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Molecular dissection of seedling-vigor and associated physiological traits in rice

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Abstract seedling-vigor is important for crop establishment. There have been reported quantitative trait locus (QTL) analyses on seedling-vigor related morphological traits. However, physiological understanding of these detected QTLs is rather limited. In this study, we employed a recombinant inbred population to detect QTLs for seedling-vigor traits and physiological traits related to seedling-vigor. Germination rate and seedling growth were measured to quantify seedling-vigor. Total amylase activity, α-amylase activity, reducing sugar content, root activity and seed weight were determined. Correlations were observed between the seedling-vigor and physiological traits. QTL analysis reveals that the intervals of RG393-C1087-RZ403 on chromosome 3, C246-RM26-C1447 and R830-R3166-RG360-C734b on chromosome 5, and the interval of Waxy on chromosome 6 are the four main chromosomal regions controlling seedling-vigor. Several QTLs for amylase activities, reducing sugar content and root activity were localized in the similar regions as the QTLs for seedling-vigor. The results suggest that these traits were under the control of pleiotropic and/or closely linked QTLs. The implications of the results in the understanding of the physiological basis of seedling-vigor were discussed.

Keywords *Oryza sativa* L. · Seedling vigor · Physiological traits · Molecular marker · Quantitative trait locus (QTL)

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Introduction

Rice cultivars with strong seedling-vigor are desirable for crop establishment in the direct-seeded culture system and in temperate rice-growing areas where low temperature retards early seedling growth (Redoña and Mackill 1996b). Germination rate and early seedling growth are the major seedling-vigor related traits. Rapid shoot and root growth were observed to be closely associated with seedling-vigor (Williams and Peterson 1973; Sasahara et al. 1986). Redoña and Mackill (1996a) surveyed genetic variation in seedling-vigor of rice cultivars by measuring shoot weight, shoot length, and coleoptile length across three environments, and found significant genetic differences for all seedling-vigor traits. They also found that seedlings of temperate japonica and indica varieties were more vigorous than tropical japonica types.

It is generally considered that carbohydrates for early seedling growth before seedlings gain the ability of photoautotrophy are provided by breakdown of the starch stored in the endosperm. Thus, functionally, it should be expected that amylase activities are correlated with germination rate and early seedling growth in rice, as was reported previously (Williams and Peterson 1973; Sasahara et al. 1986). However, more study is needed to understand the genetic basis of such an association.

The application of molecular-marker technology and QTL mapping has facilitated understanding of the genetic basis of many agriculturally important quantitative traits and phenomena, such as yield, heterosis, tiller number, plant height, heading date and root distribution in rice. However, QTL analyses have largely been used for agronomic and morphological traits.

Physiological traits are more closely related to gene expression than agronomic traits. In recent years, there are also increasing interests in mapping QTLs for physiological traits (Causse et al. 1995; Prioul et al. 1997; Mitchell-Olds and Pedersen 1998; Quarrie et al. 1999; Sanguineti et al. 1999). For most agronomic and morphological traits, however, information about physiological- and process-level is extremely limited. Therefore, it

is often difficult and even impossible to explain QTLs detected by molecular mapping (McMullen et al. 1998). In rice, for example, several reports have been published on mapping QTLs controlling seed dormancy (Lin et al. 1998), seedling-vigor (Redoña and Mackill 1996b) and root characteristics (Champoux et al. 1995; Ray et al. 1996; Yadav et al. 1997; Price et al. 2000). But a physiological explanation for those detected QTLs was not provided by these studies.

In the study reported in this paper, a recombinant inbred population derived from the parents of an elite hybrid was employed to map the loci underlying five seedling-vigor related traits and several associated physiological characters. The objective was to explore physiological understandings underlying QTLs for seedling-vigor traits.

Materials and methods

Genetic materials

A set of 241 F_{10} recombinant inbred lines (RILs) derived from a cross between Zhenshan 97 (*Oryza sativa* ssp. *indica*) and Minghui 63 (*Oryza sativa* ssp. *indica*), the parents of Shanyou 63, an elite rice hybrid, was used as the experimental materials. Seeds of F_{10} RILs used in the study were harvested from plants grown in the dry season of 1999 in the same field under identical cropping management and cultivation conditions on the farm of the International Rice Research Institute, the Philippines.

Measurement of seedling-vigor traits

After breaking dormancy at 50 °C for 4 days, 120 seeds of each RIL were soaked in 1% NaClO for 30 min, and placed in a Petri dish. Tap water-saturated filter paper (Whatman) was placed in the bottom of the Petri dish. All Petri dishes were covered and placed in an incubator in the dark at 30 ± 1 °C. Seeds were considered to have germinated when their axes had broken through the seed coat. Seed germination rate 24 h after soaking was calculated as the percentage of seeds that were germinated.

