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Identification of quantitative trait loci associated with drought tolerance traits in rice (*Oryza sativa* L.) under PEG and field drought stress

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Abstract Two recombinant inbred line F_{10} rice populations (IAPAR-9/Akihikari and IAPAR-9/Liaoyan241) were used to identify quantitative trait loci (QTLs) for ten drought tolerance traits at the budding and early seedling stage under polyethylene glycol-induced drought stress, and two traits of leaf rolling index (LRI) and leaf withering degree (LWD) under field drought stress. The results showed that the drought-tolerance capacity of IAPAR-9 was stronger than that of Akihikari and Liaoyan241. Thirty-four

QTLs for 12 drought tolerance traits were detected, and among them, in the IAPAR-9/Akihikari population, *qLRI9-1* and *qLRI10-1* for LRI were repeatedly detected in RM3600-RM553 on chromosome 9 and in RM6100-RM3773 on chromosome 10, respectively, at two times points of July 31 and August 13 in 2014. The two QTLs are stable against the environmental impact, and *qLRI9-1* and *qLRI10-1* explained 6.77–13.66% and 5.01–8.32% of the phenotypic variance, respectively, at the two times points. *qLWD9-2* for LWD in the IAPAR-9/Liaoyan241 population contributed 8.73% of variation was detected in the same marker interval with the *qLRI9-1*, and *qLRI1-1* for LRI and *qLWD1-1* for LWD were located in the same marker interval RM11054-RM5646 on chromosome 1, which contributed 18.82 and 5.78% of phenotype variation respectively. *qGV3*

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for germination vigor and *qRGV3* for relative germination vigor at the budding stage were detected in the same marker interval RM426-RM570 on chromosome 3, which explained 14.98 and 16.30% of the observed phenotypic variation respectively, representing major QTLs. The above-mentioned stable or major QTLs regions could be useful for molecular marker assisted selection breeding, fine mapping, and cloning.

Keywords Rice · PEG and field drought stress · Quantitative trait locus · Simple sequence repeat · Recombinant inbred line

Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops and provides food to over half of the population worldwide. Drought stress results from environment changes, water shortage, and population expansion worldwide, and severely influences rice crop fertility and yield stability (Basu et al. 2016; Zhou et al. 2016a, b; Hu and Xiong 2014; Suji et al. 2012). To secure a sustained and steady supply of food, it is necessary to carry out drought stress tolerance studies in crops (Kudo et al. 2017). Exploring drought-tolerance genes and breeding drought-tolerant varieties is critical to solve the drought problem.

Plants have evolved an array of resistance strategies to confront drought stress (Basu et al. 2016). For example, root architecture and leaf traits in crops are vital to resist adverse environments (drought, cold, and salt) (Lee et al. 2017; Sandhu et al. 2016; Li et al. 2016; Zhang et al. 2015; Yang et al. 2014). Roots can absorb water and nutrients, detect soil information, and perceive and pass drought stress signals to the rest of the plant, which initiate morphological, physiological, biochemical, and molecular responses (Agrawal et al. 2016; Moumeni et al. 2011). Upon drought perception, plants undergo morphological modifications, such as root length, root number, and seedling height (Lee et al. 2017; Uga et al. 2013; Jeong et al. 2013; Redillas et al. 2012; Mao et al. 2012), Root length, root number, and seedling height are very important drought-tolerance indexes among all the root morphology characteristics (Lee et al. 2017; Courtois et al. 2009). Liu et al. (2013) used F$_{2:3}$ families to show that root number and seedling height

were inhibited to some extent, while root length (RL) increased slightly under polyethylene glycol (PEG)-6000 stress in rice. In addition, the responses of seed germination vigor and coleoptiles length under abiotic stress have been studied. Liu et al. (2013) found the coleoptiles length was inhibited in rice under PEG-6000 stress, and Hu et al. (2006) demonstrated that the coleoptiles length trait is correlated positively with the drought-tolerance index under low water potential stress in rice. Plant leaves can determine the efficiency and intensity of photosynthesis and transpiration, and can rapidly respond to environmental changes (drought, salt, and cold). Leaf rolling is considered as a defensive measure to confront adverse conditions by decreasing water loss in plants (Zhang et al. 2015; Kadioglu et al. 2012; Kadioglu and Terzi 2007). In drought-tolerance studies, the leaf rolling index (LRI) and leaf withering degree (LWD) are regarded as the most important drought tolerance criteria at all stages of rice growth (Yang et al. 2016; Li et al. 2016; Zhang et al. 2015; Richards et al. 2002). Xiao et al. (2012) examined leaf rolling and leaf dying among different varieties, and found the LRI and LWD could reflect drought-tolerance capacity, which can be used as an evaluation index in drought stress research.

