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ORIGINAL PAPER

Evaluation of near-isogenic lines for drought resistance QTL and fine mapping of a locus affecting flag leaf width, spikelet number, and root volume in rice

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Abstract Drought stress is a major limiting factor for crop production and breeding for drought resistance is very challenging due to the complex nature of this trait. Previous studies in rice suggest that the upland *japonica* variety IRAT109 shows better drought resistance than the lowland *indica* variety Zhenshan 97. Numerous quantitative trait loci (QTL) have been previously mapped using a recombinant inbred line population derived from these two genotypes. In this study, near-isogenic lines (NILs) for 17 drought resistance-related QTL were constructed and phenotypic variations of these NILs were investigated under drought and normal conditions. Fourteen of these NILs showed significant phenotypic differences relative to the recurrent parent under at least one of the conditions and nine NILs showed significant differences under both conditions. After eliminating the effect of heading date on drought resistance, only four NILs carrying seven QTL (four for the same grain yield-related traits and three for the same or similar root traits QTL) showed differences consistent with the original QTL mapping results. One of these lines (N19) contains *qFSR4*, a QTL on chromosome 4 controlling root volume per tiller and co-segregating with flag leaf width and spikelet number per panicle. Using a population derived from N19, *qFSR4* was mapped to a 38-kb region containing three open reading frames including the previously characterized *NARROW LEAF 1* (*NAL1*) gene. *NAL1*, which controls leaf width and also affects vein patterning and polar auxin transport, is the most promising candidate genes for *qFSR4*. Our results underscore the importance of the development of NILs to confirm the identification of QTL affecting complex traits such as drought resistance.

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Introduction

As the world population continues to expand, food shortages will become increasingly severe. Rice (*Oryza sativa*) is one of the most important crops and is a staple food for a large segment of the world population. Therefore, continuing to increase rice yields is vital to relieving the growing pressure for greater food production. Because of the reduction of available water resources and frequent unexpected meteorological events, such as absence or uneven distribution of rainfall, drought stress is becoming one of the major limiting factors for rice production (Luo 2010). Understanding the mechanisms underlying yield formation and drought resistance is crucial for breeding rice varieties with greater yield potential and drought resistance under adverse environmental conditions.

Drought resistance is a complex trait that is affected by many morphological and physiological factors. Genetic analysis of drought resistance has resulted in the identification of a large number of quantitative trait loci (QTL) for drought resistance-related traits in rice, maze, and sorghum (reviewed by Ashraf 2010). In rice, many QTL for drought resistance-related traits (e.g. cell membrane stability, osmotic adjustment, yield- and root-related traits) have been identified (reviewed by Bernier et al. 2008,

Kamoshita et al. 2008). For example, by using a recombinant inbred line (RIL) population derived from a cross of lowland *indica* rice Zhenshan 97 and upland *japonica* rice IRAT109 and a sprinkler system that enables creation of a water gradient from well-watered condition to drought stress within a block, 32 QTL for grain yield and its component traits (e.g. spikelet fertility, spikelet number per panicle, 1,000-grain weight, and panicle number) were identified by Zou et al. (2005). Among them, 18 and 14 QTL were detected under normal irrigation and drought stress conditions, respectively. Using the same RIL but a different drought treatment method in which rice plants were individually planted in polyvinyl chloride (PVC) pipes and stressed with drought to the similar extent at the same developmental stage, Yue (2005) detected 60 QTL for grain yield and its component traits. Of these QTL, 32 and 28 were detected under the normal irrigation and drought stress conditions, respectively, and only 9 were detected under both the normal and drought conditions.

Commonly accepted mechanisms for drought resistance include drought escape, drought avoidance, drought tolerance, and drought recovery (Nguyen et al. 1997). Drought avoidance, mainly via thick and deep root system, may play more important role than the other mechanisms of drought resistance for rice grown in sandy soil condition, while drought tolerance may be the major genetic basis of drought resistance for rice grown in paddy soil condition (Yue et al. 2005). In order to distinguish the contributions from drought avoidance and drought tolerance, Yue et al. (2006a) employed the PVC pipe system that enables plants to be individually stressed to the similar degree at the same developmental stage and to be easily measured for root-related traits. Their results suggest that, with regard to the chromosomal locations of QTL for drought resistance at the reproductive stage, the genetic basis of drought tolerance is largely different from that of drought avoidance.

Although numerous QTL for drought resistance-related traits have been reported by using different primary populations in rice, very few have been confirmed (reviewed by Bernier et al. 2008 and Kamoshita et al. 2008). Co-location or overlapping analysis of drought resistance-related QTL obtained from different research groups or populations revealed that only four QTL intervals for the traits were reproducibly detected (Kamoshita et al. 2008). Steele et al. (2006) created near isogenic lines (NILs) of rice by marker-assisted back-crossing for five root trait-related QTL and evaluated their root traits in different field experiments, however, only one root-related QTL was confirmed (Steele et al. 2006).

To further understand the genetic basic of drought resistance of rice, some of the drought resistance-related QTL reported by Yue (2005), Yue et al. (2006a), and Zou et al. (2005) were selected for developing NILs in this study. Seventeen NILs were generated to verify the phenotypic effects of the corresponding 17 QTL. Fourteen NILs showed significant differences in various traits related to drought resistance and yield potential. One NIL (N19) showing phenotypic differences in root volume per tiller (RVT) as well as flag leaf width (FLW) and spikelet number per panicle (SPP) was characterized and the corresponding QTL interval on chromosome 4 (RM241-MRG4503) was mapped to a 38-kb interval containing three open reading frames.

Materials and methods

Construction of NILs

Previously, QTL mapping was conducted for drought resistance-related traits in an RIL population derived from a cross of a paddy *indica* rice Zhenshan 97 (ZS97) and an upland *japonica* rice IRAT109 (Yue 2005; Yue et al. 2006a; Zou et al. 2005). Seventeen representative QTL intervals for both drought tolerance- and drought avoidance-related traits were selected to develop NILs (Table 1). For accelerating the development of NILs containing QTL associated with drought resistance, crosses were made between ZS97 and selected RILs with >72% of genetic background of ZS97 but containing the target allelic region from IRAT109, or between IRAT109 and selected RILs with >72% of genetic background of IRAT109 but containing the target allelic region from ZS97. The resulting F_1 plants were backcrossed with ZS97 or IRAT109 as female parents. Plants with heterozygous genotype for the target regions in the progenies were selected by SSR markers and backcrossed to ZS97 or IRAT109. NILs for drought resistance-related QTL were obtained through continuous four backcrosses with ZS97 or IRAT109 as recurrent (female) parent and selection of the genotypes of target regions by SSR markers.

Genotyping NILs and molecular marker development

Leaf sample were harvested from the BC_4F_3 and BC_4F_4 NIL plants at tillering stage for genotyping. The DNA samples were extracted by the cetyltrimethylammonium bromide (CTAB) method (Murray and Thompson 1980). The genotype for each plant of the 17 NIL families was identified by checking the SSR markers flanking the QTL targeted intervals. SSR analysis was performed as described previously (Wu and Tanksley 1993). For background checking, a mixed DNA sample from each NIL family was surveyed using 120 SSR markers evenly distributed over the 12 rice chromosomes.

