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Genes/QTLs affecting flood tolerance in rice

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Abstract The adaptation of deepwater rice to flooding is attributed to two mechanisms, submergence tolerance and plant elongation. Using a QTL mapping study with replicated phenotyping under two contrasting (water qualities) submergence treatments and AFLP markers, we were able to identify several genes/QTLs that control plant elongation and submergence tolerance in a recombinant inbred rice population. Our results indicate that segregation of rice plants in their responses to different flooding stress conditions is largely due to the differential expression of a few key elongation and submergence tolerance genes. The most important gene was *QIne1* mapped near *sd-1* on chromosome 1. The Jalmagna (the deepwater parent) allele at this locus had a very large effect on internal elongation and contributed significantly to submergence tolerance under flooding. The second locus was a major gene, *sub1(t)*, mapped to chromosome 9, which contributed to submergence tolerance only. The third one was a QTL, *QIne4*, mapped to chromosome 4. The IR74 (non-elongating parent) allele at this locus had a large effect for internal elongation. An additional locus that interacted strongly with both *QIne1* and *QIne4* appeared near RG403 on chromosome 5, suggesting a complex epistatic relationship among the three loci. Several QTLs with relatively small effects on plant elongation and submergence tolerance were also identified. The genetic aspects of these flooding tolerance QTLs with respect to patterns of differential expression of elongation and submergence tolerance genes under flooding are discussed.

Key words Elongation ability · Submergence tolerance · *Oryza sativa* L. · Epistasis · Abiotic stress · Molecular markers · Differential gene expression

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Introduction

Rice is the staple food for more than one-third of the world's population and is grown under a wide range of agroclimatic conditions in which it is subjected to diverse biotic and abiotic stresses. Among the various ecosystems where rice is grown are 12 million hectares of flood-prone land in South and Southeast Asia, where stagnant water frequently covers the land to depths varying from 50 cm to more than 2 m (Datta and Banerjee 1972). Local rice types adapt to these environments through two basic mechanisms, namely, submergence tolerance and elongation ability. Submergence tolerance is the ability of the rice plant to survive in water under completely submerged conditions for 10 days or more, while elongation ability is defined as the ability of a rice plant to elongate as water rises to keep the leaf canopy above the water surface.

Inheritance of elongation ability and its related phenotypes in rice is complex, and results based on earlier studies have varied considerably depending largely on the parental lines used. Hamamura and Kupkanchanakul (1979) reported that partially dominant genes with additive effects are involved in the elongation of internodes in deepwater rice. Thakur and Hillerislambers (1988) reported that plant and internodal elongation ability is controlled by two dominant complementary genes, one for plant height and the other for elongation. Each gene separately has the ability for elongation, but the presence of both causes greater elongation ability. In another study using the cross Tan-ginbozu [a non-deepwater rice and gibberellic acid (GA)-deficient mutant] × Aswina (deep-

water rice), internodal elongation was explained in terms of complementary genes, one controlling GA production and the other responsiveness to ethylene (Suge 1988). Saha et al. (1994) reported that stem elongation ability showed segregation ratios of 13:3 and 15:1 in F_2, suggesting digenic inheritance with dominant epistasis as well as duplicate dominant epistasis. Based on an analysis of several crosses between South and Southeast Asian floating rices, Mishra et al. (1996) observed an overdominance effect of elongation and a positive trangression for plant height.

Classical genetic studies on submergence tolerance in rice have indicated the involvement of both major and minor genes (Suprihatno and Coffman 1981; Mohanty and Khush 1985; Haque et al. 1989). This is consistent with recent gene/QTL mapping studies which revealed a dominant gene, *Sub1(t)*, located on chromosome 9 and a few quantitative trait loci (QTLs) controlling submergence tolerance in rice (Xu and Mackill 1996; Nandi et al. 1997).

We report here an effort to map genes/QTLs associated with the elongation ability and submergence tolerance under flooding in a rice recombinant inbred mapping population using amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism (RFLP) markers.

Materials and methods

Plant materials

The parental lines were two indica cultivars, IR74 and Jalmagna. IR74 is a high-yielding, semidwarf, photoperiod-insensitive, and non-elongating cultivar developed at IRRI. Jalmagna is a traditional, tall, low-yielding, photoperiod-sensitive, and rapid-elongating rice from India. The mapping population consisted of 165 F_8 recombinant inbred lines (RILs) derived by single-seed descent from the cross between IR74 and Jalmagna.

