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Relationship Between Coleoptile Length and Drought Resistance and Their QTL Mapping in Rice

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Abstract: By using a set of recombinant inbred line (RIL) population involving in 195 lines derived from a cross of Zhenshan 97B (lowland variety) and IRAT109 (upland variety), the correlation analysis between coleoptile length (CL) and drought resistance index (DRI) and their QTL identification were conducted. There existed a significantly positive relationship between CL and DRI with the correlation coefficient of 0.2206** under water stress conditions. Under normal and water stress conditions, a total of eleven and four QTLs for CL and DRI, respectively, were detected on chromosomes 1, 2, 4, 5, 6, 7, 9, 11 and 12 by using a linkage map including 213 SSR markers, which explained 4.84% to 22.65% of phenotypic variance. Chromosomes 1 and 9 possessing the QTLs for DRI harbored simultaneously QTLs for CL, and *qCL9* shared the same chromosome location with *qDRI9* (RM160-RM215). Comparing the QTLs related to drought resistance in other studies, QTLs for CL and DRI were located in the same or adjacent marker interval as those related to root traits, such as number, dry weight, depth, and length of root. Moreover, sixteen and three pairs of epistatic loci for CL and DRI were found, which accounted for 56.17% and 11.93% of the total variation in CL and DRI, respectively.

Key words: recombinant inbred lines; coleoptile length; drought resistance index; quantitative trait loci; rice

In plant, coleoptile is an organ to provide protection to the developing shoot as it is passing through soil, which is very important at the early growth stage of cereal crops [1-5]. Previously, most of the studies on coleoptile were focus on the mechanism of elongation and physiological responses [6-9]. Wang et al [10-12] found a significant relation between coleoptile length (CL) and drought resistance index (DRI) in wheat under drought, and suggested that the CL can be used to evaluate drought tolerance (DT) in wheat and to screen drought-tolerant genotypes at early generation in wheat breeding. Though some reports have shown that longer coleoptile genotype is suitable for direct seeding, further studies on the relationship between drought tolerance and coleoptile length were scarce in rice [13]. In order to understand the molecular mechanism and the relationships between CL and DRI in rice and then to clone drought resistance genes, the CL and DRI in rice were investigated under drought stress and normal conditions and their QTLs were mapped by using a RILs population.

MATERIALS AND METHODS

Rice materials and treatments

Population construction and drought treatment were conducted according to description by Liu et al [14]. A set of recombinant inbred lines (RILs) population involving in 195 lines (F$_9$) was derived from the cross of Zhenshan 97B and IRAT109 by single-seed descent method. Zhenshan 97B, considered to be drought susceptible, was an improved indica rice variety adapted to the lowland ecosystem in China, while IRAT109, considered to be drought tolerant, was an upland japonica rice variety originally developed by the Africa Rice Center (WARDA). Recombinant inbred lines population and their parents were planted in a facility especially designed for drought tolerance screening in Shanghai, China. Seeds were directly

sown into soil in two-row plots, with about 5 seeds each hill. There were 15 hills in each row with a space of 20 cm between rows and 18 cm between hills. After seedling emergence the seedlings were thinned to one per hill. Materials were divided into three groups and sown on May 10, 17, and 24 in 2003, respectively, to synchronize flowering time. The groups in the population and lines in each group were arranged randomly in the field with three replications (blocks).