Table 1 Information of 17 QTL for drought resistance and genetic background of NILs

NILs[a]	Chr[b]	Interval markers		Trait[c]	RIL parent[d]	Recurrent parent	Genetic background (%)
N01	1	RM237	RM403	DIDRV	R51	ZS97	95.3
N04	2	RM29	RM341	DIRD, SPP	R181	IRAT109	98.5
N06	2	RM526	RM221	DRVC, KGW	R47	ZS97	97.3
N08	2	RM573	RM318	DIDRV, SF	R116	ZS97	96.1
N09	2	RM240	RM166	GY	R116	ZS97	96.3
N11	3	RM523	RM489	RVD, GY, SF	R96	IRAT109	98.2
N12	3	RM473	RM487	DRVD, RGDC	R46	IRAT109	98.3
N15	4	RM335	RM307	DRVD, MRDD	R46	IRAT109	96.5
N19	4	RM241	MRG4503	RVD, SPP	R206	ZS97	99.5
N20	4	RM349	RM127	RVC, RVD	R51	ZS97	99.3
N23	5	RM421	RM274	MRDC, KGW	R202	ZS97	97.4
N24	7	RM125	MRG4499	RVD, KGW, SF	R135	ZS97	97.1
N29	9	RM316	RM219	DRVC, SF	R38	ZS97	98.4
N30	9	RM219	RM296	DRVD, SF	R38	ZS97	98.5
N36	11	RM287	RM229	DRVC, MRDC	R14	IRAT109	96.7
N37	12	MRG2483	RM453	RSN	R46	IRAT109	97.2
N38	12	RM235	MRG5454	FP	R181	IRAT109	98.2

[a] Systematic nomenclature of NILs developed corresponding to the target intervals

[b] Chromosome in which the target interval is located

[c] Major QTL for drought resistance-related traits from Yue (2005), Yue et al. (2006a) and Zou et al. (2005), with the abbreviations of the traits that were not described in this study are as follows: *DIDRV* deep root rate in volume induced by drought (%), *DIRD* drought induced root growth in depth (cm), *DRVC* Deep root rate in volume under control (%), *DRVD* deep root rate in volume under drought (%), *FP* fertile panicles, *MRDC* maximum root depth under control (cm), *MRDD* maximum root depth under drought (cm), *RGDC* root growth rate in depth under control (cm/day), *RSN* relative spikelet number per panicle (%), *RVC* root volume under control (mL), *RVD* root volume under drought (mL)

[d] RIL selected for backcross with Zhenshan 97 or IRAT109

SSR markers were identified from the Gramene database (http://www.gramene.org/). The SSR primers were designed according to the public database (McCouch et al. 2002; Temnykh et al. 2001). For fine mapping, five novel InDel markers were developed on the basis of the publicly available *japonica* and *indica* rice genome sequences (http://www.rgp.dna.affrc.go.jp; http://www.rise.genomics.org.cn/) (Supplemental Table 1).

Evaluation of NILs

To evaluate the NILs for their agronomic performance under normal irrigation conditions, the two parents (ZS97 and IRAT109) and NIL families (BC_4F_3 or BC_4F_4) were planted in filed in Wuhan (East longitude 114°, North latitude 30°). The plants were grown in plots with distance of 16.5 cm between plants within a row and 25 cm between rows following a randomized complete block design with three replicates. Field management followed the normal rice cultivation practices as described previously (Xing et al. 2008).

To evaluate the drought resistance of the NILs, drought stress treatment was performed according to Yue et al. (2006a). Forty plants of each NIL, including 20 plants with homozygous allele of ZS97 and 20 plants with homozygous allele of IRAT109 for the target interval, were planted in PVC pipes. The PVC pipe, 20 cm in diameter and 100 cm in length, was designed with a plugged hole through the pipe wall at the bottom and was placed under a rain-out shelter. Each PVC pipe was loaded with a plastic bag with mixed soil including two parts of clay and one part of sand. The drought treatment was individually applied to each plant in the PVC pipes. When all leaves of the stressed rice plant became completely rolled (with soil water content at about 11.5%), water was applied to the full capacity of the pipe and the second round of drought stress was applied until all leaves became completely rolled again, then watering was resumed for the rest of the life cycle.

For investigating the phenotypic variation of the NILs, eight traits (6 above-ground traits for drought [PVC] and normal [field] conditions and 2 root traits for drought

condition) were scored in this study. Plants were measured for the traits related to fitness and productivity, including plant height (PH, in centimeters), grain yield (GY, in grams), number of tillers per plant (TPP), spikelet number per panicle (SPP), 1,000-grain weight (KGW, in grams), and spikelet fertility (SF, in percentage). GY and the yield-related traits (TPP, SPP, KGW, and SF) were measured for all plants under drought stress and the normal conditions. After maturation, rice plants were harvested individually and the following traits were measured: GY as the total weight of the grains from whole plant, SPP as the total number of spikelets of the whole plant divided by its total number of panicles, SF as the number of filled grains divided by the total number of spikelets of the entire plant, and KGW as yield divided by the number of filled grains then multiplied by 1,000. The two root traits, including the total root volume (RV, in milliliters) and the root volume per tiller (RVT, in milliliters), were measured at the seed maturity stage of the plants in PVC pipes. The RV was measured in a cylinder using the water-replacing method (Price and Tomos 1997). RVT was measured as RV divided by TPP. Paired *t* test was used to analyze the difference between the two homozygotes (i.e. ZS97 and IRAT109 alleles for the target interval) of each NIL.

Fine-mapping of N19-targeted QTL

Because flag leaf width (FLW) co-segregated with RVT and SPP in the progeny testing of N19, FLW was adopted for fine-mapping of N19-targeted QTL. To determine the flag leaf width, three to five fully expanded flag leaves were measured in the middle (widest part) of the leaves at grain filling stage (~20 days after heading), and the average value was used as FLW of the plant. The selfing progenies (BC_4F_3 plants) that were heterozygous for the target region and contained the least genetic background (<5%) from the other parent were identified using SSR markers. A total of 146 BC_4F_4 plants, derived from one BC_4F_3 plant heterozygous for the target region, were grown in PVC pipes for genetic analysis. Progeny testing was performed for all three traits. Genetic linkage map was conducted by using Mapmaker/EXP 3.0 and the genetic distance was calculated by Kosambi function (Lincoln et al. 1992). For fine mapping, a BC_4F_4 population (F_2 progeny of heterozygous BC_4F_3 plant) consisting of >12,000 plants was planted and pre-screened using the FLW trait which is easier to assess than using SPP or RVT. A subset of 1295 plants showing recessive phenotype (narrow flag leaf, FLW ≤1.60 cm) was screened for recombinants using DNA markers flanking the QTL interval. Forty BC_4F_5 plants from each recombinant were investigated for RVT, SPP and FLW variation.