Phenotyping for elongation ebility

The parents and RILs were evaluated for elongation ability and submergence tolerance in two experiments. In test 1, all lines were evaluated, using single plant harvests, for elongation under slowly increasing water levels in the greenhouse conditions. This test was aimed at evaluating the elongation ability of the RILs under a slow-rising water condition. The experiment was conducted in concrete tanks in the greenhouse. The RILs and parents were seeded in plastic pots (5.5 × 5.5 ×5.5 cm) with 1 plant per pot and 15 pots per entry. Heights of 10 plants and initial internode lengths of 2 plants for each entry were measured 18 days after seeding. The trays were then transferred to concrete tanks, submerged by raising the water level at a rate of 5 cm day $^{-1}$ up to 120 cm, and maintained there for 7 days before draining. After draining, the final plant height and total internode length of 10 plants for each entry were measured.

In test 2, all lines were assayed, using bulk harvests, for elongation ability under slowly rising water levels (about 5 cm day^{-1}) under the field condition, which is the typical flooding pattern in most deepwater areas. The experimental procedure was similar to test 1 but with the following modifications: (1) the test was conducted with two replications in a randomized complete block (RCB) design; (2) initial plant height of ten hills and internode length of five main culms for each entry were measured 30 days after transplanting; the water level was increased to 120 cm above the soil surface at a rate of 5 cm day^{-1}; (3) a maximum water level of 120 cm was maintained for 7 days, then drained; the final plant height and total internode length of 10 plants (main culms) of each entry were measured. The elongation ability of each individual measured in the two tests was calculated based on increments in plant height, internode length, and leaf length as follows: plant height increment after flooding (PHI, in cm) = $b - a$; actual internode length increment after flooding (INLI, in cm) = $d - c$; and leaf length increment after flooding (LLI, in cm) = $(b - d) - (a - c)$; where, a = initial plant height (IPH, in cm), b = plant height after flooding (cm), c = initial total internode length (cm), and d = internode length after flooding (cm). For tolerance to submergence in both tests, lines that survived the flooding treatment were scored as 1, and those that died were scored as 0.

Genotyping with AFLP and RFLP markers

Genomic DNA of the 165 RILs, parents and three additional checks – FR 13A, IR54, and Azucena – was extracted from fresh leaf tissues using a modification of the method of Dellaporta et al. (1991). AFLP analysis including the restriction of genomic DNA, ligation of adapters to restricted DNA fragments, preamplification, selective amplifiation, and denaturing gel electrophoresis, was conducted following the protocol described previously (Maheswaran et al. 1997). Of the polymorphic AFLP bands identified by 23 primer pair combinations, only the clear and unambiguous bands were scored. Because AFLP markers are dominant in nature, markers were scored for the presence or absence of corresponding bands. The parents of a reference RI population (IR74 and FR13A) from which a previous AFLP map was constructed were used to find common polymorphic markers with the mapping parents, IR74 and Jalmagna (Nandi et al. 1997). Those common markers were used as anchors for constructing the AFLP map using the program MAPMAKER (Lander et al. 1987). To validate the AFLP map, we used 29 RFLP markers located on the 12 rice chromosomes in the genotyping experiment following standard procedures. An isozyme marker (Adh1) and a morphological marker, *sd-1*, were also assayed for all RILs.

Data analyses

MAPMAKER/EXP 3.0 (Lander and Bolstein 1989) was used to construct the AFLP linkage map using the 29 previously mapped RFLP markers and 27 AFLP markers as anchors. QTLMAPPER v1.0 based on the mixed linear model approach (Wang et al. 1999) was used to interval map main-effect and epistatic QTLs affecting plant elongation. A comprehensive background genetic variation control of both main and epistatic effects of QTLs with the initial inclusion probability of 0.001 and the exclusion probability of 0.002 and the stepwise regression was used. The threshold of 3.0 LOD or greater was chosen for claiming putative main-effect and epistatic QTLs. For submergence tolerance, the likelihood chi-square statistic G^2 was used to detect associations between individual markers and the submergence tolerance (0 and 1 scores) using SAS PROC FREQ (SAS 1989).

Results

Segregation of AFLP markers and construction of the AFLP map

Twenty-three *Pst*I and *Mse*I primer combinations were used to detect DNA polymorphism between IR74 and Jalmagna; 155 (12.6%) distinct scorable bands were produced, with 90 bands inherited from IR74 and 65 from Jalmagna. Using 27 anchor AFLP markers (Maheswaran