Many quantitative trait loci (QTLs) related to root and leaf morphology traits have been identified. QTLs associated with rice root system architectures can be directly used to improve both water and fertilizer use efficiency in rice and enhance biomass and yields. For instance, Zhou et al. (2016b) identified 38 root morphology QTLs using sequencing-based chromosome segment substitution lines and found that qMRL11.1 for maximum root length (MRL) was located on the same chromosome segment with the QTL that controlled shoot biomass detected by Kamoshita et al. (2002). Obara et al. (2010) identified a major QTL for root length under drought conditions and found that QTL qRL6.1 can greatly facilitate root lengthening under different NH4$^+$ concentrations, which enhances rice yield in molecular breeding programs. Uga et al. (2011) mined a major QTL, *Dro1*, for the ratio of deep rooting (RDR) and found that *Dro1* gene significantly increased RDR, which is an important strategy for avoiding drought stress in rice under upland field condition. Salunkhe et al. (2011) detected QTLs on chromosome 1 for the LRI and LWD through bulk segregation analysis (BSA) of extreme phenotypes in the recombinant inbred lines

(RILs). These QTL are essential for improving drought tolerance and maintaining stability of rice yields. Yue et al. (2006) examined QTLs for leaf rolling and leaf drying score under two different environments and found that: four QTLs for leaf rolling overlapped with QTLs for root traits; and one QTL for leaf-drying overlapped with the QTL for relative spikelet number that affects deep-root rate. Price et al. (2002) used a Bala/Azucena population to examine QTLs for LRI and LWD and found one QTL reducing leaf rolling from Bala and five QTLs reducing leaf dying come Azucean. These QTLs for LRI and LWD have been associated with drought tolerance, which can be used as the target QTLs in drought stress research.

Through many quantitative trait loci (QTLs) related to root and leaf morphology traits have been identified, to further exploring the genetic foundation of rice root and leaf development and identified available genes associated with root and leaf traits under drought-stress condition, we constructed two RIL populations under hydroponic conditions to identify QTLs for key measures of root length, root number, seeding height, germination vigor, coleoptiles length, leaf rolling trait and leaf drying traits, and to test their association with drought stress. These can provide a valuable contribution to the genetic analysis of root and leaf traits and theoretical foundation for marker-assisted breeding for stress tolerance in rice.

Materials and methods

Mapping population

Two recombinant inbred line (RIL) populations were used in this study, which were developed using the single seed descent (SSD) method. One F10 RIL population, containing 231 lines, was derived from the cross between two rice varieties IAPAR-9 and Akihikari, and the other F10 RIL population, containing 228 lines, was derived from the cross between rice varieties IAPAR-9 and Liaoyan241. IAPAR-9 is a tropical *Japonica* upland rice variety from Brazil, known for its drought tolerance ability through a strong root system and leaf structure (Sheng et al. 2000). Akihikari is a *Japonica* lowland rice variety from Japan, and Liaoyan241 is a *Japonica* lowland rice variety from the institute of saline-alkali land use

of Liaoning Province, China (Xu et al. 1997). The leaf DNA of the parents and the two RIL populations were extracted using the modified cetyltrimethyl ammonium bromide (CTAB) method (John 1992) and simple sequence repeat (SSR) marker analysis was carried out using 8% polyacrylamide gel electrophoresis. The phenotypes of the two RILs and the SSR makers were used to construct a linkage map and to conduct the QTL studies.

Hydroponic trials under PEG stress

Polyethylene glycol (PEG)-simulated drought stress trials were conducted in the rice germplasm resources experimental laboratory of the Chinese Academy of Agricultural Sciences (CAAS) in 2014. PEG-6000 (20%) was used to treat the two RIL populations and their parents. Thirty grains of plump seeds from each strain were selected and disinfected for 20 min using 0.1% NaClO solution, washed three times using distilled water, and placed in 7.5 cm-diameter petri dishes covered by two layers of filter paper at the bottom. The treatment and control groups comprised two replicates. The treatment groups were treated using 10 mL of 20% PEG-6000 solution, the control groups were treated with 10 mL of distilled water. Lids were placed on the treatment and control group petri dishes, which were incubated at 25–30 °C; the PEG and distilled water were replaced daily. After 4 days, the germination vigor was recorded, and after 6 days, the root length, root number, seeding height, and coleoptiles length were determined in the control and treatment groups.

Field drought stress trials

Field drought stress trials were carried out in the experimental fields of the Ningxia Academy of Agriculture and Forestry Sciences (NAAFS) in 2014. The two RIL populations and their parents were sown on April 12, and transplanted on May 17. The transplanting standard was 26.7 cm × 13.3 cm with two lines; the row length was 1.86 m, and 15 seedlings were transplanted in each row with two replications. Water irrigation was stopped on June 25 (38 days after transplanting) at the tillering stage and drought stress treatment commenced. Other cultivation management, such as extermination of disease and insect pest, and weed out, followed general field production

methods. On July 10, the field had lost sufficient water for the earth to be visible. From the 17 of July, the LRI and LWD traits were recorded once every 7 days. From July 17 to September 24, eight LRI records and nine LWD trait records were measured at 11:00–12:00 am. The classification and evaluation standards were in accordance with Zhou et al. (2006) (Supplementary Tables S1 and S2).

