic effect of the traits.

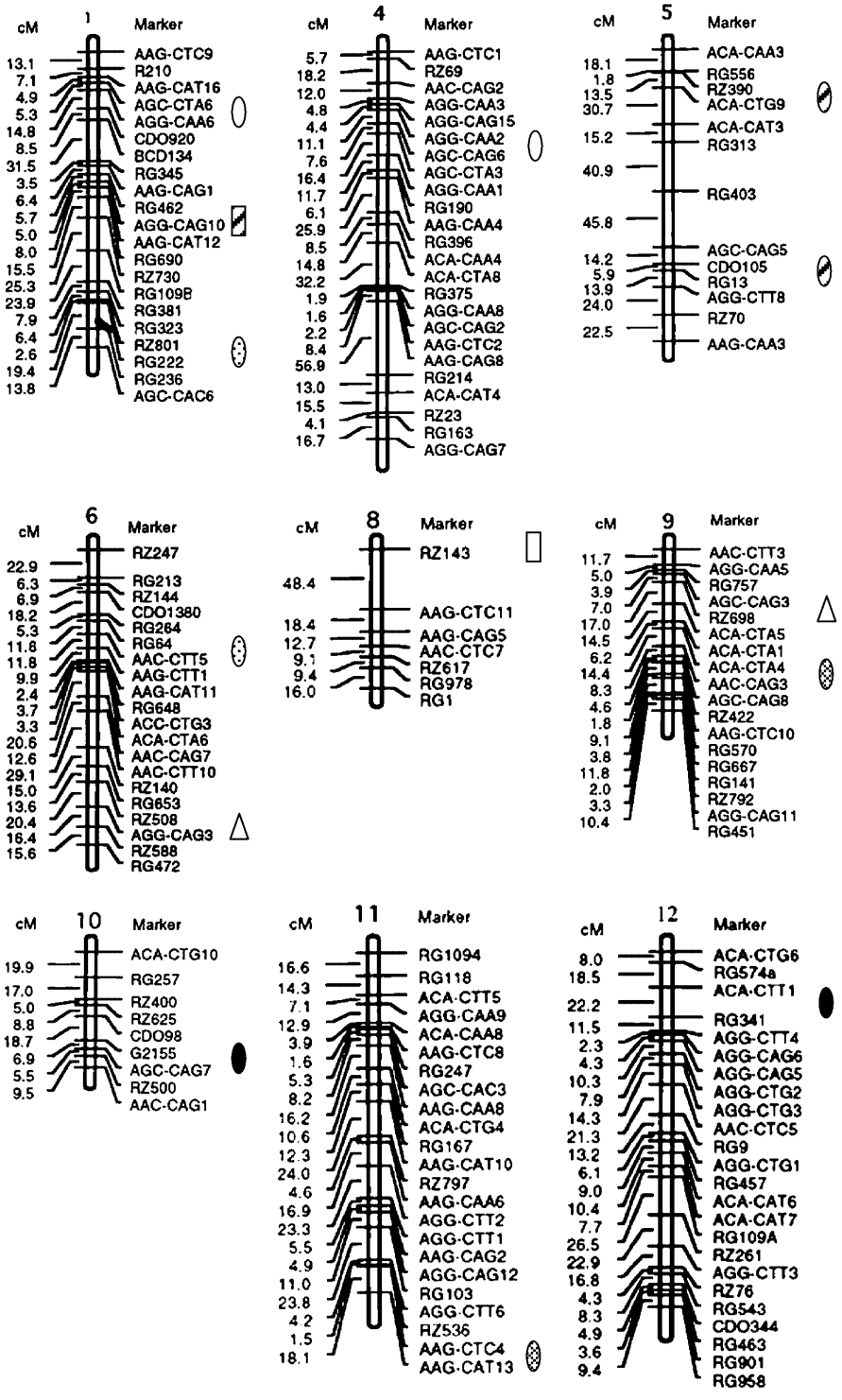

Fig. 3 The most-likely location of QTLs and epistasis loci for SRLS and SRLP in the RI population derived from a cross between IR1552 and Azucena. The *symbols* for different parameters are shown above. The *designation on the right* is marker name, and *on the left* is the map distance based on the Kosambi function

Discussion

Roots were difficult to extract intact from the soil in order to measure in detail. We developed a paper-scanning system to expediently and rapidly observe root system growth under the upland condition. This system resolved the problem that main roots could be stranded when sampling from solid medium.

In the present study, two QTLs were detected for seminal root length under different water supply conditions in the RI population consisting of 150 lines with 207 molecular markers mapped on 12 chromosomes. Among the two QTLs, one for SRLS was detected on chromosome 8. Price and Tomos (1997) reported ten QTLs for rice maximum root length (LOD≥2.4) after 3, 7, 14, 21 and 28 days of growth in a hydroponic system,

but none of them were on chromosome 8. The seminal root is usually the maximum root before the third-leaf period. But we were unable detect the QTLs for seminal root length which Price and Tomos (1997) reported in our RI population in solution culture, whereas Azucena was the common parent both used in both ours and Price and Tomos' population. This may be because of an allelic/non-allelic difference at the QTL locations between the other parent. Moreover, the existence of non-allelic interactions in our case might affect the common QTLs detected between the two populations (see below). One QTL for SRLP was identified on chromosome 1 and flanked by RZ730 and RG109B. Yadav et al. (1997) found one QTL for maximum root length (MRL) on chromosome 1 associated with RG381 and RG690 under upland conditions. The MRL was the longest nodal root in their study. In our map, RZ730 and RG109B were in the marker interval of RG381 and RG690. As some markers used were common between these two populations, aligning chromosomes was practicable, and these two QTLs were very similar if not identical. It can be suggested that the QTL on chromosome 1 is expressed to control the root length (of both seminal and nodal roots) under upland conditions. Since this QTL (flanked by RZ730 and RG109B on chromosome 1) has an additive effect on root length, it may be phenotypically selectable in a breeding program using paper culture.

Epistasis, or interlocus interaction, is a kind of gene interaction whereby one gene interferes with the phenotypic expression of another non-allelic gene. A considerable body of classical evidence has strongly suggested the prevalence of an epistatic effect on quantitative traits in genetic populations (Spickett and Thoday 1966; Allard 1988). Two QTLs involved in root development with large effects were mapped on chromosomes 2 and 6, and an epistatic interaction was found between these two loci (Kreike et al. 1996). Price et al. (1997) observed that the non-allelic interactions were important for maximum root length (MRL) in a hydroponic system. Yadav et al. (1997) reported 16 pairs of interaction between marker loci for MRL under drought conditions in a IR64×Azucena population. In the present study, an epistatic effect accounted for about 60% of the phenotypic variation of SRLS and 20% of SRLP, but no identical pairs were found to be responsible for the two traits. These results suggested that different epistatic systems control seminal root elongation under different water supply conditions, and that epistasis explained a considerable portion of the total genotypic variances of seminal root length.

Three epistatic loci detected in our study were involved in the reported QTLs for rice root length mapped on chromosome 1 (Redoña and Mackill 1996) and chromosome 5 (Price and Tomos 1997). One epistatic locus for MRL (Yadav et al. 1997) flanked by RZ19 on chromosome 1 was in the marker interval of the QTL for SRLP, in our case on chromosome 1. It has been assumed that QTLs and epistatic effects can be exchanged in different genetic backgrounds and environments where they are identified (Li et al. 1997). Since epistatic genes which influence quantitative phenotypes remain to be resolved by molecular genetics, more related studies need to be carried out for understanding the interactions between the non-allelic loci of complex quantitative traits.

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