Screening facility for drought tolerance (DT) and water management

A north-south oriented rainproof DT screening facility with a powerful water control system was established in Shanghai in 2002. The plastic roof could prevent the rainfall from entering into the facility and could be opened in sunny days. The field contained two plots, 76 m long and 6 m wide for each. A sprinkler irrigation system was installed above the plots while a drip irrigation system was installed on a north-south orientation in the middle of each plot. Plots were surrounded by a 2-m deep canal. Holes were designed on the north-south oriented wall of the canal at the same intervals so that the soil water could be leaked into the canal. Surface irrigation was applied during the vegetative growth period. Additional water was provided via a sprinkler system to keep the plants growing under well water conditions. When 60% lines reached the early stage of panicle initiation, sprinkler irrigation was stopped. Water level in the canal was decreased from the normal ground water level to 1.8 m below the soil surface. Consequently, the soil water was leaked into the canal through the holes. Since the rates of water loss in the middle and the edge of plots were different, a water gradient was produced. In order to enhance the soil water gradient, drip irrigation was applied everyday for several hours to supply additional water during the drought duration. As a result, the edge of plots close to the canal was under water stress conditions while the middle of plots close to drip irrigation was under normal water conditions.

Measurement

Calculation of drought resistance index (DRI)

The plants under stress and normal conditions were separately harvested to measure the grain yields (October 15, 2003), and the DRI was calculated by the formula:

DRI = The grain yield under stress / The grain yield under normal conditions.

Measurement of coleoptile length (CL)

The optimum concentration of PEG-6000 for simulating drought stress was noted at 0.75 mol/L (15%) of PEG-6000 by seed germination experiment among a series of concentrations of PEG-6000 (0-50% with a interval of 5%), at which most of the tested rice materials were stressed significantly but still be in survival. Fifty grains of each line was washed with water and soaked in 20 mL PEG-6000 solution (0.75 mol/L) in a plastic box (10 cm×10 cm×5 cm). All materials were kept in an incubator at 25℃ and relative humidity of 90% for 7 d. CL was measured with a ruler. Three replications were arranged during experiments with pure water as control treatment.

QTL mapping method

ANOVA and phenotypic correlation analysis were performed by using S-Plus for Windows V6.1 (Insightful Corporation, 2001). QTL analysis was conducted by using QTLMapper V1.0 [7] based on a mixed model approach. A threshold of $P \leqslant 0.05$ was used to select significant effect QTL, while the QTL with a LOD value beyond 3.0 was retained as the main effect QTL only. For digenic epistatic QTL detection, the threshold was increased to $P \leqslant 0.001$. Contribution rate (R^2) was estimated as percentage of variance explained by each locus or epistatic pair in relation to total phenotypic variance. Main effect QTLs were named following the popular nomenclature [7], but using alphabetic order for QTLs on the same chromosome [14].

RESULTS

Frequency distribution of CL and DRI in RILs population

Under both water stress and normal conditions, a significant difference was found in CL between two parents, i.e. female parent Zhenshan 97B was 4-5 mm
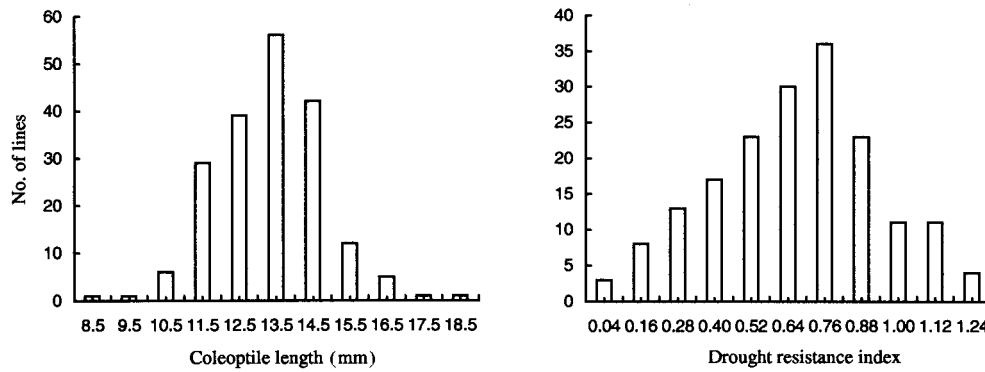
Fig. 1. Frequency distribution of coleoptile length and drought resistance index of RILs.

shorter than male parent IRAT109 in CL (Table 1). The water stress treatment significantly decreased the CL compared with the normal treatment. The CL of RILs showed a transgressive segregation under two treatments. The mean of CL in RILs population was between the CLs of the parents under water stress conditions, but slightly lower than the low CL parent Zhenshan 97B under normal conditions.