Measure of phenotypes

Plants were sampled at the budding stage in the simulated trials and at the seedling stage in the field trails. In the PEG-simulated trials, ten traits including germination vigor (GV), relative germination vigor (RGV), root number (RN), relative root number (RRN), root length (RL), relative root length (RRL), seedling height (SH), relative seedling height (RSH), coleoptile length (CL), and relative coleoptile length (RCL) were evaluated in the PEG treatment and control groups. Five random samples were measured and the average value was regard as a statistical unit. In the field trails, two traits, LRI and LWD were evaluated. For LRI and LWD, we used the two data points attained when the stress effect was the most obvious as the statistical unit. Analysis of variance (ANOVA) was performed to estimate the genetic variation for all the traits among the RIL lines. The frequency distributions of all the traits were calculated to test the skewness of the traits towards the parents. Pearson correlation was computed among the traits using the trait mean value.

Genotyping and linkage map

SSR markers distributed throughout the genome were screened for polymorphisms between the parents IAPAR-9 and Akihikari, IAPAR-9 and Liaoyan241. The amplified PCR products of these markers were checked using 8% polyacrylamide gel electrophoresis. A marker that was the same as that of the female IAPAR-9 was recorded as "2"; a marker that was the same as that of the male Akihikari and Liaoyan241 was recorded as "0"; missing makers were recorded as "− 1". The segregation of each marker was scored after genotyping 231 RILs of IAPAR-9/Akihikari and 228 RILs of IAPAR-9/Liaoyan241. Linkage analysis was performed using the IciMapping 4.0 software using the Kosambi mapping function. Markers were

placed into linkage groups using the group command, with a minimum logarithm of the odds (LOD) score of 3.0 and a maximum recombination fraction of 0.5 (Wang 2009; Li et al. 2008; Kosambi et al. 1994). We constructed one linkage map using 178 SSR (Supplementary Table 6) markers in IAPAR-9/Akihikari RIL population; meanwhile, we constructed another linkage map using 174 SSR (Supplementary Table 7) makers in IAPAR-9/Liaoyan241 RIL population. These linkage maps were used for the QTL analysis.

QTL analysis

QTLs were identified and validated by inclusive composite interval mapping (ICIM), using IciMapping 4.0. Stepwise regression was applied to identify the most significant regression variables. One-dimensional scanning or interval mapping were conducted to detect additive QTLs, and a two-dimensional scanning was conducted to detect digenic epistasis. The minimal LOD value required to declare a QTL was obtained empirically from 1000 permutation tests, with a walk speed of 1 cM. A LOD threshold of 3.0 in this population and an experiment-wide significance level of 0.05 were set. The QTL naming convention was according to the method of McCouch et al. (1997).

Results

Phenotypic evaluation under PEG stress

Under 20% PEG-6000 stress, the 10 phenotypic traits of the parents and two RIL populations were all inhibited to some extent. The data of the two RIL populations showed a normal, or approximately normal, distribution (Fig. 1; Supplementary Table S3). Phenotypic differences among the parents and the range of variation of the two RIL populations were evident. The GV (73.33%), CL (0.64 cm), RN (2.80), RL (3.58 cm) and SH (1.22 cm) values of IAPAR-9 were all apparently higher than those of Akihikari (43%, 0.62 cm, 1.40, 2.64 cm and 1.10 cm) and Liaoyan241 (20%, 0.60 cm, 1.30, 1.85 cm and 0.68 cm). Among them, the GV, RN, and RL values of the parents showed significant differences. The GV value of IAPAR-9 was higher (70.4–266.5%) than that of Akihikari and Liaoyan241. The RN value of IAPAR-9 was 1–1.15 times than that of Akihikari
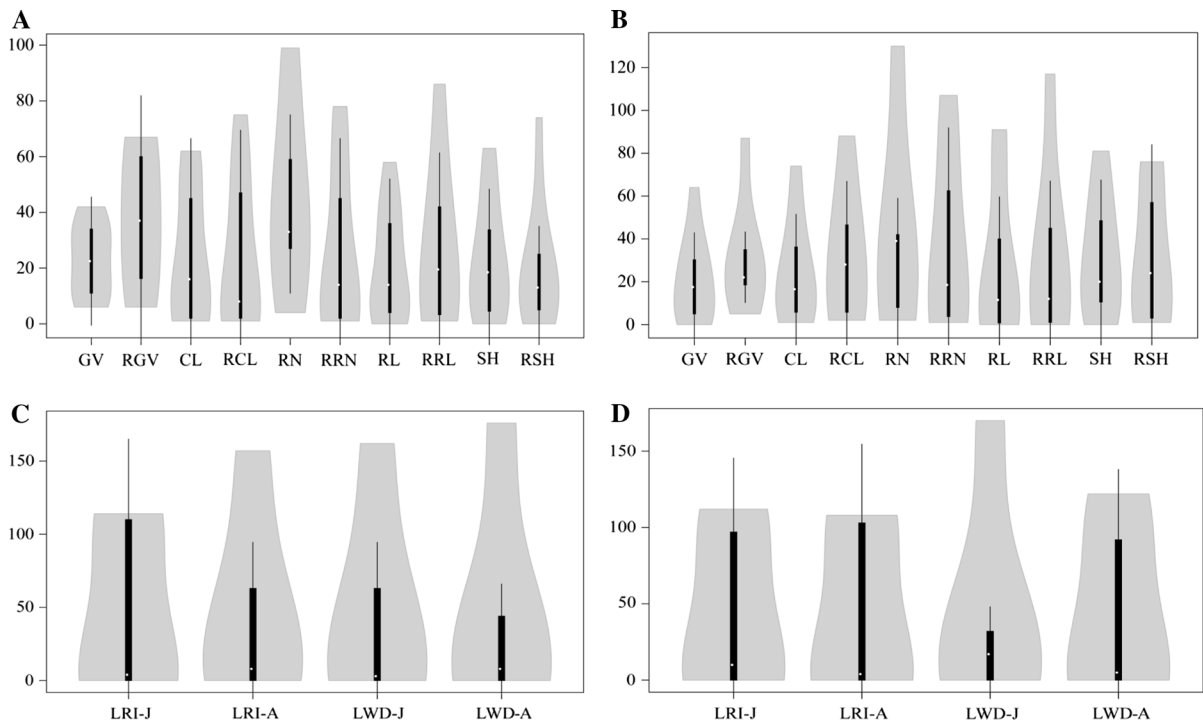
Fig. 1 Violin plots of frequency distributions for each trait in the recombinant inbred line (RIL) populations (Spitzer et al. 2014). **A** Distribution of the number of lines for drought tolerance traits for the IAPAR-9/Akihikari RIL population under polyethylene glycol (PEG)-simulated drought stress. **B** Distribution of the number of lines for drought resistance traits for the IAPAR-9/Liaoyan241 RIL population under PEG-simulated drought stress. **C** Distribution of the number of lines for leaf rolling degree and leaf withering degree in the IAPAR-9/ Akihikari RIL population under the field drought stress. **D** Distribution of the number of lines for leaf rolling degree and leaf withering degree in the IAPAR-9/Liaoyan241 RIL population under the field drought stress. (LRI-J, LWD-J: the data from July 31, 2014, LRI-A, LWD-A: the data from August 13, 2104)