After continuous incubation for 6 days, at which time almost all the seeds were germinated, the Petri dishes were removed from the incubator and 15 seedlings were randomly sampled from each Petri dish. Shoots and roots were removed from seedlings and only seeds were used to obtain crude extracts for the determination of total amylase, α-amylase activities and reducing sugar content. The rest of the seedlings were allowed to grow continuously under room temperature with the light intensity set at approximately 300 μmol PAR m^{-2} s^{-1}. After growing for 5 days, 15 seedlings were randomly sampled from each Petri dish to measure the maximum root-lengths of each seedling. The average of the maximum root-lengths over the 15 seedlings was considered as the maximum root-length of each line. Then all roots were cut for determining root activity. Another set of 15 seedlings was taken to measure shoot, root dry weight and total seedling dry weight after placing samples in an oven at 70 °C for 3 days, which were expressed as mg per seedling.

The entire experiment was repeated one more time under the same laboratory conditions.

Enzyme and reducing-sugar extraction

For crude extraction and determination of enzyme activity, all operations were processed on ice unless otherwise specified. Fifteen seeds, which were incubated for 6 days and with their roots and shoots removed (see previous paragraph), were placed in a prechilled mortar and pestle, and 3 ml of ice-cold Na-phosphate buffer (pH 7.0, 0.1 M) was added to the mortar. The seeds were homogenized and rinsed with 3 ml of the same buffer. After centrifugation at 10,000 g for 15 min at 4 °C, the supernatant was collected as a crude extract for determination of total amylase, α-amylase activity and reducing sugar content.

Determination of total amylase activity

The total amylase activity was determined colormetrically by measuring the disappearance of starch substrate as described by Rood and Larsen (1988) with a slight modification. Exactly 0.3 ml of crude extract was added to a test tube as the reaction tube, followed by 0.3 ml of 0.1 M Na-phosphate buffer (pH 7.0) and 0.5 ml of a 1% soluble-starch solution. The tube was incubated for 10 min at 30 °C, after which 0.5 ml of iodine solution (0.1 g of iodine, 0.3 g of potassium iodide in 100 ml of distilled water) was added. Optical density (*D*) at 620 nm was determined using a spectrophotometer for measuring the remaining starch (μQuant, BIO-TEK Instruments, Incorporated, USA). The same procedure was conducted with 0.5 ml of 0.1 M Na-phosphate buffer in place of the 0.5 ml of 1% soluble-starch solution for determining the amount of starch in the crude extract. The amount of starch left in the reaction tube and the amount of starch in the crude extract were determined by comparison with starch standards. The amount of hydrolyzed starch was calculated as the summation of added starch and the starch in the crude extract minus the remaining starch in the reaction tube. Total amylase activity was expressed as the hydrolyzed soluble starch (mg) during 10 min.

Determination of α-amylase activity and reducing-sugar content

The crude extract was incubated for 15 min at 70 °C to inactivate β-amylase. Alpha-amylase activity was determined by measuring the amount of reducing sugar as described by Yang and Zhang (1990) with slight modification. Exactly 0.3 ml of the above heat-treated extract was added to two labeled test tubes followed by adding 0.5 ml of 0.1 M Na-phosphate buffer (pH 6.5) to each tube. Then, 0.5 ml of 1 M NaOH was added to the first tube to inactivate α-amylase, and 0.5 ml of 1% soluble-starch solution was added to each of the two tubes. After incubation at 40 ± 1 °C for 20 min, the two tubes were immediately placed on ice. The reaction in the second tube was stopped by adding 0.5 ml of 1 M NaOH, after which 0.3 ml of 1% 3,5-dinitrosalicylic acid was added to each tube. Both tubes were incubated in boiling water for 5 min. After cooling, the *D* at 520 nm was measured using a spectrophotometer. The *D* at 520 nm in the first tube was used as the measurement of the reducing-sugar content of germinating seeds. Alpha-amylase activity was calculated as the difference in *D* at 520 nm between the two tubes.