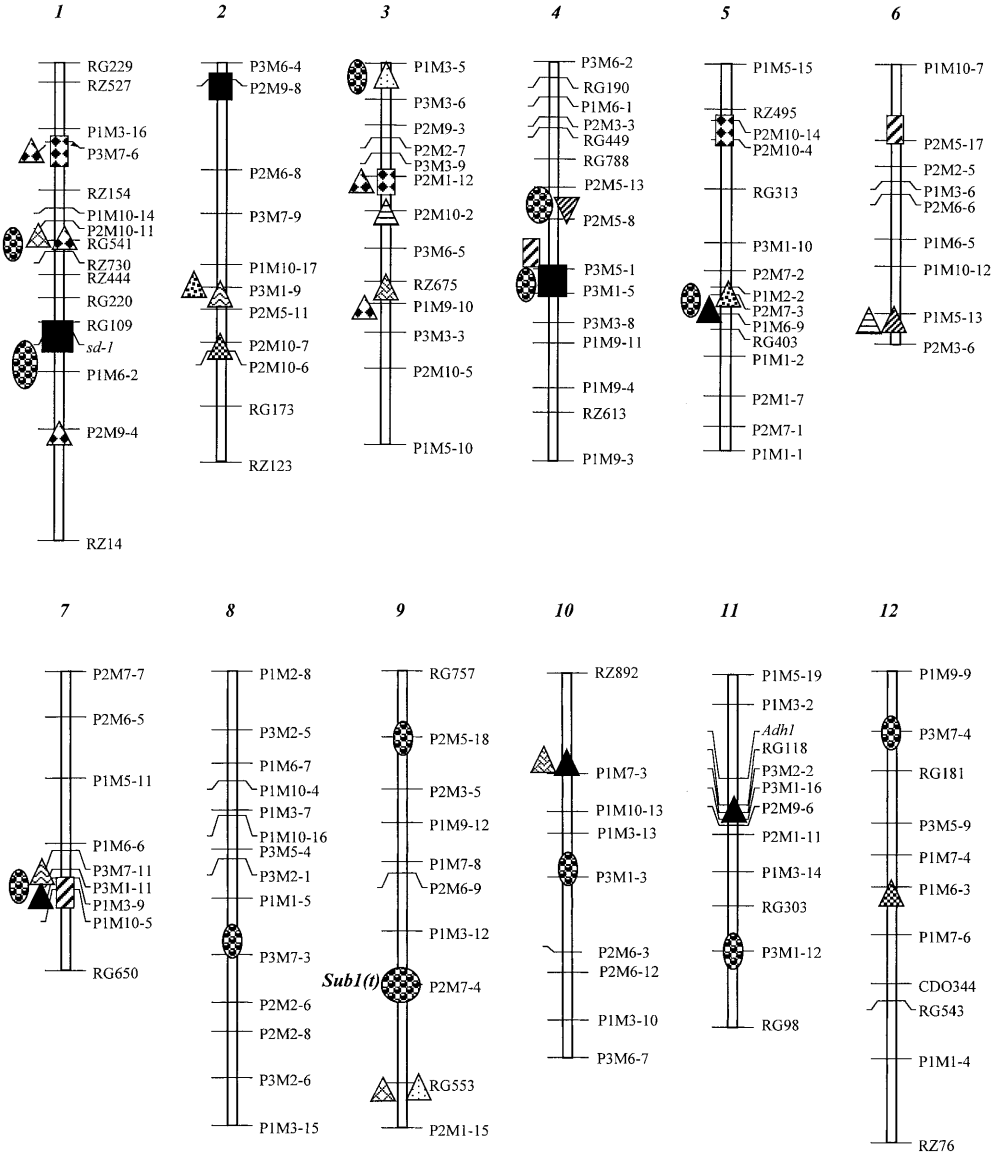

Fig. 1 Genomic locations for main-effect QTLs and epistatic loci affecting plant elongation of the IR74/Jalmagna RI population. ■ Main-effect QTLs affecting internode elongation, ▨ main-effect QTLs affecting leaf elongation, ▲,◬,◭,◬,◬ pairwise epistatic loci affecting plant elongation, ◬ epistatic and main-effect QTLs affecting initial plant height, ◍ markers associated with submergence tolerance

et al. 1997) and 29 anchor RFLP markers (Causse et al. 1994), 113 of the 155 polymorphic AFLP markers were assigned to the 12 linkage groups. Two genes, *sd-1* (semidwarf) and *adh-1* (acohol dehydrogenase), were also included. Together, the map contained 144 markers covering a total length of 2,339.9 cM, with an average interval of 17.9 ± 10.3 cM between markers. The anchor markers on each chromosome have the same order and alignment as the previous AFLP and RFLP maps (Maheswaran et al. 1997; Nandi et al. 1997) with a few exceptions. The number of markers for each chromosome ranged from 9 to 17, with chromosome 11 having the smallest mean distance (13.7 cM) and chromosome 9 the largest distance (23.3 cM) between markers (Fig. 1). Gaps larger than 35 cM were observed in all chromosomes except chromosomes 4, 5, and 11. Of the 144 markers mapped, only 28 (19%) fit the expected 1:1 ratio; 100 (68%) were significantly skewed towards the non-elongation parent, IR74, and the remaining 18 (13%, mostly on chromosome 2) towards the elongating parent, Jalmagna. Overall, 64% of the genome consisted of IR74 alleles.