and Liaoyan241. The RL value increased by 35.6–93.5% compared with than that of Akihikari and Liaoyan241. The CL and SH values of the parents showed little difference. The CL value of IAPAR-9 was only higher (3.22–6.67%) than that of Akihikari and Liaoyan241, and the SH was only higher 10.9% than that of Akihikari. According to the relative phenotypic value, the RGV (83%) value of IAPAR-9 was apparently higher than that of Akihikari (46%) and Liaoyan241 (24%); the other values showed only slight differences. The coefficient of variation of GV and RGV were 53.66 and 54.17% in the IAPAR-9/ Akihikari population, respectively, while the coefficients of variation of GV and RGV had maximum values of 82.16 and 82.41%, respectively, in the IAPAR-9/Liaoyan241 population, which suggested that GV and RGV showed higher genetic variation. However, the coefficient of variation of CL and RCL had minimum values of 25.71 and 26.12%,

respectively, in the IAPAR-9/Akihikari population, while the coefficient of variation of CL and RCL had minimum values of 14.12 and 20.20%, respectively, in the IAPAR-9/Liaoyan241 population, which suggested that CL and RCL showed lower genetic variation. The means of the phenotypic values of most budding stage traits were closer to those of the male parent IAPAR-9 (Supplementary Table S3).

Evaluation of drought tolerance under field drought stress

Under field drought stress, data for the LRI and LWD in the two populations were attained at the high stress intensity time points of July 31 and August 13 2014. The phenotypic traits in the two RIL populations showed a normal, or approximately normal, distribution (Fig. 1; Supplementary Table S3). The LRI values of IAPAR-9 were all 5 degrees and those of

the parents Akihikari and Liaoyan241 were all 7 degrees on July 31 and August 13 in 2014, while the LWD values were all 3 and 5 degrees for the IAPAR-9 when investigated on July 31 and August 13 of 2014, respectively, and those of the parents Akihikari and Liaoyan241 were 5 and 7 degree when investigated on July 31 and August 13 of 2014, respectively (Supplementary Table S3). The above results suggested that the drought tolerance capacity of IAPAR-9 is stronger than that of parents Akihikari and Liaoyan241. In the IAPAR-9/Akihikari population, the variation range of LRI and LWD values investigated on July 31 were 5–9 and 1–5 degrees, while the variation range of LRI and LWD values investigated on August 13 were 5–9 and 3–7 degree, respectively. In the IAPAR-9/Liaoyan241 population, the variation range of LRI and LWD values investigated on July 31 were 3–7 and 1–5 degree, respectively, while the variation range of LRI and LWD values investigated on August 13 were 5–9 and 3–7 degree, respectively (Supplementary Table S3), which suggested that the influence of drought stress on August 13 was stronger than that on July 31. The above results showed that with increasing of drought stress time, the degree of drought stress damage became more serious, and the LRI and LWD gradually increased.