Determination of root activity

For determining root activity, all roots were placed in a 25-ml test tube before adding 5 ml of 0.1 M Na-phosphate buffer (pH 7.0) and 5 ml of 50 ppm α-naphthylamine. One microliter of solution was taken from the test tube after shaking for 30 min and 3 h, respectively. Then, 1 ml of 1% ρ-aminobenzene sulfonic acid and 1 ml of 0.01% $NaNO_2$ were added to the two samples. The *D* at 510 nm was measured after incubation for 10 min at 30 °C. Meanwhile, a control without roots was used to determine the auto-oxidized α-naphthylamine content. The α-naphthylamine concentrations in the tube after shaking for 30 min and 3 h were determined by comparison with α-naphthylamine standard solutions. The oxidized α-naphthylamine was calculated as the decrease in the amount of α-naphthylamine from 30 min to 3 h. Root activity was expressed as oxidized α-naphthylamine (μg) minus auto-oxidized α-naphthylamine in the control per g of fresh root per h.

Table 1 Phenotypic values of five seedling-vigor traits of the parents and RILs

Item	Germination rate (%)	Total dry weight (mg/seedling)	Shoot dry weight (mg/seedling)	Root dry weight (mg/seedling)	Max. root length (mm)
Minghui 63	11.0	7.5	4.7	2.8	69
Zhenshan 97	32.9	10.5	6.7	3.8	113
Shanyou 63	50.5	9.8	6.0	3.8	82
LSD	10.10[a]	1.39[b]	0.90[a]	0.25[b]	13.14[b]
H_B^2	89.9	85.0	82.5	83.5	64.8
	Recombinant inbred lines				
Mean ± SD	30.7±9.4	8.9±0.5	5.4±0.3	3.4±0.3	80±10
Range	0–91.0	6.5–11.9	3.8–7.3	2.4–4.6	45–132

[a] $LSD_{0.05}$ for the trait
[b] $LSD_{0.01}$ for the trait

Table 2 Phenotypic values of four physiological traits associated with the seedling-vigor of the parents and RILs

Item	Total amylase activity[a]	α-Amylase activity[b]	Reducing sugar content[c]	Root activity[d]	Seed weight[e]
Minghui 63	0.11	0.24	1.16	97.5	27.3
Zhenshan 97	0.32	0.46	1.09	59.3	25.4
Shanyou 63	0.22	0.46	1.28	69.4	27.6
LSD	0.14[f]	0.21[f]	0.40[f]	25.16[g]	0.41[g]
H_B^2	66.2	39.6	71.9	69.2	95.0
	Recombinant inbred lines				
Mean ± SD	0.26±0.08	0.32±0.13	1.06±0.19	76.6 ± 23.0	26.1±2.7
Range	0.05–0.45	0.11–0.95	0.62–1.73	33.7–152.5	19.8–32.1

[a] Total amylase activity: mg of hydrolyzed soluble starch during 10 min
[b] α-Amylase activity: the increase in *D* at 520 nm 20^{-1} min
[c] Reducing sugar content: *D* at 520 nm
[d] Root activity: μg of α-naphthylamine per g of fresh root per h
[e] Seed weight: mg per grain
[f] $LSD_{0.05}$ for the trait
[g] $LSD_{0.01}$ for the trait

For each trait, two subsamples were taken from each Petri dish. The measurements of total amylase activity, α-amylase activity and reducing-sugar content were repeated twice for each subsample. Seed weight was determined after drying in an oven at 70 °C for 3 days.

Data analyses

All the basic statistical analyses were done using the SAS package (SAS institute 1990). Broad-sense heritability (H_B^2) was computed on the basis of the RIL population using the formula $H_B^2 = \sigma^2 g/(\sigma^2_g + \sigma^2_e/n)$, where σ^2_g is genetic variance, σ^2_e is error variance and n is the number of replicates of the trial.

The molecular-marker linkage map and the markers were described by Xing et al. (2002). This map consisted of 221 marker loci and covered a total of 1,796 cM. The average values of each line from the two replicates of the trial were used in detecting QTLs. The chromosomal locations and the number of putative QTLs were determined using QTLMapper (version 1.0) which was based on a mixed-model approach (Wang et al. 1999). QTLs were declared with a LOD threshold of 2.7 corresponding to a probability of 0.001 for the detection.

Results

Performances of morphological and physiological traits

The two parents, Minghui 63 and Zhenshan 97, showed significant differences in germination rate, total dry weight, shoot dry weight, root dry weight and maximum root length based on LSD values (Table 1). It was observed that Zhenshan 97 was the high-value parent for all the five traits, suggesting that it had greater seedling-vigor than Minghui 63. Significant differences in all the related physiological traits, except reducing-sugar content, were also observed between the two parents (Table 2). Zhenshan 97 had higher total amylase activity and α-amylase activity, but lower root activity and seed weight than Minghui 63.

Among the ten traits, the germination rate and dry weight showed high heritabilities, respectively, while α-amylase activity showed a relatively low heritability (Tables 1 and 2). Transgressive segregation for all seedling-vigor traits and related physiological traits was observed in the RIL population (Tables 1 and 2).