Phenotypic variation of the RILs for elongation ability and submergence tolerance

Table 1 shows the summary statistics of the measured traits under the two test conditions. Before flooding, the parents and RILs in test 2 had significantly greater mean values and variation for plant height than their counterparts in test 1. After flooding, Jalmagna elongated very quickly and survived under both tests conditions. For instance, Jalmagna reached a final plant height of 152.3 and 171.8 cm, elongated internodes grew by 87.5 cm and 104.7 cm, and elongated leaves by 21.4 cm and 7.1 cm in the two tests, respectively. IR74 did not survive in test 2. It showed no internodal

Table 1 Summary statistics of the traits associated with plant elongation segregating in the IR74/Jalmagna recombinant inbred population

Trait[a]	Recombinant inbred lines				IR74 Mean	Jalmagna Mean
	Mean	SD	Minimum	Maximum		
IPH (1)	38.1	8.1	23.4	60.9	37.6	43.4
IPH (2)	52.0	9.4	32.2	80.3	39.4	60.0
FPH (1)	89.9	27.7	38.3	152.0	78.3	152.3
FPH (2)	124.7	30.4	61.5	181.8	dead	171.8
PHI (1)	42.6	27.9	0.0	103.4	40.7	108.9
PHI (2)	48.2	38.5	0.0	114.1	dead	111.8
INLI (1)	17.1	19.5	0.0	80.0	0.0	87.5
INLI (2)	41.1	33.0	0.0	92.4	dead	104.7
LLI (1)	33.7	13.3	6.4	74.6	40.7	21.4
LLI (2)	10.9	11.0	0.0	60.9	dead	7.1

[a] IPH, FPH, PHI, INLI, and LLI Initial plant height, final plant height, plant height increment, internode length increment, and leaf length increment, respectively (all in centimeters). The numbers 1 and 2 in parenthesis represent tests 1 (slow-rising water condition in the greenhouse) and 2 (slow-rising water under field conditions)

elongation but did have significantly elongated leaves by 40.7 cm, in test 1 (clear water conditions).

Considerable variation for both elongation ability and submergence tolerance was present in the RILs. In tests 1 and 2, 25 and 50 lines, respectively, died, and the surviving RILs segregated considerably for elongation ability (Table 1, Fig. 2). The frequency distributions of the 165 RILs for plant height and internode, and leaf length increment under both tests were continuous but skewed toward the non-elongating parent, IR74 (Fig. 2). Thirty-five and fifty lines showed no elongation, similar to IR74, for plant height and internodes under tests 1 and 2, respectively, but none of the RILs exhibited the same elongation ability as Jalmagna.

Plant elongation of the RILs was largely determined by internodal elongation. In the two tests, the correlation between plant increment and internode length increment was 0.81 and 0.97, respectively, but was only 0.51 and 0.43, respectively, between plant and leaf length increments. Internodal elongation was not correlated with leaf elongation in both tests (r = –0.09 and –0.01). These two components of plant elongation also behaved very differently in the two tests. In test 1, the parents and RILs had much greater mean values and variation for internodal elongation than in test 2. The reverse was true for leaf elongation. Results across the two tests were more consistent for plant height increment and internode increment (r = 0.64 and 0.56 than for leaf length increment (r = 0.34).