Results

Construction of NILs for drought resistance-related QTL

To verify the drought resistance-related QTL previously detected in the ZS97/IRAT109 RIL population (Yue 2005; Yue et al. 2006a, Zou et al. 2005), a total of seven NILs with a genetic background of IRAT109 and ten NILs with a genetic background of ZS97 were obtained (Table 1). SSR marker analysis suggested that these NILs showed >95% similarity of genetic background compared to their corresponding recurrent parents (Table 1). For example, except three heterozygous markers in the target QTL interval of NIL N19, only one heterozygous marker was detected in the genome (Supplemental Fig. 1) but this marker did not co-segregate with the traits associated with the interval present in N19.

Phenotyping NILs under normal irrigation conditions

Before phenotyping, the genotypes of 17 NIL families (BC_4F_3) were assayed with SSR markers. The plants of each NIL family were classified into three groups: homozygous for ZS97 allele (NIL-ZS), homozygous for IRAT109 allele (NIL-IR), and heterozygous (NIL-ZI) at the targeted QTL.

Grown under the normal irrigation condition in the field, ZS97 showed significantly higher trait values than IRAT109 in grain yield (GY), number of tillers per plant (TPP) and spikelet fertility (SF), but IRAT109 had higher traits values in plant height (PH), spikelet number per panicle (SPP), and 1,000-grain weight (KGW) (Table 2). Ten NILs showed a significant difference in at least one of the yield component traits, including TPP, SPP, SF, and KGW, or GY between the two homozygous near-isogenic groups (NIL-ZS and NIL-IR, hereafter referred as NIL pair) (Table 2). Two NIL pairs showed a significant difference in PH. The average value of GY between NIL pairs was significant different for seven NIL families, of which four NIL pairs also showed significant variation in TPP. Five NIL pairs showed significant differences in SPP, of which four were related to GY. Six NIL pairs showed significant differences in KGW, three of which were also related to GY. Only one NIL pair showed a significant difference in SF. Notably, the N11 NIL pairs showed significant differences for all six traits.

Phenotyping NILs under drought stress conditions

When drought stress was applied in PVC pipes at the reproductive stage, the paddy rice ZS97 had significantly higher TPP and SPP, but lower PH, GY, KGW, total root

Table 2 Phenotypic performance of two parents and 17 NIL pairs under normal conditions

NILs	PH (cm)	TPP	SPP	SF (%)	GY (g)	KGW (g)
ZS97	87.34 ± 6.96	7.57 ± 2.27**[b]	93.34 ± 17.81	0.91 ± 0.06**	15.72 ± 5.41*	24.71 ± 2.02
IRAT109	103.95 ± 7.58**	5.85 ± 2.13	134.18 ± 24.94**	0.67 ± 0.08	14.45 ± 7.11	31.28 ± 2.14**
N01[a]	78.67 ± 8.69	7.33 ± 2.34	87.88 ± 11.49	0.86 ± 0.10	13.34 ± 5.31	24.07 ± 2.04
	77.40 ± 6.85	7.00 ± 1.89	88.44 ± 13.37	0.85 ± 0.08	11.94 ± 3.36	23.03 ± 2.31
N04	100.38 ± 5.44	5.62 ± 1.71	112.74 ± 22.31	0.65 ± 0.05	12.68 ± 4.55	30.49 ± 1.43
	99.75 ± 5.56	5.50 ± 2.38	98.57 ± 11.93	0.70 ± 0.06	12.00 ± 6.46	31.05 ± 0.77
N06	89.89 ± 3.72	8.11 ± 2.20	88.47 ± 10.75	0.93 ± 0.02	16.42 ± 4.90	24.62 ± 1.22
	88.44 ± 5.27	7.22 ± 2.48	88.97 ± 13.85	0.96 ± 0.02	15.51 ± 4.65	25.87 ± 1.29*
N08	87.60 ± 3.44	6.80 ± 2.49	91.27 ± 7.55	0.91 ± 0.07	13.72 ± 2.83	25.33 ± 1.65
	86.78 ± 2.86	7.22 ± 2.59	91.82 ± 12.07	0.91 ± 0.07	14.53 ± 4.38	24.73 ± 1.73
N09	86.71 ± 4.72	6.57 ± 1.72	88.30 ± 12.85	0.91 ± 0.04	12.93 ± 3.27	24.74 ± 1.03
	88.86 ± 5.37	9.14 ± 2.54*	85.01 ± 5.26	0.93 ± 0.02	17.73 ± 4.44*	24.79 ± 0.91
N11	112.86 ± 5.98**	7.00 ± 0.82**	128.03 ± 7.26**	0.72 ± 0.05*	20.63 ± 3.92**	31.74 ± 2.33*
	100.89 ± 4.51	4.85 ± 1.28	103.06 ± 16.52	0.62 ± 0.10	9.53 ± 3.51	30.12 ± 0.89
N12	100.71 ± 6.85	5.14 ± 1.57	94.19 ± 24.56	0.73 ± 0.07	10.39 ± 3.27	29.31 ± 1.34
	96.44 ± 8.28	4.78 ± 1.39	89.93 ± 24.64	0.69 ± 0.05	9.93 ± 5.34	32.19 ± 1.79*
N15	111.14 ± 3.72	6.29 ± 2.14	135.30 ± 14.08	0.69 ± 0.04	17.97 ± 5.62	31.15 ± 1.38
	111.17 ± 6.52	6.00 ± 1.67	126.88 ± 17.38	0.70 ± 0.04	16.10 ± 5.60	30.21 ± 3.51
N19	86.00 ± 6.58	8.87 ± 3.18	86.93 ± 12.41	0.93 ± 0.04	18.09 ± 7.78	24.87 ± 1.09**
	87.64 ± 5.72	7.36 ± 1.95	112.02 ± 8.13**	0.90 ± 0.07	17.62 ± 4.82	23.92 ± 1.10
N20	88.00 ± 3.74	8.14 ± 2.41	87.97 ± 9.37	0.93 ± 0.03	16.46 ± 4.82	24.71 ± 0.96
	85.00 ± 3.74	8.25 ± 3.30	93.72 ± 12.87	0.95 ± 0.02	18.75 ± 8.31	25.72 ± 1.34
N23	85.00 ± 3.71	8.22 ± 2.28	74.86 ± 11.47	0.92 ± 0.06	14.43 ± 3.76	25.91 ± 1.73
	86.63 ± 3.16	9.13 ± 3.09	87.81 ± 12.15*	0.93 ± 0.03	19.43 ± 5.54*	26.72 ± 1.18
N24	86.43 ± 4.12	7.29 ± 2.36	87.93 ± 18.48	0.81 ± 0.07	12.16 ± 3.75	24.27 ± 0.54
	102.50 ± 3.57**	6.10 ± 1.91	133.99 ± 18.83**	0.83 ± 0.06	17.45 ± 4.77*	26.36 ± 0.72*
N29	87.52 ± 5.32	7.43 ± 2.30	91.27 ± 13.85	0.94 ± 0.03	16.19 ± 4.97*	25.55 ± 1.10**
	83.50 ± 3.94	6.67 ± 1.07	89.44 ± 13.68	0.90 ± 0.04	12.57 ± 3.64	23.12 ± 1.35
N30	87.67 ± 5.36	7.44 ± 2.07	92.70 ± 8.25	0.90 ± 0.04	15.47 ± 5.23	24.56 ± 1.42
	86.33 ± 4.21	8.33 ± 1.73	91.83 ± 14.8	0.90 ± 0.05	16.64 ± 3.68	24.27 ± 0.67
N36	103.33 ± 3.33	5.33 ± 1.03	123.85 ± 16.67	0.68 ± 0.03	14.77 ± 3.28	33.02 ± 0.98
	103.89 ± 5.51	5.44 ± 2.19	119.31 ± 13.92	0.67 ± 0.05	14.18 ± 5.74	32.66 ± 1.68
N37	102.70 ± 6.67	5.91 ± 1.92	98.4 ± 17.00	0.72 ± 0.08	13.72 ± 5.17	32.64 ± 1.68
	108.00 ± 4.64*	7.33 ± 1.66*	129.05 ± 26.25**	0.66 ± 0.05	20.20 ± 6.59*	32.40 ± 0.99
N38	106.86 ± 4.81	5.29 ± 1.98	124.09 ± 10.26	0.64 ± 0.06	12.69 ± 4.69	30.46 ± 1.51
	109.71 ± 9.18	7.71 ± 1.98*	138.95 ± 21.06	0.69 ± 0.05	22.16 ± 5.21**	30.88 ± 1.94