Correlations among various traits

Significant correlations among the five seedling-vigor traits (germination rate, total dry weight, shoot dry weight, root dry weight and maximum root length) were observed (Table 3). The correlations among the five physiological parameters were relatively weak compared with the correlations among the seedling-vigor traits. Total amylase activity and α-amylase activity were negatively correlated with reducing sugar and root activity. Total amylase activity was positively correlated with seed weight. Reducing-sugar content was also highly and positively correlated with seed weight, but negatively correlated with root ac-

Table 3 Phenotypic correlations among ten morphological and physiological traits related to seedling-vigor

Traits	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)[a]
Germination rate (1)	0.22**	0.21**	0.19**	0.13	0.12	0.18*	–0.24**	–0.29**	–0.21**
Total dry weight (2)		0.95**	0.89**	0.35**	0.35**	0.16*	0.36**	–0.44**	0.52**
Shoot dry weight (3)			0.70**	0.27**	0.33**	0.14*	0.31**	–0.33**	0.52**
Root dry weight (4)				0.40**	0.31**	0.16*	0.36**	–0.52**	0.43**
Maximum root length (5)					0.25**	0.11	–0.02	–0.21**	0.13
Total amylase activity (6)						0.42**	–0.17*	–0.16*	0.24**
α-Amylase activity (7)							–0.17*	–0.31**	0.02
Reducing sugar content (8)								–0.23**	0.64**
Root activity (9)									–0.09

*, **Significant at 0.05 and 0.01 probability levels, respectively
[a] Seed weight

Table 4 The putative QTLs for seedling-vigor traits

Traits	QTL[a]	Chr.[b]	Marker interval	LOD[c]	Add[d]	Var%[e]
Germination rate	qGR3-1	3	C944-R321	3.4	–3.43	3.4
	qGR3-2	3	RG393-C1087	5.2	–4.50	5.8
	qGR3-3	3	C63-RM232	4.3	4.12	4.9
	qGR5-1	5	C246-RM26	15.9	–7.80	17.4
	qGR5-2	5	RG360-C734b	6.4	5.37	8.3
	qGR6-1	6	RZ667-RG424	5.1	–4.70	5.6
	qGR6-2	6	Waxy-C1496	4.9	–4.41	6.3
	qGR7-1	7	RG128-C1023	2.7	3.25	3.0
						Total: 54.7
Total dry weight	qTDW1-1	1	RG236-C112	7.7	–0.26	7.9
	qTDW1-2	1	G359-RG532	3.8	–0.17	3.3
	qTDW3-1	3	RG393-C1087	4.2	0.19	4.0
	qTDW5-1	5	RM26-C1447	16.2	–0.39	17.4
	qTDW5-2	5	R3166-RG360	11.5	–0.30	10.3
	qTDW6-1	6	R2549-C962	3.0	0.16	3.1
	qTDW6-2	6	Waxy-C1496	5.0	–0.20	4.4
	qTDW9-1	9	C734-R1164	8.2	0.26	7.6
						Total: 58.0
Shoot dry weight	qSDW1-1	1	RG236-C112	8.4	–0.18	9.7
	qSDW3-1	3	RG393-C1087	7.2	0.16	8.2
	qSDW5-1	5	RM26-C1447	7.9	–0.17	8.6
	qSDW5-2	5	R360-C734b	4.7	–0.15	6.6
	qSDW6-1	6	R2549-C962	3.0	0.10	3.0
	qSDW6-2	6	Waxy-C1496	8.7	–0.18	10.3
	qSDW9-1	9	C734-R1164	5.7	0.20	6.2
						Total: 52.7
Root dry weight	qRDW1-1	1	G359-RG532	2.8	–0.07	2.9
	qRDW5-1	5	C246-RM26	19.4	–0.22	24.1
	qRDW5-2	5	R3166-RG360	10.4	–0.16	13.2
	qRDW10-1	10	C677-RM258	5.6	–0.12	7.3
						Total: 47.5
Maximum root length	qMRL1-1	1	C2340-C86	9.8	4.54	11.4
	qMRL1-2	1	G359-RG532	4.1	–3.03	5.1
	qMRL5-1	5	RM26-C1447	11.0	–4.87	13.1
	qMRL6-1	6	C952-Waxy	12.4	–5.17	15.0
						Total: 44.6

[a] QTL nomenclature follows that of McCouch et al. (1997a)
[b] Chromosome on which the QTL is located
[c] Log-likelihood value
[d] Additive effect, the negative value indicates that the allele from Zhenshan 97 increases phenotypic value
[e] Variation explained by each QTL

tivity. There was no significant correlation between seed weight and α-amylase activity or root activity.