The correlation analysis showed that DRI was significantly related with CL in RILs population under water stress conditions (r=0.2206**, Fig. 1). For RILs population, the CL showed a normal distribution under two treatments, with similar range, mean and kurtosis but different skew values in the two treatments, while DRI displayed an approximate normal distribution under water stress conditions (Fig. 1), indicating that these two traits were quantitative ones suitable to QTL mapping.

QTLs for CL and DRI

A total of 11 QTLs for CL and 4 for DRI under normal and stress conditions were detected on all the 12 chromosomes except the chromosome 3,8 and 10 (Table 2, Fig. 2).

Table 1. Phenotypic values of coleoptile length in RILs population and their parents.

| Treatment | IRAT109 | Zhenshan 97B | RILs population | | | | |
	Mean±SD (mm)	Mean±SD (mm)	Range	Mean±SD (mm)	Kurtosis	Skew	F value
Water stress condition	15.07±1.52	11.07±0.88	8.47–18.04	13.11±0.10	0.7274	0.0766	68.63**
Normal water condition	18.11±0.85	13.65±0.70	8.06–18.11	12.16±0.12	0.7123	0.4494	

Table 2. Putative QTLs for CL and DRI of RILs (Zhenshan 97B×IRAT109) under different conditions.

| Trait	QTL	Treatment	Chromosome	Interval	LOD	R^2%	Additive
CL	qCL2a	Stress	2	RM145–RM438	5.00	9.53	0.3759
	qCL2b	Stress	2	RM262–RM263	7.90	18.69	-0.5266
	qCL6	Stress	6	RM539–RM136	7.85	11.46	-0.4123
	qCL9	Stress	9	RM160–RM215	4.19	7.62	0.3361
	qCL1a	Normal	1	RM572–RM23	8.16	12.77	0.6374
	qCL1b	Normal	1	RM476B–RM315	4.40	5.06	-0.4013