*,** Significant at $P = 0.05$ and $P = 0.01$ level, respectively

[a] For each NIL, the top and bottom rows are the trait values of homozygous Zhenshan 97 (ZS) genotype (NIL-ZS) and IRAT109 (IR) genotype (NIL-IR), respectively, at the target locus. Values are mean ± SD ($n \geq 20$)

[b] The difference between the two different genotypes in each NIL pair or between the parents was evaluated by *t* test

volume (RV), and root volume per tiller (RVT) than the upland IRAT109. There was no significant difference in SF between ZS97 and IRAT109. 12 NIL pairs showed significant differences in GY or at least one yield component traits under drought stress conditions (Table 3). Among them, seven NIL pairs were significant for GY and five of them were also significant for TPP. Eight NIL pairs showed significant differences in SPP and four of them were also significant for GY. For KGW, eight NIL pairs showed significant differences and six of them were significant for GY. For SF, six NIL pairs showed significant differences and five were significant for GY. These results suggest that most of the loci for yield component traits also have significant effects on GY under drought stress. Two NIL pairs (N11 and N24) showed significant differences in PH. Under drought stress conditions, eight NIL pairs showed

Table 3 Phenotypic performance of two parents and 17 NIL pairs under drought stress

NILs	PH (cm)	TPP	SPP	SF (%)	GY (g)	KGW (g)	RV (mL)	RVT (mL)
ZS97	84.81 ± 6.63	31.84 ± 8.12**[b]	75.44 ± 12.57**	0.47 ± 0.10	21.83 ± 7.40*	19.69 ± 2.27	31.26 ± 13.15**	1.26 ± 0.47
IRAT109	95.33 ± 6.63**	20.13 ± 5.94	72.14 ± 16.51	0.46 ± 0.20	20.55 ± 10.14	31.36 ± 3.84**	27.82 ± 12.43	1.84 ± 0.70**
N01[a]	81.47 ± 6.87	28.59 ± 3.97	71.05 ± 10.09	0.48 ± 0.08	18.21 ± 3.58	18.86 ± 1.00	13.71 ± 6.63	0.76 ± 0.33
	79.19 ± 7.65	26.19 ± 6.55	71.10 ± 10.36	0.48 ± 0.07	17.05 ± 4.26	19.36 ± 1.31	16.06 ± 10.15	0.87 ± 0.38
N04	93.50 ± 4.18	20.67 ± 5.43	76.12 ± 12.23*	0.55 ± 0.20	25.46 ± 10.74	30.89 ± 2.33	20.56 ± 7.53	1.48 ± 0.50
	94.69 ± 4.70	21.00 ± 4.65	67.88 ± 11.33	0.56 ± 0.19	24.83 ± 9.01	32.45 ± 1.68*	22.23 ± 6.23	1.77 ± 0.55
N06	79.35 ± 5.96	31.53 ± 7.24	68.54 ± 6.74	0.47 ± 0.08	20.75 ± 3.41	19.91 ± 0.80	18.50 ± 8.17	0.86 ± 0.31
	82.36 ± 5.97	31.27 ± 9.35	75.84 ± 5.06**	0.53 ± 0.10	26.28 ± 5.47**	21.20 ± 1.44**	24.36 ± 8.82*	1.20 ± 0.32**
N08	84.64 ± 2.94	31.64 ± 8.57	72.69 ± 10.51	0.50 ± 0.09	23.29 ± 5.86	20.47 ± 0.91	43.70 ± 9.66*	2.21 ± 0.70
	86.73 ± 3.34	29.27 ± 6.72	83.29 ± 13.85*	0.47 ± 0.10	21.90 ± 5.50	19.76 ± 1.35	36.05 ± 9.13	1.95 ± 0.41
N09	84.64 ± 4.80	34.21 ± 11.08	71.44 ± 12.96	0.47 ± 0.03	22.12 ± 5.78	20.02 ± 1.35	23.71 ± 9.13*	0.91 ± 0.26
	80.53 ± 6.93	29.29 ± 7.90	74.26 ± 7.37	0.45 ± 0.08	19.71 ± 7.36	19.77 ± 2.09	18.63 ± 6.02	0.80 ± 0.19
N11	114.50 ± 8.46**	13.00 ± 1.90	134.50 ± 16.6**	0.73 ± 0.16*	36.85 ± 9.65**	29.30 ± 2.59	39.00 ± 9.92**	3.06 ± 0.90*
	97.20 ± 3.30	20.27 ± 5.90**	72.69 ± 13.86	0.55 ± 0.14	24.02 ± 5.94	31.88 ± 2.03*	26.85 ± 8.41	1.99 ± 0.68
N12	92.67 ± 4.30	21.78 ± 3.19	56.91 ± 5.39	0.74 ± 0.09**	28.76 ± 5.27	31.32 ± 1.24	19.67 ± 6.26	1.38 ± 0.43
	92.89 ± 4.28	23.63 ± 4.76	67.00 ± 10.32**	0.55 ± 0.16	26.76 ± 7.43	32.29 ± 2.76	39.08 ± 10.55**	2.36 ± 0.58**
N15	99.36 ± 7.42	17.27 ± 5.71	79.26 ± 16.95*	0.34 ± 0.15	13.39 ± 9.90	28.28 ± 4.01	30.27 ± 11.12	2.20 ± 0.83
	98.88 ± 6.40	20.71 ± 4.31*	68.56 ± 13.84	0.47 ± 0.13*	19.35 ± 4.94*	30.47 ± 2.91*	23.35 ± 14.56	1.74 ± 0.79
N19	87.60 ± 5.47	36.13 ± 6.09	74.36 ± 8.32	0.49 ± 0.05	23.86 ± 3.10	18.34 ± 0.93	37.94 ± 11.28	1.40 ± 0.42
	86.22 ± 6.26	35.00 ± 4.97	86.85 ± 7.31**	0.45 ± 0.06	24.22 ± 4.89	17.71 ± 1.06	57.70 ± 10.6**	1.96 ± 0.41**
N20	89.88 ± 4.16	31.06 ± 9.23	81.27 ± 13.66	0.47 ± 0.06	21.15 ± 5.15	18.53 ± 1.97	35.61 ± 12.21	1.35 ± 0.40
	88.38 ± 3.99	31.85 ± 7.19	75.13 ± 13.64	0.47 ± 0.07	20.72 ± 6.23	18.43 ± 1.88	34.62 ± 9.84	1.36 ± 0.42
N23	80.00 ± 5.10	31.81 ± 6.73	68.93 ± 12.99	0.45 ± 5.28	20.62 ± 5.28	21.23 ± 1.74	26.00 ± 10.55	0.97 ± 0.36
	78.09 ± 5.28	38.20 ± 8.57*	68.42 ± 8.52	0.46 ± 0.13	26.85 ± 7.49*	23.05 ± 1.17**	34.73 ± 9.57*	1.10 ± 0.20
N24	86.24 ± 5.46	32.24 ± 7.76*	71.51 ± 9.94	0.43 ± 0.08	18.84 ± 5.20	19.20 ± 1.41	26.13 ± 10.32	1.02 ± 0.31
	95.86 ± 2.54**	26.86 ± 2.12	104.18 ± 16.12**	0.78 ± 0.12**	46.87 ± 6.46**	21.94 ± 1.02**	55.14 ± 6.07**	1.84 ± 0.21**
N29	87.82 ± 4.66	36.78 ± 7.68**	76.21 ± 9.86	0.47 ± 0.07*	26.45 ± 5.23**	20.33 ± 1.71**	39.63 ± 13.07	1.74 ± 0.68
	87.00 ± 4.13	30.63 ± 7.41	78.37 ± 7.98	0.43 ± 0.08	18.82 ± 5.23	18.58 ± 1.34	33.95 ± 13.96	1.61 ± 0.63
N30	88.48 ± 6.79	27.65 ± 8.18	73.11 ± 10.98	0.46 ± 0.08	17.21 ± 5.67	18.98 ± 1.27	29.75 ± 6.83	1.16 ± 0.28
	85.45 ± 6.64	28.82 ± 7.15	76.25 ± 5.67	0.46 ± 0.08	18.60 ± 5.04	18.51 ± 1.70	30.33 ± 6.36	1.34 ± 0.25
N36	92.91 ± 5.79	17.55 ± 5.99	75.92 ± 7.07	0.40 ± 0.18	15.45 ± 7.32	30.23 ± 1.73	21.73 ± 8.43	1.83 ± 0.76
	94.67 ± 5.68	20.83 ± 5.97	70.28 ± 10.97	0.41 ± 0.22	19.08 ± 10.58	32.1 ± 3.61*	24.5 ± 12.24	1.81 ± 0.82
N37	92.00 ± 4.97	20.43 ± 4.24	69.63 ± 12.87	0.41 ± 0.20	18.48 ± 9.64	32.44 ± 1.93	26.71 ± 5.12	1.78 ± 0.43
	96.42 ± 5.66	21.42 ± 6.83	62.77 ± 11.68	0.44 ± 0.21	18.82 ± 9.82	31.17 ± 2.81	25.00 ± 12.05	1.69 ± 0.77
N38	91.83 ± 8.20	21.10 ± 5.88	74.86 ± 12.77	0.27 ± 0.12	13.50 ± 6.77	31.74 ± 3.08	23.00 ± 12.44	1.59 ± 0.78
	94.33 ± 7.43	21.13 ± 6.55	69.7 ± 10.86	0.45 ± 0.10**	19.58 ± 5.22**	31.16 ± 2.35	22.56 ± 10.06	1.58 ± 0.65