There were also significant correlations between the seedling-vigor traits and physiological traits (Table 3). Total amylase activity was positively correlated with all the seedling-vigor traits except germination rate. Alpha-amylase activity had a much weaker correlation with the seedling dry weight than total amylase activity. Reducing-sugar content was positively correlated with seedling dry weight, but negatively correlated with germination rate. A significant and negative correlation was observed between root activity and seedling-vigor. Seed weight was highly positively correlated with seedling dry weight, but negatively correlated with germination rate.

Table 5 The putative QTLs detected for physiological traits associated with seedling-vigor

Traits	QTL[a]	Chr[b]	Marker interval	LOD[c]	Add[d]	Var%[e]
Total amylase activity	qTAA3-1	3	C316-C63	3.5	0.02	5.5
	qTAA5-1	5	R830-R3166	2.7	–0.02	4.0
	qTAA6-1	6	Waxy-C1496	6.8	–0.03	11.0
						Total: 20.5
α-Amylase activity	qAAA6-1	6	RG653-G342	2.9	0.01	5.7
	qAAA6-2	6	Waxy-C1496	4.1	–0.02	8.7
						Total: 14.4
Reducing sugar content	qRS3-1	3	RG393-C1087	15.2	0.07	18.4
	qRS5-1	5	R3166-RG360	12.7	–0.06	13.0
	qRS6-1	6	RZ667-RG424	6.7	0.05	9.0
	qRS6-2	6	Waxy-C1496	4.5	0.04	5.2
	qRS10-1	10	C1633-C667	3.5	–0.03	3.4
	qRS11-1	11	RM254-Clone4	2.7	–0.03	2.9
						Total: 51.9
Root activity	qRA5-1	5	C246-RM26	7.8	6.99	13.0
	qRA5-2	5	R830-R3166	2.8	4.14	4.6
	qRA9-1	9	RM257-RM242	5.1	6.00	9.6
						Total: 27.2
Seed weight	qSW1-1	1	RG236-C112	6.5	–0.47	3.2
	qSW1-2	1	C2340-C86	7.9	0.61	5.3
	qSW1-3	1	G359-RG532	11.0	–0.73	7.7
	qSW3-1	3	C746-C944	10.7	0.70	7.2
	qSW3-2	3	C1087-RZ403	18.1	0.97	13.7
	qSW5-1	5	R3166-RG360	23.4	–1.05	15.9
	qSW8-1	8	R1394-G2132	7.0	–0.52	3.8
	qSW9-1	9	RM257-RM242	6.4	–0.52	4.0
	qSW11-1	11	G44-G257	5.3	0.53	4.1
						Total: 64.9

[a] QTL nomenclature follows that of McCouch et al. (1997a)
[b] Chromosome on which the QTL is located
[c] Log-likelihood value
[d] Additive effect, the negative value indicates that the allele from Zhenshan 97 increases phenotypic value
[e] Variation explained by each QTL

QTLs for seedling-vigor traits

A total of 31 QTLs were detected for the five seedling-vigor traits (Table 4), of which eight were for germination rate, eight for total dry weight, seven for shoot dry weight, four for root dry weight and four for maximum root length, accounting for 54.7%, 58.0%, 52.7%, 47.5% and 44.6% of the phenotypic variations, respectively. The putative QTLs, qGR3-2, qTDW3-1 and qSDW3-1, shared the same interval of RG393-C1087 on chromosome 3. The QTLs, qGR5-1 for germination rate and qRDW5-1 for root dry weight were both located in the interval of C246-RM26 on chromosome 5, while qTDW5-1, qSDW5-1 and qMRL5-1 shared the same interval of RM26-C1447. Similarly, another chromosomal region (R3166-RG360-C734b) on chromosome 5 was also identified to control germination rate, total dry weight, shoot dry weight and root dry weight. Table 4 also shows that the C952-Waxy-C1496 on chromosome 6 governed germination rate, total dry weight, shoot dry weight and maximum root length. In most of the cases, alleles of the putative QTLs that increased seedling-vigor came from Zhenshan 97, while Minghui 63 with low seedling-vigor also provided alleles for increasing the phenotypic score in some cases.