	qCL2c	Normal	2	RM492–RM145	5.19	5.89	0.4328
	qCL2d	Normal	2	RM526–RM525	16.56	22.65	0.8488
	qCL7	Normal	7	RM481–RM125	9.75	11.48	0.6042
	qCL11	Normal	11	RM20B–RM167	10.53	12.16	0.6220
	qCL12	Normal	12	RM453–RM247	5.33	4.84	-0.3925
DRI	qDRI1	–	1	RM104–RM414	10.52	19.79	0.1095
	qDRI4	–	4	RM273–RM252	5.44	9.43	0.0756
	qDRI5	–	5	RM574–RM169	4.78	7.83	-0.0689
	qDRI9	–	9	RM160–RM215	3.70	6.82	-0.0643
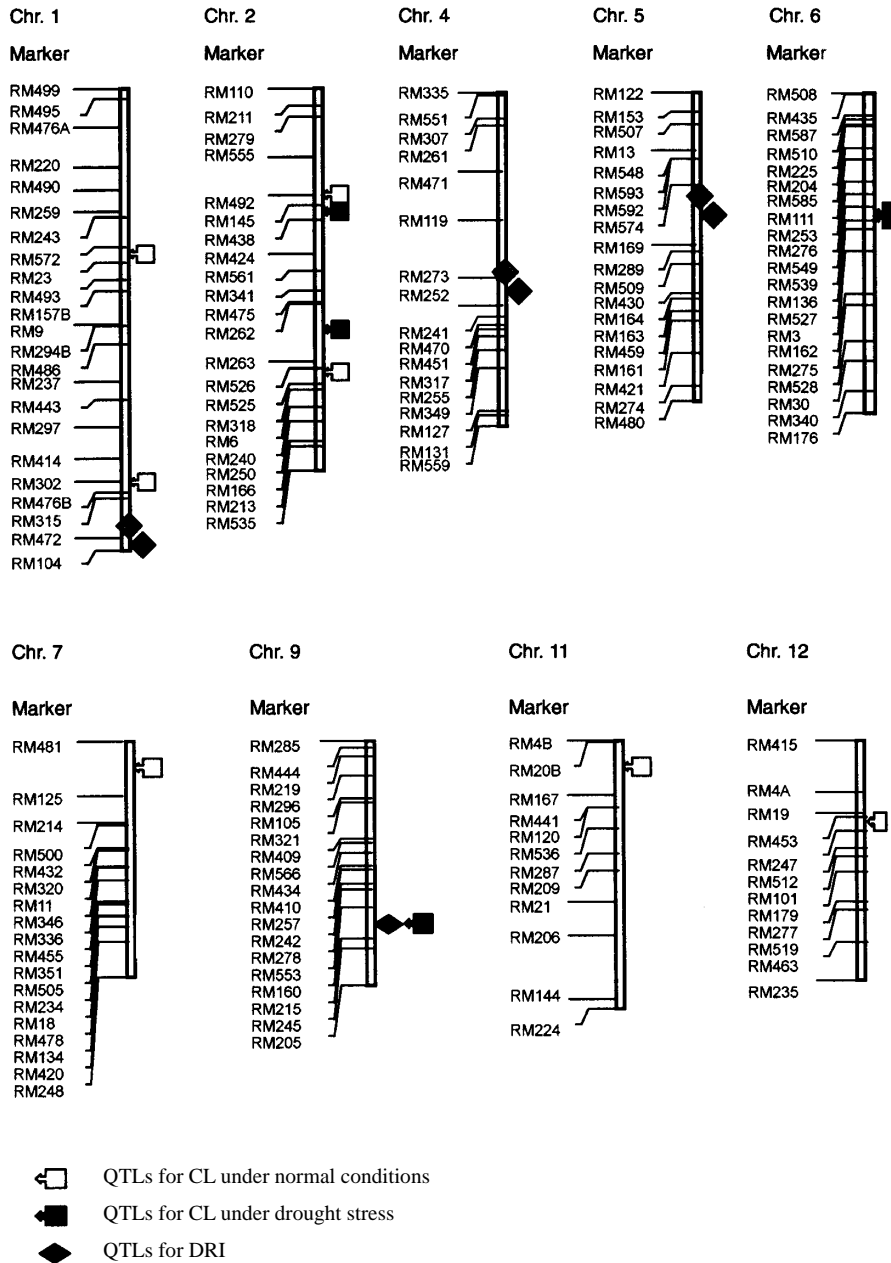
Fig. 2. Mapping of QTLs for coleoptile length (CL) and drought resistance index (DRI) on the rice linkage map.

QTLs for CL

Eleven main effect QTLs were detected under normal and stress conditions. Under the stress, four QTLs for CL, explaining 47.30% of total variation (each from 7.62 to 18.69%), were located on the chromosomes 2, 6 and 9; while under normal water conditions, seven QTLs for CL, explaining 74.85% of total variation (each from 4.84 to 22.65%), were detected on the chromosomes 1, 2, 7, 11, 12. It could

be noted that under normal or stress conditions, two QTLs (*qCL2a* and *qCL2c*) were detected on the same or adjacent loci on chromosome 2 (RM492–RM145–RM438) with positive alleles from IRAT109, which could increase the CL by 0.3759 and 0.4328 mm, respectively.

QTLs for DRI

A total of four QTLs with different effects were detected on the chromosomes 1, 4, 5, 9, which explained individually 6.82-19.79% of total variation

Table 3.   Putative epistasis loci for CL and DRI of RILs(Zhenshan 97B×IRAT109) under different conditions.