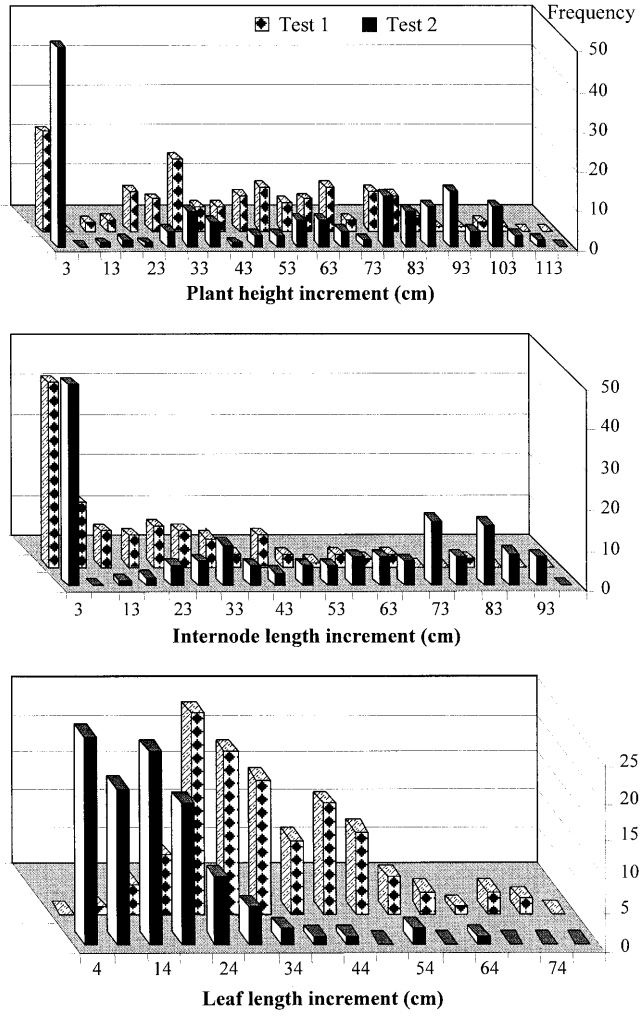

Fig. 2 Frequency distribution of elongation ability of the IR74/Jalmagna recombinant inbred lines. *Tests 1* and *2* represent the complete submergence condition: slow-rising water condition in the greenhouse (*Test 1*) and slow-rising water under the field condition (*Test 2*)

Genes/QTLs affecting elongation ability

Six main-effect QTLs and 14 pairs of digenic epistatic loci affecting plant elongation were identified in the IR74/Jalmagna RI populations (Tables 2 and 3, Fig. 2). These QTLs explained the majority of the total phenotypic variation in plant elongation under the two tests. In addition, 13 genomic regions showed significant associations with submergence tolerance (Table 4).

Table 2 Main-effect QTLs affecting plant elongation detected in the IR74/Jalmagna recombinant inbred population

Trait	QTL	Chromosome	Marker interval	Test 1 (greenhouse)			Test 2 (field)		
				LOD	*a* (cm)[b]	R^2 (%)	LOD	*a* (cm)[b]	R^2 (%)
IPH	*QPh1a*	1	RG109 ~ *sd1*	10.1	–3.8	17.3	4.1	–3.2	12.6
IPH	*QPh1b*	1	P3M7–6 ~ RZ154				6.6	–3.5	14.9
IPH	*QPh3*	3	P2M1–12 ~ P2M10–2				4.6	3.4	14.1
PHI	*QIne1*	1	RG109 ~ *sd1*	10.5	–13.6	33.8	21.5	–23.2	29.6
PHI	*QIne2*	2	P290–8 ~ P2M6–8				7.4	–14.2	11.2
PHI	*QIne4*	4	*P3M1–5* ~ P3M5–1				12.6	21.8	25.6
INI	*QIne1*	1	RG109 ~ *sd-1*	7.0	–8.7	20.1	18.9	–18.2	33.1
INI	*QIne2*	2	P2M9–8 ~ P2M6–8				5.1	–9.6	8.6
INI	*QIne4*	4	*P3M1–5* ~ P3M5–1				10.7	21.8	36.7
LLI	*QLe4*	4	*P3M5–1* ~ P2M5–8	6.2	–6.2	14.2			
LLI	*QLe6*	6	P1M10–7 ~ *P2M5–17*	2.5	4.9	9.4			
LLI	*QLe7*	7	P1M3–9 ~ P1M10–5				3.7	–3.5	12.0

[a] IPH, PHI, INI, and LLI, Initial plant height, plant height increment, internode increment, and leaf length increment, respectively
[b] The gene effect, *a*, is the phenotypic effect due to substitution of the Jalmagna allele by the IR74 allele

Table 3 Digenic epistatic QTLs affecting plant elongation ability identified in the IR74/Jalmagna recombinant inbred population

Trait[a]	Chromosome	Interval *i*	Chromosome	Interval *j*	LOD	a_i (cm)[b]	a_j (cm)[b]	aa_{ij} (cm)	R^2
IPH (1)	1	P3M7-6 ~ RZ154	3	P3M3-9 ~ P2M1-12	5.89	2.7*		5.4***	31.3
	1	P2M9-4 ~ RZ14	3	P1M9-10 ~ P3M3-3	4.01			–2.3***	5.6
	3	RZ675 ~ P1M9-10	10	P1M7-3 ~ RZ892	4.47			2.4***	6.1
IPH (2)	1	RG541 ~ RZ730	8	P2M2-8 ~ P3M2-6	5.77			–4.3***	14.6
	3	P1M3-5 ~ P3M3-6	9	P2M7-4 ~ RG533	5.87			–4.6**	16.7
	7	P1M10-5 ~ RG650	11	P3M2-2 ~ RG118	6.92			4.3***	14.8
PHI (1)	4	*RG109 ~ sd-1*	5	*RG403 ~ P1M1-2*	12.98	–6.1*	8.2*	–10.5**	13.7
	2	P3M1-9 ~ P2M5-11	7	P1M6-6 ~ P3M7-11	6.01	4.0**		–9.2***	10.2
	2	P2M10-7 ~ P2M10-6	12	P1M6-3 ~ P1M7-6	5.21	4.3**		–9.0***	9.8
	4	P2M5-8 ~ P2M5-13	6	P1M5-13 ~ P2M3-6	5.60			–9.1***	10.0
PHI (2)	1	P2M10-11 ~ RG541	9	RG533 ~ P2M1-15	4.09			–9.1***	6.2
	2	P1M10-17 ~ P3M1-9	5	P1M2-2 ~ P2M7-3	3.60			5.9**	5.1
	3	P2M10-2 ~ P3M6-5	6	P1M5-13 ~P2M3-6	3.60			8.4***	5.9
	7	P1M10-5 ~ RG650	10	P1M7-3 ~ RZ892	6.26	5.1**		5.8***	4.8
INLI (1)	2	P3M1-9 ~ P2M5–1	7	P3M7-11 ~ P3M1-11	5.60			–7.9***	19.4
	4	P3M1-5 ~ P3M5-1	5	*RG403 ~ P1M1-2*	10.83		–21.2***	23.7***	35.5
INLI (2)	2	P1M10-17 ~ P3M1-9	5	P1M2-2 ~ P2M7-3	3.99			8.0**	6.4
	3	P2M10-2 ~ P3M6-5	6	P1M5-13 ~ P2M3-6	4.68			8.6***	7.4
	7	P1M10-5 ~ RG650	10	P17-3 ~ RZ892	7.32	5.1**		8.8***	7.8
LLI (1)	1	*RG109 ~ sd-1*	5	*RG403 ~ P1M1-2*	10.80	–17.9***		12.7***	25.0