QTLs for physiological traits

Three and two QTLs were detected for total amylase activity and α-amylase activity respectively, explaining 20.5% and 14.4% of the total phenotypic variations (Table 5). Two QTLs, qTAA6-1 and qAAA6-2, were located in the same interval of Waxy-C1496, accounting for the largest phenotypic variations for total amylase activity (11.0%) and α-amylase activity (8.7%), respectively. One QTL for total amylase activity, qTAA3-1, located on chromosome 3, was closely linked to qGR3-3 and the other QTL for total amylase activity, qTAA5-1, was located in the interval of R830-R3166 on chromosome 5. The remaining QTL for α-amylase activity, qAAA6-1, was located in the interval of RG653-G342 on chromosome 6.

Six QTLs were identified for reducing-sugar content (Table 5). One QTL, qRS3-1, explaining 18.4% of the total variation, was located in the interval of RG393-C1087, in which QTLs for germination rate, total dry weight, and shoot dry weight were also detected. Another QTL, qRS5-1 was located near qTAA5-1 for total amylase activity on chromosome 5. Interestingly, qRS6-2 was located in the same interval of Waxy-C1496 as that of qAAA6-2 for α-amylase activity, qTAA6-1 for total amylase activity, and also QTLs for four seedling-vigor traits.

Three QTLs were detected for root activity, explaining 27.2% of the total variation. The QTL with largest

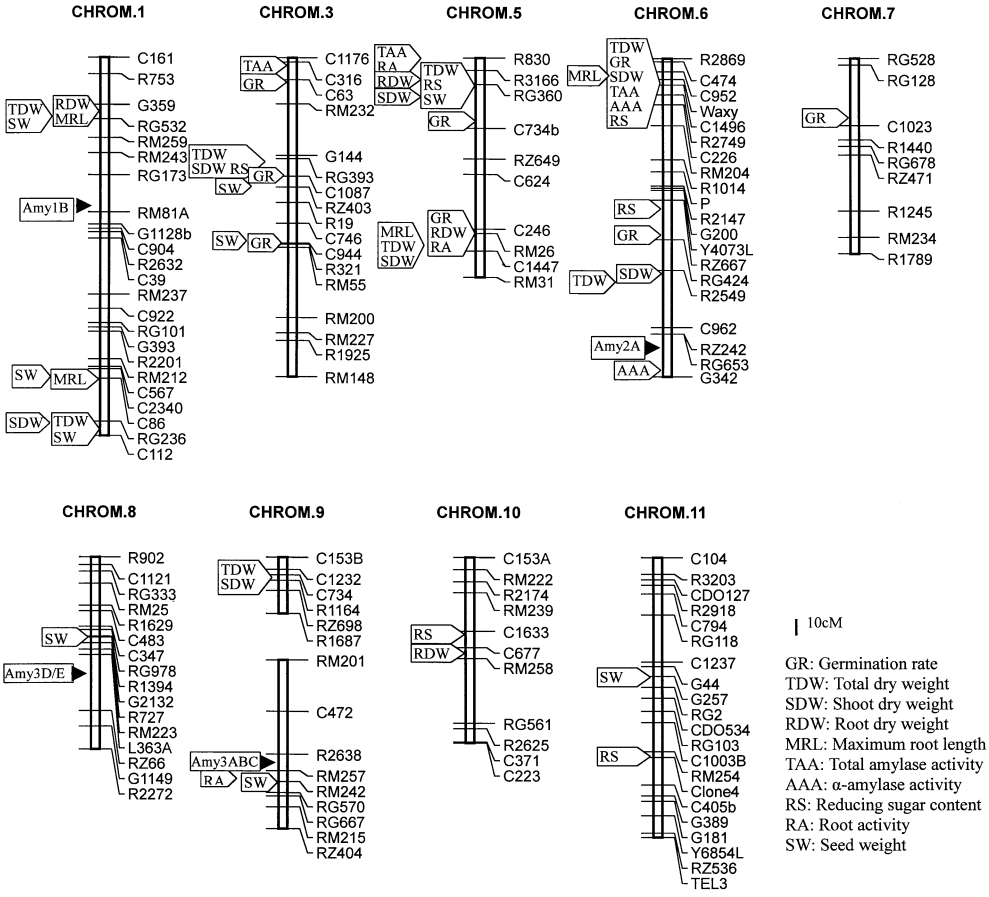

Fig. 1 Quantitative trait loci (QTLs) for seedling-vigor traits and associated physiological traits. The *black arrows* on chromosomes 1, 6, 8 and 9 indicate the approximate locations of α-amylase according to McCouch et al. (1997b) and Huang et al. (1997)

effect, qRA5-1, was also located in C246-RM26, where several QTLs for seedling-vigor traits were detected. qRA5-2 was located between R3166 and R830, where two QTLs for total amylase activity and reducing-sugar content traits were also detected. Alleles of the three QTLs for increasing root activity all came from Minghui 63.