Trait	Treatment	Marker interval (i)	Chromosome	Marker interval (j)	Chromosome	LOD score	R^2 (%)	Epistatic effect(%)	P-value
CL	Stress	RM490–RM259	1	RM245-RM205	9	3.91	1.82	0.4992	0.0004
	Stress	RM294B–RM486	1	RM216-RM311	10	3.64	2.29	0.5634	0.0001
	Stress	RM145–RM438	2	RM21-RM206	11	3.25	2.99	0.5219	<0.0001
	Stress	RM318–RM6	2	RM531-RM80	8	3.41	1.85	-0.3499	0.0003
	Stress	RM231–RM489	3	RM444-RM219	9	3.54	6.68	-0.5550	<0.0001
	Stress	RM592–RM574	5	RM539-RM136	6	3.12	6.04	-0.3428	<0.0001
	Stress	RM289–RM509	5	RM25-RM544	8	3.82	5.93	-0.6128	<0.0001
	Stress	RM585–RM111	6	RM512–RM101	12	4.18	4.54	0.5373	<0.0001
	Stress	RM455–RM351	7	RM152–RM52	8	4.04	4.07	0.5514	<0.0001
	Normal	RM110–RM211	2	RM342–RM515	8	3.11	4.08	-0.4674	<0.0001
	Normal	RM145–RM438	2	RM545–RM517	3	3.48	2.36	0.2000	<0.0001
	Normal	RM526–RM525	2	RM105–RM321	9	3.51	4.99	0.2739	<0.0001
	Normal	RM156–RM16	3	RM339–RM342	8	4.39	2.06	0.7429	0.0001
	Normal	RM289–RM509	5	RM25–RM544	8	2.99	1.79	-0.4437	0.0002
	Normal	RM508–RM435	6	RM547–RM404	8	3.63	1.92	-0.5233	0.0001
	Normal	RM455–RM351	7	RM152–RM52	8	3.94	2.76	0.6138	<0.0001
DRI	–	RM220–RM490	1	RM287–RM209	11	3.57	5.32	-0.1010	<0.0001
	–	RM104–RM414	1	RM18–RM478	7	4.87	4.11	-0.0719	<0.0001
	–	RM349–RM127	4	RM228–RM591	10	3.86	2.50	-0.0983	0.0003

(totally 39.99%).

By comparing the QTLs for CL and DRI, it could be noted that chromosomes 1 and 9 possessing the QTLs for DRI simultaneously harbored QTLs for CL, especially *qCL9* shared the entirely same location with *qDRI9* on the chromosome 9 (RM160－RM215) (Fig. 2). The positive alleles of *qCL9* were from IRAT109 with 0.3361 mm of additive effect under the stress, explaining 7.62% of total phenotypic variation for CL, while the positive alleles of *qDRI9* from Zhenshan 97B with -0.0643 of additive effect, explaining 6.82% of total variation for DRI.

Epistatic loci for CL and DRI

Epistasis is the phenotypic effects of interactions among alleles at multiple loci [15], which played an important role in the genetic basis of quantitative traits [16-18]. During the experiment a total of 19 pairs of epistatic loci were detected, each pair of epistatic loci explained 1.79% to 6.68% of total variations (Table 3).

For CL

Sixteen epistatic interactions were detected for CL, involving 30 loci dispersed on all chromosomes except for chromosome 4. Four interactions each involved a main effect QTL and a non-main effect QTL (the locus that did not have a significant effect). The other twelve interactions occurred between two non-main effect QTLs. Although the epistatic loci affecting the CL of rice were distributed on eleven chromosomes, most of epistasis occurred among the loci on chromosomes 2 and 8.

Under normal water conditions, seven epistatic interactions were found on seven chromosomes (chromosomes 2, 3, 5, 6, 7, 8 and 9), with total contribution of 19.96% to the phenotypic variation. Three epistatic interactions had the positive effects, while the other four showed negative effects.