*,**,*** P <0.05, 0.01, and 0.001, respectively, based on *t*-test
[a] IPH, PHI, INI and LLI, Initial plant height, plant height increment, internode increment, and leaf length increment, respectively; 1 and 2 in parenthesis represent results from tests 1 (greenhouse) and 2 (field), respectively
[b] a_i and a_j are main effects associated with epistatic QTLs *i* and *j*, respectively
[c] a_{ij} is the epistatic effect between QTLs *i* and *j*

QTLs for initial plant height

In test 1, a single main-effect QTL and 3 pairs of epistatic loci affecting the initial plant height before flooding were detected and mapped to chromosomes 1, 3, and 10. Together, these QTLs explained 60.3% of the total phenotypic variance. In test 2, 3 main-effect QTLs and 3 pairs of epistatic loci were identified and mapped to chromosomes 1, 3, 7, 8, 9 and 11. Together, these QTLs explained approximately 90% of the total phenotypic variance. The only QTL detected in both tests was mapped to *sd-1*, wich explained 17.3% and 12.6% of the total phenotypic variance, respectively.

QTLs affecting internode elongation

Three main-effect QTLs affecting internodal and plant elongation were identified. Of these, only 1 QTL was detected in test 1 and all 3 QTLs were detected in test 2. These main-effect QTLs explained 33.8% and 20.1% of the total phenotypic variation for plant and internodal

Table 4 Genomic regions showing significant association with submergence tolerance detected in the IR74/Jalmagna (Jal.) recombinant inbred population

Chromosome	Marker[a]	G^2 [b]	Test 1					G^2	Test 2				
			IR74 allele frequency		Jal. allele frequency		Tolerant allele		IR74 allele		Jalmagna allele		Tolerant allele
			Obs.[c]	Exp.[c]	Obs.	Exp.			Obs.	Exp.	Obs.	Exp.	
1	*P1M6-2*							23.2	40	26.2	10	23.8	Jal.
1	*RG541*	6.4	19	13.6	5	10.4	Jal.	5.13	34	27.5	15	21.5	Jal.
3	*P1M3-5*							15.7	2	10.5	48	39.5	IR74
4	*P2M5-8*							19.7	47	36.4	3	13.6	Jal.
4	*P3M1-5*							5.92	19	14.5	5	9.5	Jal.
5	*P1M6-9*	7.2	26	21.9	0	3.1	Jal.	9.4	44	36.4	6	13.6	Jal.
7	*P1M3-9*							5.2	44	38.7	6	11.3	Jal.
8	*P3M7-3*	6.9	22	16.8	3	8.2	Jal.	11.9	43	33.9	7	16.1	Jal.
9	P2M1-15	5.1	7	12.1	18	12.9	IR74	58.3	6	27.6	44	22.4	IR74
9	P2M5-18	6.6	22	16.9	3	8.1	Jal.						
10	P3M1-3							13.0	45	36.1	5	13.9	Jal.
11	P3M1-12							8.0	44	37.3	5	11.7	Jal.
12	P3M7-4							6.0	41	34.5	9	15.5	Jal.