Nine QTLs were detected for seed weight, explaining 64.9% of the phenotypic variation (Table 5). Two QTLs of large effect, qSW3-2 and qSW5-1, accounted for 13.7% and 15.9% of the variations, respectively. The former was located in the interval of C1087-RZ403, and was also linked to qGR3-2, qTDW3-1, qSDW3-1 and qRS3-1. The latter was located in the interval of R3166-RG360, sharing the interval with qTDW5-2, qRDW5-2 and qRS5-1, and was also linked to qGR5-2, qSDW5-2, qTAA5-1 and qRA5-2.

Discussion

Jones and Peterson (1976) and McKenzie et al. (1980) reported that seedling traits, like seedling height, measured under controlled laboratory conditions, were correlated with seedling vigor, measured as a percentage of emergence and the emergence index, under field conditions. Therefore, germination rate and seedling-vigor were determined under laboratory conditions in the study.

Relationships between seedling-vigor traits

Significant correlations among five seedling-vigor traits were observed, suggesting that germination rate and early seedling growth were interrelated. Rapid germination favors seedling establishment. Pleiotropic effects or close linkage of gene(s) are the main causes for correlations among traits (Aastveit and Aastveit 1993). Several QTLs associated with seedling-vigor traits were localized in the interval of RG393-C1087-RZ403 on chromosome 3, C246-RM26-C1447 and R3166-RG360-C734b on chromosome 5 and C952-Waxy-C1496 on chromosome 6 (Fig. 1). This co-localization or close linkage of several QTLs provides a genetic basis underlying the correlation among the traits. The two intervals on chromosome 5 show effects on all five traits of seedling-vigor, suggesting that the interval may contain genes that interactively affect seed germination and seedling growth.

Relationships between physiological traits

Total amylase activity, α-amylase activity and reducing sugar were measured 6 days after germination, which was determined according to the fact that α-amylase activity in germinated cereal seeds reaches a maximum level in 6 to 8 days after germination (Deng 1988; Yang

and Zhang 1990). Two large effect QTLs (qTAA6-1 for total amylase activity and qAAA6-2 for α-amylase activity) were detected to share the similar chromosomal region (Waxy-C1496) with qRS6-2 for reducing sugar and had opposite effects. This is consistent with the fact that reducing sugar was significantly and negatively correlated with total amylase activity and α-amylase activity, respectively. Those findings provide the genetic basis for correlations among the traits. Yu (1999) showed that α-amylase genes were suppressed by sugar via a feedback control mechanism in the embryo and via an osmotic control mechanism in the aleurone cells. Karrel and Rodriguez (1992) also reported the suppression of an α-amylase gene by sugars. The interval of Waxy-C1496, therefore, might be a chromosomal region responsible for interaction between sugar and amylase activity.

Physiological explanation of seedling-vigor performances

At the initial stage of seedling growth, growth or dry matter accumulation is largely dependent on the seed reserve (Yoshida 1981). Significant and positive correlations observed between seed weight and seedling dry weight in the present study suggest role of seed reserve in seedling-vigor. By genetic analysis, QTLs for seed weight and seedling dry weight were closely linked or else shared the same chromosomal regions such as RG236-C112 and G359-RG532 on chromosome 1, RG393-R1087-RZ403 on chromosome 3 and R3166-RG360-C734b on chromosome 5. Overall, our results indicated that several chromosomal regions related to physiological traits were also associated with seedling-vigor performances. These results give a picture about the relationship between physiological and seedling-vigor performances. Close correlations among seed weight, reducing sugar and seedling dry weight suggest that large seed size may supply more sugar for seedling growth, which fuels rapid early growth. López-Castañeda et al. (1996) also suggested that the early vigor of cereals could be increased by sowing larger seeds. In that study, however, the parent Zhenshan 97, that did not have a larger seed size or more reducing sugar in germinated seeds, showed higher seedling-vigor than Minghui 63. This might be due to a high amylase activity and/or the rapid utility of reducing sugar by rapid dry matter accumulation.

Amylase activities were strongly correlated with seedling dry weight, which was consistent with results of Williams and Peterson (1973) and Sasahara et al. (1986). Karrel et al. (1993) considered expression of α-amylase as a possibly limiting factor in determining seedling-vigor. In that study, the interval of C952-Waxy-C1496 on chromosome 6 controlled four seedling-vigor traits, amylase activity and reducing-sugar content. It also shows the association of amylase activity with seedling-vigor. Our results suggest that the regions near waxy on chromosome 6 and RM26 on chromosome 5 might play an important role in cereal grain-germination and early seedling growth and also suggest that the pleiotropy of QTLs should be the genetic basis for a relationship between amylase and seedling vigor.