Genetic map construction

We chose SSR markers to explore polymorphisms among parents IAPAR-9, Akihikari, and Liaoyan241. Most SSR markers in the two populations were verified to conform to Mendelian inheritance with regard to the χ^2-test; a few markers were skewed to one of the parents. Lower recombination and miscarriage of gametes and zygotes can cause segregation distortions. In the RIL populations, segregation distortion can be relevant to the environment and artificial selection from one generation to another. For co-dominant markers, skewed markers have little effect upon assessment of the recombination rate. Therefore, the skewed markers were used to for the QTL analysis. Finally, 178 markers in the IAPAR-9/Akihikari population and 174 SSR markers in the IAPAR-9/Liaoyan241 population were used to construct the linkage map. The 178 SSR markers (Supplementary Table S6) were even distributed on the 12 chromosomes and comprised a total length 2353.45 cM, at an average distance of 13.22 cM. In

the IAPAR-9/Liaoyan241 population, the 174 SSR markers (Supplementary Table S7) were evenly distributed on the 12 chromosomes, with a total length of 2345.08 cM at an average distance of 13.47 cM (Figs. 2 and 3). The high level of linkage information might have an important role in identifying useful markers for rice mutation-assisted breeding (MAB).

QTLs identification for all traits

Under PEG-6000 simulated drought stress (Table 1; Figs. 2 and 3), in the IAPAR-9/Akihikari population, 10 QTLs for traits associated with drought tolerance at the budding and early seedling stage were detected on chromosomes 1, 3, 5, 9, and 11, which explained 5.2–18.5% of the observed phenotypic variation. Among them, QTL *qRSH1* controlling RSH, was detected in interval RM265-RM3482 on chromosome 1, which led to the largest effect and explained 18.28% of the observed phenotypic variation, representing a major QTL. In addition, *qSH5*, controlling SH, and *qRSH5*, controlling RSH, were found in intervals RM2010-RM267 and RM7118-RM430 on chromosome 5, respectively, which explained 15.61 and 14.38% of the observed phenotypic variation, respectively, representing two major QTLs. QTL *qGV3*, associated with GV, and *qRGV3*, related to RGV, were detected in interval RM426-RM570 on chromosome 3, which explained 14.98 and 16.30% of the observed phenotypic variation, respectively, representing two major QTLs. The additive alleles of these five major QTLs all originated from IAPAR-9 (Table 1). The remaining five QTLs contributed 5.20–9.37% of the observed phenotypic variation and were distributed on chromosomes 1, 9, and 11. Under PEG-6000 simulated drought stress (Table 1; Figs. 2 and 3), in the IAPAR-9/Liaoyan241 population, 10 QTLs for traits associated with drought tolerance at the budding stage and early seedling stage were found on chromosomes 1, 3, 4, 9, and 11, which explained 7.25–12.38% of the observed phenotypic variation. Among them, *qSH9* and *qSH11*, controlling SH, were found in intervals RM24424-RM3600 on chromosome 9 and RM260-RM441 on chromosome 11, respectively, which explained 12.38% of the same observed phenotypic variation. Besides, QTLs *qRL3*, associated with RL, and *qSH3*, related to SH, were detected in interval RM15072-RM15164 on chromosome 3, which explained 11.12 and 10.71% of the observed
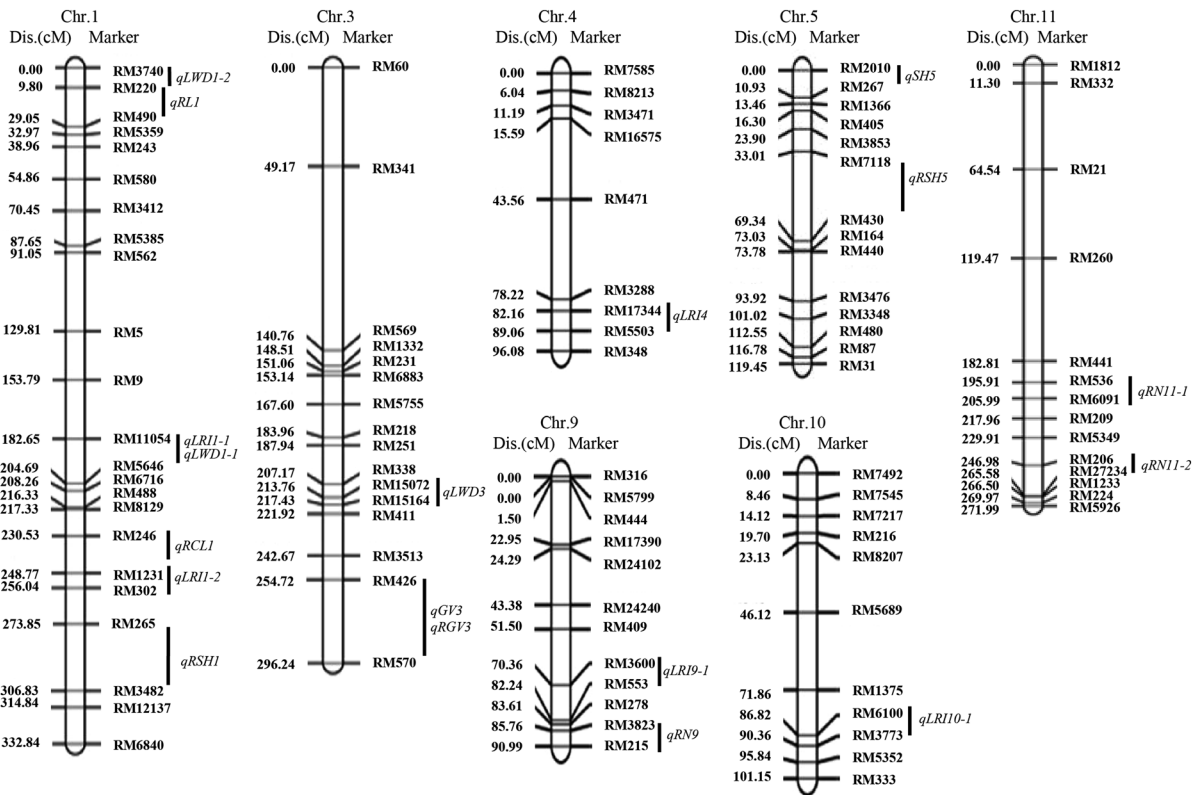
Fig. 2 Quantitiative trait locus (QTL) analysis of budding and early seedling stage traits and leaf rolling index (LRI) and leaf withering degree (LWD) for IAPAR-9/Akihikari RIL populations under PEG and field drought stress. The horizontal black short lines on the left and right of each chromosome indicate to the relative genetic position and marker of each QTL, respectively; the vertical black bold short lines on the right of each chromosome represent the identified QTLs