[a] Underlined markers are associated with the detected QTLs affecting internodal or plant elongation

[b] G^2 is the likelihood ratio chi-square statistic (Fienberg 1977). The significant values of G^2 at P = 0.05, 0.01 and 0.001 are 3.79, 6.63, and 11.60, respectively

[c] Obs. and Exp. are the observed and expected (based on the assumption of independence) allelic frequencies, respectively

elongation in test 1, and 66.4% and 78.4% in test 2, respectively. Two of these QTLs were important. The Jalmagna allele at *QIne1* near *sd-1* on chromosome 1 resulted in 13.6 cm (R^2=33.8%) and 8.7 cm (R^2=20.1%) plant and internodal elongation in test 1, and 23.2 cm (R^2=29.6%) and 18.2 cm (R^2=33.1%) in test 2. The other QTL, *QIne4*, mapped between P3M1–5 and P3M5–1 on chromosome 4, was detected only in test 2. The allele from the non-elongating IR74 at *QIne4* had a very large effect (21.8 cm, R^2 = 36.7%) on elongated internodes. An additional QTL, *QIne2*, detected only in test 2, was mapped to the interval between markers P2M9–8 and P2M6–8 on chromosome 2, and the Jalmagna allele at *QIne2* caused elongated internodes.

In addition to the main-effect QTLs, 8 pairs of digenic epistatic QTLs affecting internode and plant elongation were identified in the two tests (Table 3). These epistatic QTLs collectively explained 54.9% and 43.7% of the total variances of internodal and plant elongation in test 1, and 21.6% and 22.0% in test 2. All epistatic effects were negative in test 1, indicating that the recombinant type of alleles at the interacting loci resulted in increased elongation, while the parental digenic genotypes were associated with reduced elongation. In test 2, all parental-type interactions except one (between RG541 of chromosome 1 and RG553 on chromosome 9) resulted in increased elongation. The epistatic effect between this QTL pair was approximately 8 cm on internodal and plant elongation. The most notable locus involved in epistasis was the one flanked by RG403~P1M1–2 on chromosome 5. In test 1, alleles at this locus affected both leaf and internode elongation through strong interactions with the main-effect QTLs, *QIne1* and *QIne4* (Table 4). However, its epistatic effect varied depending on the other loci. For instance, when interacting with *QIne1*, it had a large epistatic effect on leaf and plant elongation, and under this condition the main-effect of *QIne1* became detectable. When interacting with *QIne4*, however, it showed very large main and epistatic effects on internodal elongation.

QTLs affecting leaf elongation

Two main-effect QTLs and 1 epistatic QTL pair affecting leaf elongation were identified in test 1 (Tables 2 and 3). Together, these QTLs explained 48.6% of the total phenotypic variation. The Jalmagna allele at 1 QTL (chromosome 1) resulted in leaf elongation, while the IR74 allele at the other QTL (chromosome 6) caused leaf elongation. The interaction was detected between the main-effect QTL near *sd-1* on chromosome 1 and a QTL flanked by RG403 and P1M1-2 on chromosome 5. The large main effect at this QTL was detectable only when it was involved in the interaction. The epistatic effect between the QTLs was 12.7 cm and explained 25% of the total phenotypic variation. In test 2, a single main-effect QTL was identified on chromosome 7 that explained 12.0% of the total phenotypic variation. The Jalmagna allele at the QTL resulted in leaf elongation.

Genomic regions associated with submergence tolerance

Thirteen genomic regions on 10 of the 12 rice chromosomes showed significant associations with submergence tolerance based on the likelihood chi-square G^2 (Table 2, Fig. 2). Of these, 4 regions on chromosomes 1, 5, 8, and

9 were detectable in both tests, 1 (chromosome 9) was detected only in test 1, and the remaining 8 were detected only in test 2. The Jalmagna allele at 11 of these regions was associated with submergence tolerance, while the IR74 allele at the other 2 regions contributed to submergence tolerance. Seven (chromosomes 1, 3, 4, 5, and 7) of the thirteen submergence tolerance loci were located in the QTL regions for plant elongation (Fig. 2), and in all these cases, alleles for increased plant elongation were associated with submergence tolerance. The other 6 genomic regions on chromosomes 8, 9, 10, 11, and 12 for submergence tolerance were not associated with elongation QTLs. Of these, a major gene was identified with a G^2 of 58.3 near P2M1–15 on chromosome 9. The allele for improved submergence tolerance at this locus was derived from IR74.

Discussion and conclusions

Using a QTL mapping study combined with submergence treatments of two contrasting water qualities, we were able to identify several genes/QTLs that largely controlled submergence tolerance and plant elongation in a recombinant inbred rice population. Our results indicate that segregation of rice plants with respect to their responses to different flooding stress conditions was largely due to the differential expression of elongation and submergence tolerance genes. Several general features regarding the patterns of differential gene expression under flooding were revealed in this study and merit further discussion.