*,** Significant at $P = 0.05$ and $P = 0.01$ level, respectively

[a] For each NIL, the top and bottom rows are the trait values of NIL-ZS and NIL-IR, respectively, at the target locus. Values are mean ± SD ($n \geq 20$)

[b] The difference between the two different genotypes in each NIL pair or between the parents was evaluated by t test

significant differences in RV, and two of them were also significant in TPP; five NIL pairs showed significant differences in RVT but only one NIL revealed significant TPP. Interestingly, N11 showed a significant difference in all the traits investigated.

By comparing the performance of yield-related traits under normal and drought conditions, we noticed that 14 NIL pairs showed a significant difference in at least one trait under at least one condition, and nine of them showed a significant difference in at least one trait under both conditions (Tables 2, 3). Two NIL pairs (N09 and N37) showed differences in GY under the normal irrigation condition but not under the drought condition, and four NIL pairs (N04, N08, N15, and N36) showed significant differences in GY under the drought condition but no differences under normal condition.

Comparison of the quantitative traits detected by NIL and primary QTL mapping

To estimate how many QTL for drought resistance detected in the genetic mapping studies can be detected by using NILs, we compared the phenotyping results of the 17 NILs to the QTL detected in the corresponding intervals using the RIL population (Yue 2005; Yue et al. 2006a; Zou et al. 2005). Of the 17 intervals targeted in our study, yield-related QTL were detected in 11 intervals using the RIL population. Using the NILs, we observed yield-related QTL in 14 intervals under at least one growth condition (Supplemental Table 2). Ten intervals showed significant effects on yield-related traits in both the RIL mapping and NIL analysis. However, only four and six intervals were detected with exactly the same traits in both the RIL and NIL studies under the normal irrigation and the drought stress conditions, respectively (Supplemental Table 2). Three QTL, including one for KGW in the target interval of N06, one for GY in the target interval of N11, and one for SPP in the target interval of N19, were identified in both the RIL and NIL studies and under both drought and normal conditions. For root traits, QTL were detected in 14 intervals using the RIL population whereas only eight NILs showed differences in root traits. Five intervals showed effects on the same or similar root-related traits in both the RIL mapping studies and NIL analysis.

Of note, the intervals of N11 and N24 contained the heading date gene *HD9* and *GHD7*, respectively (Lin et al. 2002; Xue et al. 2008). We observed that N11 and N24 NIL pairs exhibited significant differences in heading date under both normal irrigation and drought conditions. Because a difference in heading date influences the evaluation of drought resistance, these two NILs should not be included in the comparison of RIL mapping and NIL analysis. If N11 and N24 are excluded, only four QTL intervals for yield-related traits (SPP in N04, KGW and GY in N06, SPP in N19, and SF in N29) and three intervals for root-related traits (in N06, N12, and N19) were confirmed in the NIL analysis (Supplemental Table 2).

Characterization of N19 that shows co-segregation in multiple traits

N19 was selected for detail analysis because the NIL pair showed phenotypic differences not only in root (RVT) and yield-related (SPP) traits but also in flag leaf width (FLW). Actually, IRAT109 also showed significantly higher values than ZS97 in RVT, SPP, and FLW (Table 4). The plants in the N19-BC_4F_3 and BC_4F_4 populations were classified into three groups according to genotypes of the target interval: homozygote of ZS97 (N19-ZS), homozygote of IRAT109 (N19-IR), and heterozygote (N19-ZI). Paired *t* tests indicated that the average values of all three traits (RVT, SPP, and FLW) were significantly different between N19-ZS and N19-IR in both BC_4F_3 and BC_4F_4 generations (Table 4). On average, the value of RVT of N19-IR was 50% greater than that of N19-ZS under both drought stress (BC_4F_3) and normal conditions (BC_4F_4). N19-IR has about 25 more SPP than N19-ZS and about a 0.4-cm wider FLW than N19-ZS.