phenotypic variation, respectively. The GV-associated QTL *qGV4* was found in interval RM3471-RM16575 on chromosome 4, which explained 10.23% of the observed phenotypic variation. The five QTLs are major QTLs whose additive alleles originated from IAPAR-9. The other five QTLs contributed 7.10–9.48% of the observed phenotypic variation and were distributed on chromosomes 1, 3, 4, and 9.

Under the field drought stress (Table 2; Figs. 2 and 3), in the IAPAR-9/Akihikari population, eight QTLs for LRI and LWD investigated on July 31 and August 13 of 2014 were found, which were located on chromosomes 1, 3, 4, 9, and 10, and explained 4.32–18.82% of the observed phenotypic variation. Among them, QTL *qLRI1-1*, controlling LRI, was detected in interval RM11054-RM5646 on chromosome 1, which led to the largest effect and explained 18.82% of the observed phenotypic variation, representing a major QTL. In addition, *qLRI9-1*, controlling

LRI, was detected repeatedly in interval RM3600-RM553 on chromosome 9 on July 31 and August 13 in 2014, which explained 6.77–13.66% of the observed phenotypic variation. The LRI-associated QTL, *qLRI10-1* was detected repeatedly in interval RM6100-RM3773 on chromosome 10 on July 31 and August 13 in 2014, which explained 5.01–8.32% of the observed phenotypic variation. QTLs *qLRI9-1* and *qLRI10-1* were stable: *qLRI9-1* is a major QTL and *qLRI10-1* is micro effect QTL. The LWD-associated QTL *qLWD3* was detected in interval RM15072-RM15164 on chromosome 3, which explained 10.73% of the observed phenotypic variation, representing is a major QTL. The additive alleles of *qLRI1-1*, *qLRI9*-1, and *qLRI10-1* originated from IAPAR-9, while the additive alleles of *qLWD3* originated from Akihikari (Table 2). The remaining four QTLs contributed 4.32–8.23% of observed phenotypic variation and were distributed on
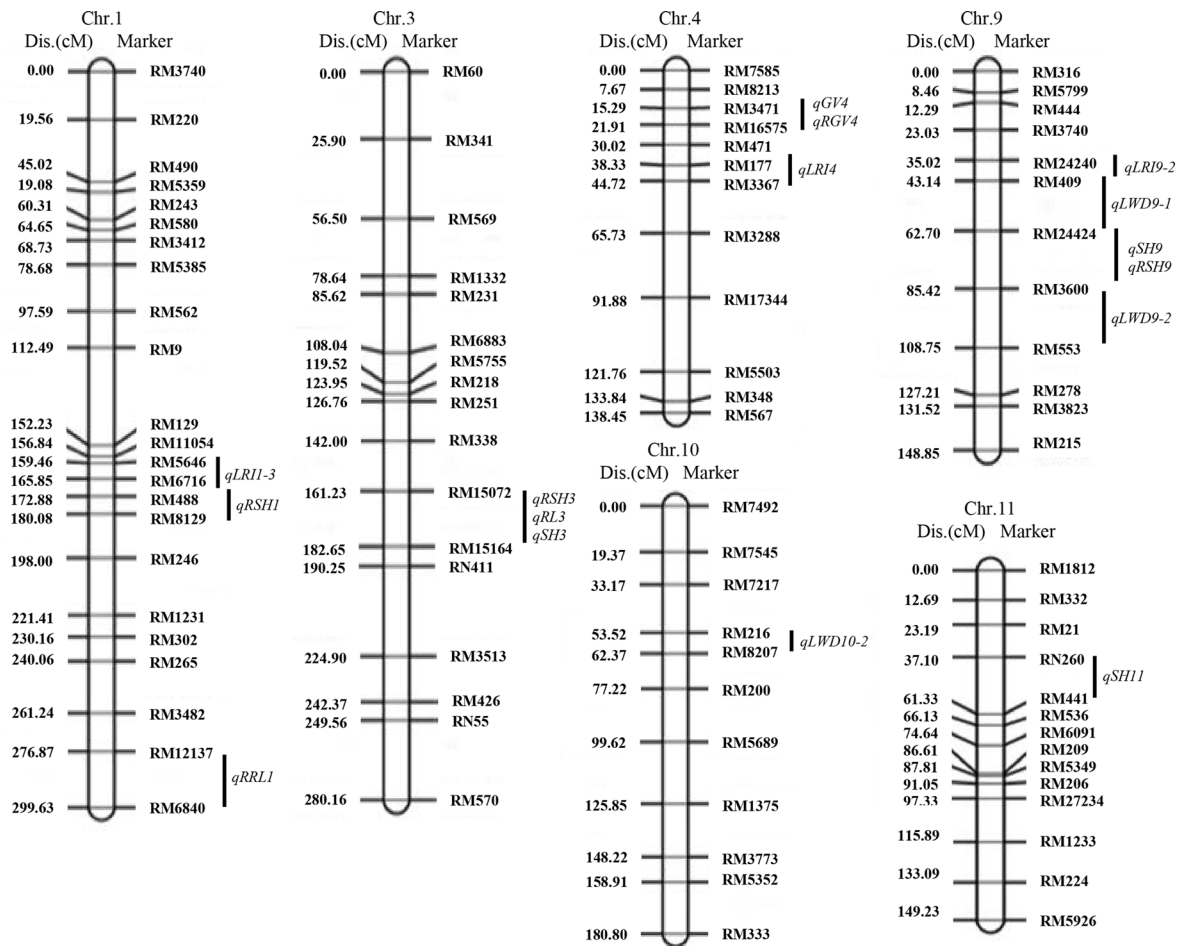