First, genes affecting the same phenotype (plant height) under flooding and non-flooding conditions often represent different sets of genes. This was supported by the observation that 2 main-effect and 3 pairs of epistatic QTLs affecting initial plant height before flooding were undetectable after flooding and that all but 1 QTL (*QIne1*) causing plant elongation under flooding were not detectable before flooding.

Second, different stress (flooding) conditions appeared to induce the expression of different sets of stress-responsive genes. For example, the major difference between tests 1 and 2 was the water quality. Our results clearly indicate that under the field flooding condition with muddy water, more internodal elongation and submergence tolerance genes were expressed. In contrast, the strong leaf elongation expressed in IR74 under clear water submergence was consistent with the mapping result that more QTLs for leaf elongation were identified in test 1, indicating that a better light condition is required for the expression of most leaf elongation genes.

Third, more severe stress (test 2) tended to induce the expression of more genes and stronger expression of the key genes (Tables 2–4). For example, 12 loci for submergence tolerance were detected in test 2, but only 5 in test 1. Under the no-stress condition (before flooding), the Jalmagna allele at *QIne1* resulted in an increase in height of approximately 3.5 cm. Under flooding, however, its effect on internodal and plant elongation increased dramatically by 3.6-(test 1) to 7.3-(test 2) fold. Its weaker effect on internodal elongation upon flooding by clear water (test 1) was compensated by its large effect (17.1 cm) on leaf elongation regulated by alleles at a second locus on chromosome 5 (Table 3). The locus with the largest effect on submergence tolerance detected in this study was mapped to approximately the same genomic location as *Sub1(t)*, a major gene for submergence tolerance reported previously (Xu and Mackill 1996; Nandi et al. 1997). In test 1, the expression of this gene was very weak, with a G^2 of 5.1. However, its expression dramatically increased in test 2, detected by a G^2 of 58.3. Both examples demonstrate that the difference between a major gene and a QTL affecting a quantitative trait may simply be due to minor differences in phenotyping environments.

Fourth, the strong expression of certain alleles at a few loci play a key role in the adaptation of rice plants to flooding. In addition to *QIne1* and *Sub1(t)* mentioned in the previous section, 2 other loci are worth mentioning. The first one of interest is *QIne4*, mapped between P3M1–5 and P3M5–1 on chromosome 4. The allele causing internal and plant elongation was from the non-elongating parent, IR74. Because *QIne4* had a very large effect and was unlinked to *QIne1*, transgressive segregation for internodal or plant elongation over Jalmagna would be expected in the RILs, which was not observed. The presence of a third locus near RG403 on chromosome 5 appeared to provide the explanation. It interacted strongly with *QIne1*, resulting in a detectable effect of *QIne1* on leaf elongation (Table 3). Alleles at this locus also interacted strongly with *QIne4* (data not shown because of a scarcity of individuals for two of the digenic genotypes), suggesting a complex epistatic relationship among the 3 loci. Because of the severe segregation distortion at this locus – few RILs had the Jalmagna alleles at these loci – it was impossible to test the trigenic epistasis at the 3 loci in a single model due to missing trigenic genotypes.

Our results are consistent with results obtained from classic genetic studies that epistasis between or among a few loci is largely responsible for plant elongation under flooding (Thakur and Hillrislambers 1988; Saha et al. 1994). Physiologically, it is known that internodal elongation in rice is regulated largely by three interacting plant hormones: abscissic acid (ABA), gibberellic acid (GA_3), and ethylene (Zarembinski and Theologis 1997). ABA inhibits the growth of submerged internodes, and GA_3 counteracts this inhibition. Ethylene promotes internode growth by increasing both the GA_3 level and the responsiveness of the internodal tissue to GA_3. If the 3 QTLs represented the loci coding for or involved in the pathways of these three hormones, an appropriate explanation for our observation could be reached. Based on its genomic location and associated phenotypes, *QIne1* is likely to be the same locus as *sd-1*, which is involved in the ethylene pathway because *sd-1* is known to be

sensitive to GA_3 (Kumar and Singh 1984). If so, the Jalmagna allele may be one of the multiple alleles at this locus that contributes to the elongation ability of rice under flooding. Knaap et al. (1997) reported a gene, *RPA1*, which codes for replication protein A1 in rice. The expression of *RPA1* in the intercalary meristem of a deepwater rice line, Pin Gaew 56, is significantly enhanced during submergence, or by GA_3 treatment. Because the genomic location of *RPA1* is unknown, the relationship between *sd-1* and *RPA1* remains to be tested. *QIne4* could be a gene involved in the pathway of GA_3 based on its large effect on internodal elongation and its suppression with the recessive mutant allele in IR74 at *sd-1*. The locus on chromosome 5 appeared to play a key role in regulating both *QIne1* and *QIne4*. How it relates to ABA's pathway remains a mystery. Molecular cloning of these QTLs will certainly provide insights into the biochemical pathways underlying the responses of rice plants to flooding.

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