Table 4 Trait performance of ZS97, IRAT109, N19-ZS and N19-IR grown in PVC

Plant	Generation	FLW (cm)	SPP	RVT (mL)
ZS97		1.55 ± 0.05	93.34 ± 17.81	1.16 ± 0.37
IRAT109		1.94 ± 0.07**	134.18 ± 24.94**	2.43 ± 0.38**
N19-ZS	BC_4F_3	1.57 ± 0.06	87.37 ± 12.78	**1.41 ± 0.42**
	BC_4F_4	1.59 ± 0.09	91.36 ± 9.25	0.83 ± 0.33
N19-IR	BC_4F_3	1.99 ± 0.05**	113.89 ± 6.55**	**1.96 ± 0.41****
	BC_4F_4	1.93 ± 0.08**	114.9 ± 12.20**	1.29 ± 0.42**

Two genotypes of N19: ZS homozygous for ZS97 allele, IR homozygous for IRAT109 allele, values are mean ± SD ($n \geq 20$), *bold values* indicate trait performance under drought stress and other under normal condition, the difference between the two different genotypes in N19 or between the parents was evaluated by *t* test;

** Significant at $P = 0.01$ level

The frequency distribution of FLW in F_2 population (146 individuals) derived from a BC_4F_3 plant that was heterozygous at the N19 interval showed bimodal distribution (Fig. 1) and the 146 plants could be separated into two distinct types, narrow flag leaves (FLW range from 1.45 to 1.75 cm, 38 families) and wide flag leaves (FLW range from 1.75 to 2.15 cm, 108 families), which fit an expected Mendelian ratio (1:3) for single locus segregation ($\chi^2 = 0.23$, $P > 0.5$). Progeny test of the 146 individuals resulted in further classification into three groups: uniform wide flag leaves (31 families), segregating width of flag leaves (77 families), and uniform narrow flag leaves (38 families) among 16 plants within each family. These three groups corresponded to the three genotypes N19-ZS, N19-ZI, and N19-IR, respectively, which also fits a Mendelian ratio (1:2:1) for single locus segregation ($\chi^2 = 1.19$, $P > 0.5$). Although the SPP and RVT of these 146 F_2 plants (self-progeny of the heterozygous BC_4F_3 plant) showed continuous and normal distributions (Fig. 1), the progeny tests confirmed that the 31 wide-flag-leaf families and the 38 narrow-flag-leaf families showed high and low RVT and SPP, respectively, whereas the other 77 families (progenies of heterozygous plants) showed variation in RVT and SPP.

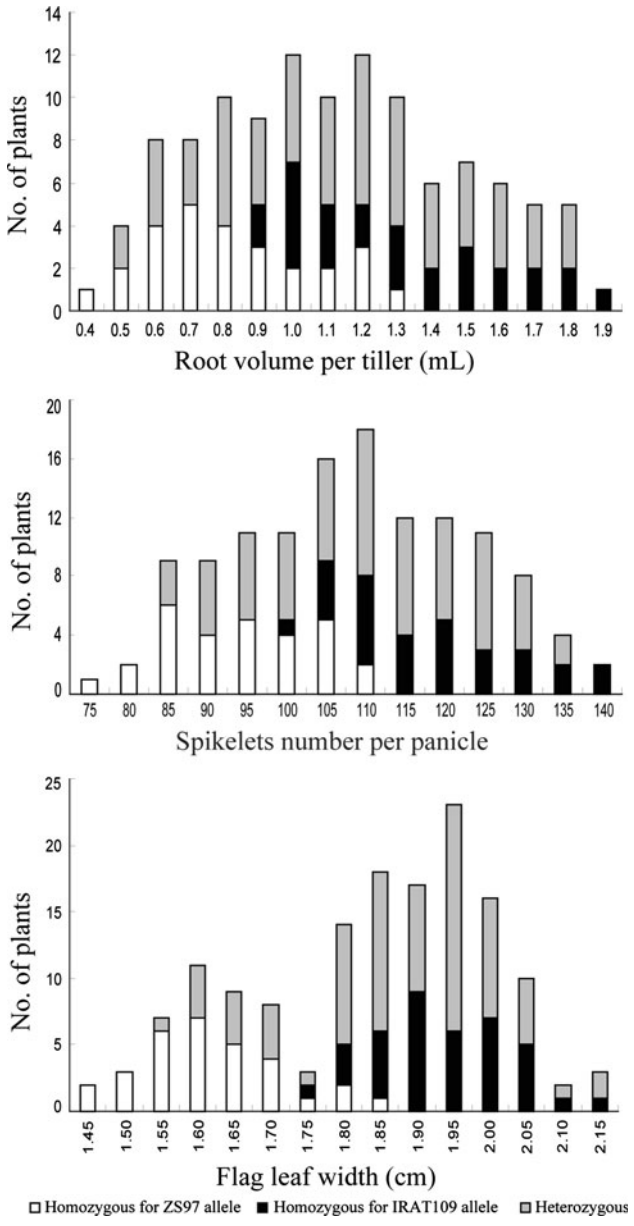

Fig. 1 Frequency distributions of the traits in the N19-BC_4F_4 population. *White*, *black*, and *gray bars* indicate the genotypes homozygous for ZS97 allele, homozygous for IRAT109 allele, and heterozygous, respectively. The three genotypes at the N19 target locus were inferred by progeny testing

Correlation analysis was performed for several traits including SF, SPP, KGW, RV, RVT, and FLW in the BC_4F_4 population of N19. Very significant positive correlations were observed among RVT, SPP, and FLW (Table 5). This result further supported the co-segregation of the three traits in the targeting interval of N19.

Fine mapping of N19-targeted QTL

Root volume is a very important trait related to drought avoidance, we initiated fine mapping of the QTL for root volume per tiller targeted by N19. This QTL also controls flag leaf width and spikelet number per panicle, and is therefore referred to as *qFSR4* hereafter. Because FLW co-segregated with RVT in the progeny test of N19 and strong correlation was observed between RVT and FLW, we used FLW as a marker trait to conduct a large scale of phenotyping for fine mapping of *qFSR4* because FLW can be easily scored.