Fig. 3 Quantitiative trait locus (QTL) analysis of budding and early seedling stage traits and leaf rolling index (LRI) and leaf withering degree (LWD) for IRPAR-9/Liaoyan241 RIL populations under PEG and field drought stress. The horizontal black short lines on the left and right of each chromosome indicate the relative genetic position and marker of each QTL, respectively; the vertical black bold short lines on the right of each chromosome represent the identified QTLs

chromosomes 1 and 4. Under field drought stress (Table 2; Figs. 2 and 3), in the IAPAR-9/Liaoyan241 population, six QTLs associated with LRI and LWD investigated on July 31 and August 13 of 2014 were detected, which were located on chromosomes 1, 4, 9, and 10 and explained 6.89–16.12% of the observed phenotypic variation. Among them, QTL *qLRI9-2*, controlling LRI, was detected in interval RM24240-RM409 on chromosome 9, which led to the largest effect and explained 16.12% of the observed phenotypic variation, representing a major QTL. The additive alleles of *qLRI9-2* originated from IAPAR-9 (Table 2). The other five QTLs contributed 6.89–8.73% of the observed phenotypic variation.

Co-location of QTLs

According to the QTL analysis (Tables 1 and 2), two co-located QTLs were repeatedly found on chromosome 9 and 10 on July 31 and August 13 of 2014, respectively, and six co-located QTLs were detected on chromosomes 1, 3, 4, and 9 using the two populations under two environments. Mostly acting as main effect QTLs, the additive alleles of these QTLs originated from the female parent IAPAR-9. Under PEG-6000 simulated stress, in the IAPAR-9/ Akihikari population, QTLs *qGV3* and *qRGV3* were co-located in interval RM426-RM570 on chromosome 3. In the IAPAR-9/Liaoyan241 population, three co-

Table 1 QTLs and their genetic effects for some traits associated with drought resistant in IAPAR-9/Akihikari and IAPAR-9/Liaoyan241 RILs under PEG-6000-simulated drought stress at the budding and early seedling stage