Root activity was negatively correlated with seedling growth in the present study. Root activity is mainly determined by the oxidative power of the root system, which is highly correlated with the respiratory activity of roots (Matsunaka 1960). Roots with high oxidative activity, therefore, may compete for carbohydrates with seedling growth before seedlings obtained the ability of photoautotrophy under laboratory conditions in the present study. This seems to be supported by the negative correlation between root activity and seedling dry weight and the observation that three chromosomal regions for root activity came from Minghui 63 with a small seedling weight. It is also supported by the observations that QTLs for root activity and seedling dry weight have similar locations on chromosome 5 with opposite effects.

Conventional breeding strategies were not successful in increasing rice seedling-vigor probably due to undesirable traits associated with seedling-vigor, such as large grain size (Li and Rutger 1980; McKenzie et al. 1994). Rice consumers have a certain requirement for grain size, and the increase in grain size may reduce the popularity of a cultivar. Our study reveals that the chromosomal regions of C246-RM26-C1447 and C952-*Waxy*-C1496, which were closely associated with seedling-vigor and α-amylase, were not linked to seed weight. Therefore, the identification of breeding lines that carry these chromosomal regions using marker-assisted selection may improve seedling-vigor without increasing grain size.

Comparison of QTLs in different populations of rice

It was reported previously that marker RG236 was closely associated with shoot length, while the interval near RZ403 was found to control mesocotyl length (Redoña and Mackill 1996b). In our study, the interval of RG236-C112 on chromosome 1 controlled seedling dry weight and the interval of RG393-C1087 near RZ403 on chromosome 3 controlled seedling-vigor related traits. Rapid mesocotyl elongation following rapid germination contributes to sprout emergence and subsequent seedling growth.

Alpha-amylase genes have been located on chromosome 1, 2, 6, 8 and 9, respectively. A gene (Amy2A) located on chromosome 6 was linked to marker RG653 and a gene (Amy1A) as an important factor in determining seedling-vigor in rice was located on chromosome 2 (Ranjhan et al. 1991; Karrel et al. 1993; Huang et al. 1997; McCouch et al. 1997b). In the present study, only one QTL for α-amylase activity was located in the region of RG653-G342 on chromosome 6, in which one α-amylase gene (Amy2A) was located. The other QTLs for α-amylase activity or total amylase activity were not located in similar chromosomal regions for the α-amylase structural genes, regardless of the high correlation between amylase activi-

ties and seedling-vigor traits. According to the results of Causse et al. (1995), QTLs for enzyme activity and its structural genes are not always located in similar chromosomal regions in mapping studies.

The waxy gene on chromosome 6 is generally considered to encode the granule-bound starch synthase. In the present study, amylase activities and reducing sugar were found to be closely associated with the region of *Waxy*. Causse et al. (1995) also found that *Waxy* and a QTL for reducing carbohydrate in the third leaf had similar locations in a segment on chromosome 9 of maize, which is syntenic to the waxy region on chromosome 6 of rice (Ahn and Tanksley 1993). Tan et al. (1999) reported that amylose content was controlled by the *Waxy* locus, or by a genomic region tightly linked to this locus, in the recombinant inbred lines employed in this study. This may imply that the region around the *Waxy* locus may pleiotropically regulate both starch synthesis and starch degradation at different stages. Moreover, the region near *Waxy* also controlled germination rate, root dry weight and maximum root length in the present study. Champoux et al. (1995) observed that the region surrounding the *Waxy* locus controlled several other root morphological traits such as root thickness, root/shoot ratio and root dry weight per tiller, which were related to drought avoidance. However, it was unclear whether the observed association of the Waxy region with these traits has resulted from pleiotropy of a gene or from the clustering of genes.

Although the mechanism for high penetration ability is not well understood, it has been associated with changes in carbohydrate levels and metabolism in impeded roots (Atwell 1993). In this study, two QTLs, qRS5-1 for reducing sugar and qRA5-2 for root activity, had a similar location (R3166-RG360) but with opposite additive effects. Several QTLs for root-penetration ability were detected near RG360, R3166 and C624 on chromosome 5 (Ray et al. 1996; Price et al. 2000). Therefore, these findings seem to imply the underlying relationships among carbohydrate, root activity and root-penetration ability. However, further studies are needed to resolve such relationships.

In summary, the study demonstrated that QTL mapping for biochemical or metabolic characteristics may provide a strategy for analyzing the physiological basis of morphological-trait variation. The co-localization of QTLs for seedling-vigor and physiological characters may be considered as the explanation for the genetic basis of the correlation between the two sets of traits in rice.

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