For fine mapping of *qFSR4*, approximately 12,000 selfing progenies of BC_4F_3 plants heterozygous for the target region and having the least genetic background from IRAT109 (<5% of the markers were heterozygous) were grown. Only 1,295 plants showing the narrowest flag leaves (FLW $\leq$ 1.60 cm), which were assumed to be *qFSR4*-ZS genotype, were used for finding recombinants with SSR markers FSR-43 and RM17492. Seven and 25

Table 5 Correlation coefficients among several agronomic traits in BC_4F_4 population for N19

Traits	SPP	SF	KWG	RV	RVT
SF	0.31				
KWG	0.21	0.25			
RV	0.54**	0.18	0.34		
RVT	0.56**	0.24	0.37	0.80**	
FLW	0.53**	0.19	−0.02	0.49**	0.49**

** Significant at the level of $\alpha = 0.01$

recombinants were identified between FSR-43 and *qFSR4*, and RM17492 and *qFSR4*, respectively. Progeny tests of the 32 recombinants suggested that 20 recombinants showed the narrow flag leaves the same as the *qFSR4*-ZS homozygote, two recombinants showed the wide flag leaves the same as *qFSR4*-IR homozygote, and 10 recombinants showed the variation of FLW just like the *qFSR4*-ZI heterozygote (Table 6). The performances of RVT and SPP were consistent with FLW in the progeny tests for 32 recombinants (Table 6, Supplemental Table 3). Three SSR markers and four novel InDel markers (FSR-60, FSR-75, FSR-78, and FSR-81, Supplemental Table 1) were used to further delimit the *qFSR4* locus. Eight recombinants between RM17487 and *qFSR4*, three between FSR-60 and *qFSR4*, four between RM3423 and *qFSR4*, two between FSR-75 and *qFSR4*, two between FSR-81 and *qFSR4*, one between FSR-78 and *qFSR4* were identified, and RM17483 was shown to co-segregate with *qFSR4*

Table 6 Marker genotypes and phenotypes of recombinant plants

Individuals	Phenotype			Marker genotype							
	RVT	SPP	FLW	FSR-43	RM3423	FSR-75	RM17483	FSR-78	FSR-60	RM17487	RM17492
WHD10-10	Z	Z	Z	Z	Z	Z	Z	Z	Z	Z	H
WHD10-19	Z	Z	Z	Z	Z	Z	Z	Z	Z	Z	H
WHD10-43	Z	Z	Z	Z	Z	Z	Z	Z	Z	Z	H
WHD10-47	Z	Z	Z	Z	Z	Z	Z	Z	Z	Z	H
WHD10-50	Z	Z	Z	Z	Z	Z	Z	Z	Z	Z	H
WHD10-62	Z	Z	Z	Z	Z	Z	Z	Z	Z	Z	H
WHD10-65	Z	Z	Z	Z	Z	Z	Z	Z	Z	Z	I
WHD10-66	Z	Z	Z	Z	Z	Z	Z	Z	Z	Z	H
WHD10-38	Z	Z	Z	Z	Z	Z	Z	Z	Z	Z	H
WHD10-39	Z	Z	Z	Z	Z	Z	Z	Z	Z	Z	H
WHD10-40	Z	Z	Z	Z	Z	Z	Z	Z	Z	Z	H
WHD10-45	Z	Z	Z	Z	Z	Z	Z	Z	Z	Z	H
WHD10-63	Z	Z	Z	Z	Z	Z	Z	Z	Z	H	H
WHD10-20	Z	Z	Z	Z	Z	Z	Z	Z	Z	H	H
WHD10-44	Z	Z	Z	Z	Z	Z	Z	Z	Z	H	H
WHD10-36[a]	Z	Z	Z	Z	Z	Z	Z	Z	H	H	H
WHD10-67	Z	Z	Z	Z	Z	Z	Z	H	H	H	H
WHD10-181	I	I	I	I	I	I	I	I	H	H	H
WHD10-74	H	H	H	I	I	H	H	H	H	H	H
WHD10-77	H	H	H	I	H	H	H	H	H	H	H
WHD10-178	H	H	H	I	H	H	H	H	H	H	H
WHD10-1	H	H	H	H	H	H	H	H	H	H	I
WHD10-34	H	H	H	H	H	H	H	H	H	H	Z
WHD10-54	H	H	H	H	H	H	H	H	H	H	Z
WHD10-173	H	H	H	H	H	H	H	H	H	H	Z
WHD10-175	H	H	H	H	H	H	H	H	H	H	Z
WHD10-72	H	H	H	H	H	H	H	H	H	Z	Z
WHD10-73	H	H	H	H	H	H	H	H	H	Z	Z
WHD10-52	Z	Z	Z	H	H	H	Z	Z	Z	Z	Z
WHD10-2	Z	Z	Z	H	H	Z	Z	Z	Z	Z	Z
WHD10-78	Z	Z	Z	H	H	H	Z	Z	Z	Z	Z
WHD10-186	I	I	I	H	I	I	I	I	I	I	I
Number of recombination events				7	4	2	0	1	3	8	25

Z, I, and H stand for the homozygous for ZS97, homozygous for IRAT109 and heterozygote genotypes measured in BC_4F_4, respectively. Phenotypes were determined based on progeny test in BC_4F_5. WHD10 means that the experiment was done at Wuhan by Ding in 2010

[a] The trait values of the lines with recombination sites closest to the QTL (indicated in bold) are presented in Supplemental Table 3

(Fig. 2). Thus, the *qFSR4* locus was narrowed down to a region of approximately 38 kb flanked by FSR-75 and FSR-78.

The 38-kb region has three predicted genes according to the rice genome of Nipponbare automated annotation database (http://www.tigr.org/tdb/e2k1/osa1/irgsp.shtml) (Supplemental Table 4). All these predicted genes have matches with full-length cDNAs. Among the three genes, one was predicted to be retrotransposon gene, one was predicted to be trypsin-like serine and cysteine proteases, and one was predicted to be lecithine cholesterol acyltransferase (Supplemental Table 4). When the cDNA sequence of these candidate genes in TIGR database was compared with that in NCBI, one gene was of the same cDNA sequence, and the other two genes were different in encoding sequences as shown in Supplemental Table 4.

Since the *qFSR4* locus controls FLW along with the RVT and SPP, we checked the published genes for leaf width in rice and found that *NARROW LEAF 1* (*NAL1*), which encodes a novel protein and affects vein patterning and polar auxin transport (Qi et al. 2008), is located in the *qFSR4* interval. However, whether there is any phenotypic difference in SPP or RVT in the *nal1* mutant was not mentioned in that report. The *NAL1* is allelic to *LOC_Os04g52479* that is very close to the marker RM17483 that co-segregates with *qFSR4*. Considering the potential association of the three traits at the developmental level, we assume that *LOC_Os04g52479* may be the most promising candidate gene for FLW and also for SPP and RVT.

Discussion

Tangibility of QTL for drought resistance-related traits

Many important agronomic traits, especially stress resistance, have a complex genetic basis and are controlled by a large number of QTL. To understand the genetic and molecular mechanisms controlling such traits, QTL analyses have been undertaken in many plant species. However, many environmental factors affect the accuracy and repeatability of phenotyping of complex traits. Although some indices for drought resistance, including root trait, leaf rolling, water-use efficiency, osmotic adjustment, yield, and relative yield, have been used to detect QTL related to drought resistance, the stability of such QTL was very low under different conditions. For example, of the 36 genomic regions reported to harbor root trait-related QTL (Yue et al. 2006a), only four had positional correspondence with previously identified QTL for root or other drought avoidance-related traits (Courtois et al. 2009; Yonemaru et al. 2010).