Under the stress, nine epistatic interactions were detected on all chromosomes with the exception of chromosome 4, each pair of epistatic loci accounted for 1.82% to 6.68% of total phenotypic variation. Five

Table 4. Comparison of rice DT's QTLs in recent time.

Marker	Chromosome	Traits in this study	Treatment	Traits in other study	Population	Type	References
RM20B–RM167	11	CL	Normal	Root depth	IR5882/IR52561	RILs	[25]
RM273–RM252	4	DRI	–	Plant height	IR64/Azucena	DH	[24]
RM273–RM252	4	DRI	–	Leaf drying	Bala/Azucena	RILs	[27]
RM574–RM169	5	DRI	–	Root volume	IR64/Azucena	DH	[26]
RM574–RM169	5	DRI	–	Root to shoot ratio	IR64/Azucena	DH	[22]

epistatic interactions possessed positive effects and the remaining four pairs showed negative effects.

Interestingly, the intervals (RM145-RM438, *qCL2a*) on chromosome 1 interacted with two loci on chromosomes 11 and 3 under the stress and normal conditions, respectively. Two epistatic interactions (RM289-RM509 on chromosome 5 with RM25-RM544 on chromosome 8, RM455-RM351 on chromosome 7 with RM152-RM52 on chromosome 8) were detected under both the stress and normal conditions.

For DRI

Three epistatic interactions were noted on chromosomes 1, 4, 7, 10 and 11, each explained 2.5% to 5.32% of total variation, and all displayed negative effects. Only one epistatic interaction involved a main effect QTL.

On the whole, the epistatic loci for CL were much more than those for DRI, suggesting relationships of the epistatic loci for CL in rice might be more complex than those for DRI.

DISCUSSION

Previously, researchers paid more attention to the character of coleoptile for direct-seeding cultivation of rice, however, they almost neglected the other roles of coleoptile in rice [14]. The current research indicated that the CL of rice was closely related with drought resistance index under the stress, and it possessed a complicated molecular genetic mechanism as a total of 11 main effect QTLs controlled this trait under normal and stress conditions. In particular, one chromosome (chromosome 9) harbored both QTLs for CL and DRI under the stress conditions. Taking

account of all the QTLs for CL under the stress and normal conditions, two chromosomes (chromosomes 1 and 9) possessing the QTLs for DRI simultaneously harbored QTLs for CL. This suggested that CL and DRI had some near relation in molecular genetic levels, implying the CL under drought stress could be used to represent drought resistance of rice in some extent.

The drought resistance in plants is a complicated issue, which was often influenced by many factors including gene and environment. To get the drought tolerance genes had been the goals of scientists. Several genes responsible for enhancing the drought tolerance of plants had been reported [23-25], but most of them controlled one or two physiological or morphological traits such as proline content, weight and length of rice and so on. Unfortunately, they couldn't better represent drought tolerance in plants. By comparing the QTLs in previous reports with our experiments, it could be noted that QTLs for CL and DRI were located in the same or adjacent marker interval with the QTLs related to root traits, such as number, dry weight, depth, and length of root (Table 4). For example, one QTLs for CL in our experiments was located at the same position on the same chromosome as QTLs for root depth detected in another experiments, and two QTLs for DRI detected in our experiments were located on the same locations with four QTLs related to the drought tolerance in another experiments (Table 4). This showed the clustering of QTLs for the root traits, coleoptile length and drought resistance, suggesting that it might be a pleiotropism or phenomenon of close gene linkage. This phenomenon has also been found in gene mapping of other traits [19-22]. Therefore, it would be important to map finely and clone these QTLs, and

verify their functions.

Moreover, two QTLs (*qCL9* and *qDRI9*) were detected on perfectly identical locus of chromosome 9 in our experiments. Whether this is a real drought tolerance gene or not should be verified by our next step work.

ACKNOWLEDGMENTS

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