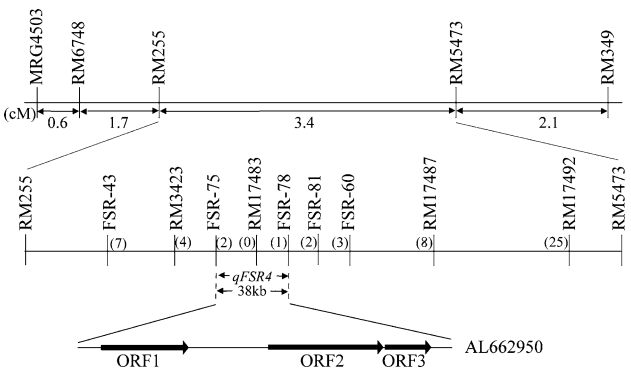

Fig. 2 High resolution genetic linkage and physical map of the N19-targeted region. *Numbers in brackets* on the right side of markers indicate the number of recombinants between *qFSR4* and the respective markers. *RM* SSR markers, *FSR* InDel markers

Because of the low reproducibility of detecting drought resistance-related QTL using RIL populations, the tangibility of these QTL was questionable. Previously, four intervals carrying QTL for root traits were targeted for generating introgression lines, but only the line targeting one of these QTL showed significantly increased root length (Steele et al. 2006). In this study, NILs were developed to examine 17 QTL identified previously (Yue 2005; Yue et al. 2006a; Zou et al. 2005). Six NILs targeting GY-related QTL showed significant phenotypic differences in the corresponding traits, and five NILs for root trait QTL showed significant phenotypic differences in the corresponding or similar traits under drought conditions. However, after the effect of heading date on drought resistance was eliminated, only four NILs for GY-related traits and three NILs for root traits showed phenotypes in agreement with the QTL identified in the original studies, and only the NILs N06 and N19 confirmed the original QTL for both GY-related and root-related traits. These results suggest that only a small proportion of the QTL for complex traits such as drought resistance can be confirmed by using NILs.

In this study, we observed that two NILs for drought resistance QTL showed significant differences between NIL-ZS and NIL-IR in the yield-related traits KGW (N06) and SPP (N19), respectively, under both normal and drought conditions. Significant differences in RV and RVT were also observed in both N06 and N19 under the PVC conditions. The N06-trageted QTL corresponds to a region controlling root penetration and maximum root length as reported by Yue et al. (2006a), and partly overlaps with a root penetration QTL identified in a different population (Steele et al. 2006). NIL was developed by Steele et al. (2006) to confirm the root penetration QTL, however, the NIL showed no significant effect on root trait but a slight phenotypic effect on both grain length and width. The target region of N19 corresponds to a region controlling both yield-related (SPP) and root-related (RVT) traits, and was therefore selected for further analysis in this study.

Pleiotropic QTL and possible candidate genes

In light of the low resolution of QTL mapping in primary populations, comparison of QTL detected in different populations often results in the finding that some chromosome regions are controlling or associated with many different traits, such as the N19-targeted region on chromosome 4. The regulation of plant development is accomplished through a complex regulation network, and changing one gene may result in changes of many different traits. Several QTL controlling multiple traits have been confirmed and the genes for some of them have been cloned in rice. For example, *GHD7*, isolated from Minghui 63 and encoding a CCT domain protein, is an important regulator of an array of traits in rice, including number of grains per panicle, PH, and heading date (Xue et al. 2008). *OsSPL14*, isolated by two independent groups, is regulated by OsmiR156 and defines plant architecture (number of tillers and plant height) and promotes panicle branching and higher grain productivity in rice (Jiao et al. 2010; Miura et al. 2010).

In this study, the N19-targeted QTL had a significant effect simultaneously on FLW, SPP, and RVT. Interestingly, QTL controlling SPP and FLW were detected in this interval in an RIL population derived from a cross between Milyang 23 and Akihikari (Kobayashi et al. 2003; Kobayashi et al. 2004). Yue et al. (2006a, b) also detected QTL controlling root-related traits, FLW, and SPP in the N19-targeted interval by using the ZS97/IRAT109 RIL population. A FLW QTL, *qFLWnpt-4*, was identified and it is located around RM17483 (Farooq et al. 2010), and this QTL overlaps with the interval of *qFSR4*. Together with our results from N19, this evidence supports that N19-targeted interval contains tightly linked, if not the same, QTL controlling RVT, SPP, and FLW.

Through fine mapping using FLW as an indicator trait, the N19-targeted QTL containing *qFSR4* was narrowed down to a 38-kb region containing 3 predicted genes. Although this region can be further narrowed by increasing the size of population for screening additional recombinants with new markers, the candidate gene(s) of *qFSR4* could be determined through exploitation of bioinformatics information just like the cloning process of *GHD7*, which was cloned by scanning a few hundred predicted genes in the interval and finding only one protein containing a CCT domain with the highest candidacy (Xue et al. 2008). To prioritize the candidate genes, we first checked whether any reported genes related to development in the *qFSR4* interval and noticed that the *NAL1* gene shows an almost identical sequence *LOC_Os04g52479*. Mutants of *NAL1* exhibit narrow leaves and reduce plant height (Qi et al. 2008). LOC_Os04g52479 was predicted to be a trypsin-like serine and cysteine protease and is closely linked with the marker RM17483 which co-segregates with *qFSR4*. NAL1 was identified as a novel protein affecting vein patterning and polar auxin transport (Qi et al. 2008). It is well known that auxin promotes cell division and growth and plays an important role in growth and development of plants. Synthesis and transport of auxin is also crucial for initiation and development of many organs, including root, leaf, and flower (Friml et al. 2004; Zhao 2010; Zhao et al. 2001). Taken together, we propose that *LOC_Os04g52479* is the most promising candidate gene for FLW and most likely affects RVT and SPP as well. Functional analysis of this gene (and the other two candidates), which is ongoing, will finally unveil the molecular basis of the *qFSR4*.

We also tried to conduct fine mapping for a few other QTL (such as those targeted by N06, N29, and N38) effective for multiple traits. None of the attempts advanced smoothly because of the same problem, that is, it is extremely difficult to screen thousands of individuals or families with adequate accuracy of phenotyping by directly using the drought resistance-related traits. For the QTL without closely linked and easily scored marker traits (such as FLW), fine mapping would require a well-controlled environment and large amounts of space.

Potential exploitation of yield and drought resistance improvement in rice

Recently, several rice genes controlling GY or its component traits have been cloned by different research groups in rice (reviewed by Xing and Zhang 2010). These genes may be exploited for improving rice yield and or grain shape-related quality. However, many challenges must be overcome to achieve the goal of increasing rice production, including diverse abiotic stresses (Zhang 2007). Because of the reduction of available water resources and unpredictable meteorological events, drought stress has become one of the major limiting factors for rice production. In this study, a root volume-related QTL *qFSR4* was selected for fine-mapping because this trait itself is very important for drought resistance and this QTL interval also has significant effects on FLW and SPP. A large and deep root system has been considered a basic mechanism for drought resistance of plants because it allows absorption of water from deep soil layers (Price and Courtois 1999). A large flag leaf area may be beneficial to increase photosynthetic assimilation efficiency and large root volume will be helpful for nutrition absorption. Through manipulation of *qFSR4* or the gene(s) of this locus, rice yield might be increased by simultaneously increasing both source (RVT and FLW) and sink (SPP).

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