Traits	Locus	Chr.	Location (cM)	Marker interval	LOD score	PVE (%)	Add	Source of allele
(1) IAPAR-9/Akihikari RILs								
GV	qGV3	3	271	RM426-RM570	2.54	14.98	8.09	IAPAR-9
RN	qRN9	9	87	RM3823-RM215	4.66	9.25	0.26	IAPAR-9
	qRN11-1	11	201	RM536-RM6091	3.05	7.03	− 0.23	Akihikari
	qRN11-2	11	262	RM206-RM27234	2.70	6.00	− 0.21	Akihikari
RL	qRL1	1	10	RM220-RM490	2.54	5.20	0.42	IAPAR-9
SH	qSH5	5	50	RM2010-RM267	2.57	15.61	0.23	IAPAR-9
RGV	qRGV3	3	272	RM426-RM570	2.63	16.30	8.97	IAPAR-9
RCL	qRCL1	1	243	RM246-RM1231	2.85	9.37	10.82	IAPAR-9
RSH	qRSH1	1	294	RM265-RM3482	3.34	18.28	8.43	IAPAR-9
	qRSH5	5	50	RM7118-RM430	2.69	14.38	7.39	IAPAR-9
(2) IAPAR-9/Liaoyan241 RILs								
GV	qGV4	4	17	RM3471-RM16575	4.60	10.23	5.32	IAPAR-9
RL	qRL3	3	168	RM15072-RM15164	3.47	11.12	0.58	IAPAR-9
SH	qSH3	3	168	RM15072-RM15164	3.90	10.71	0.21	IAPAR-9
	qSH9	9	68	RM24424-RM3600	4.92	12.38	0.23	IAPAR-9
	qSH11	11	49	RM260-RM441	3.32	12.38	0.24	IAPAR-9
RGV	qRGV4	4	17	RM3471-RM16575	4.27	9.48	5.37	IAPAR-9
RRL	qRRL1	1	299	RM12137-RM6840	3.03	7.10	− 5.08	Liaoyan241
RSH	qRSH1	1	176	RM488-RM8129	3.78	7.25	3.01	IAPAR-9
	qRSH3	3	168	RM15072-RM15164	3.08	7.81	3.44	IAPAR-9
	qRSH9	9	68	RM24424-RM3600	3.96	9.37	3.34	IAPAR-9

GV germination vigor, *RN* root number, *RGV* relative germination vigor, *RL* root length, *SH* seedling height, *RCL* relative coleoptile length, *RSH* relative seedling height, *LOD* logarithm of odds, *PVE* percent variance explained

located QTLs were found: *qGV4* and *qRGV4* were located in interval RM3471-RM16575 on chromosome 4; *qRL3*, *qSH3*, and *qRSH3* were co-located in interval RM15072-RM15164 on chromosome 3; and *qSH9* and *qRSH9* were located in interval RM24424-RM3600 on chromosome 9.

Under field drought stress, QTL *qLRI9-1* detected repeatedly in interval RM3600-RM553 on chromosome 9 on July 31 and August 13 of 2014 in the IAPAR-9/Akihikari population, and was co-located in the same marker interval with QTL *qLWD9-2* in the IAPAR-9/Liaoyan241 population. QTL *qLRI10-1* was detected repeatedly in interval RM6100-RM3773 on chromosome 10 on July 31 and August 13 of 2014, but explained a smaller phenotypic variation than that of QTL *qLRI9-1*. In addition, QTL *qLRI1-1* and *qLWD1-1* were co-located in interval RM11054-RM5646 on chromosome 1 (Tables 1 and 2).

Discussion

Phenotype variation for drought resistance traits

Plant roots can absorb water and nutrients, detect soil information, and perceive and switch drought stress signals to the whole plant, which initiate the morphological, physiological, biochemical, and molecular responses (Agrawal et al. 2016; Moumeni et al. 2011). Many studies have explored the responses of seed germination capacity, seedling growth, and coleoptiles length under abiotic stresses. Li et al. (2005) studied the water stress effect on 14 drought indices at the budding stage and seedling stage, and considered that the GV, RN, RL, and SH traits could be the indices that reflect rice drought-tolerance. In the present study, the GV, RN, RL, and SH traits were all inhibited by drought stress conditions in parents IAPAR-9,

Table 2 QTLs and their genetic effects on LRI (leaf rolling index) and LWD (leaf withering degree) for IAPAR-9/Akihikari and IAPAR-9/Liaoyan241 RILs under field drought stress

Trait	Investigated time	Locus	Chr.	Location (cM)	Marker interval	LOD	PVE (%)	Add	Source of allele
(1) IAPAR-9/Akihikari RIL population									
LRI	7.31	*qLRI1-1*	1	197	RM11054-RM5646	7.01	18.82	0.46	IAPAR-9
		qLRI1-2	1	249	RM1231-RM302	2.62	4.32	− 0.23	Akihikari
		qLRI9-1	9	71	RM3600-RM553	3.86	6.77	0.28	IAPAR-9
		qLRI10-1	10	89	RM6100-RM3773	2.93	5.01	0.24	IAPAR-9
	8.13	*qLRI4*	4	84	RM17344-RM5503	2.85	5.05	0.23	IAPAR-9
		qLRI9-1	9	74	RM3600-RM553	6.42	13.66	0.37	IAPAR-9
		qLRI10-1	10	89	RM6100-RM3773	4.71	8.32	0.29	IAPAR-9
LWD	7.31	*qLWD1-1*	1	183	RM11054-RM5646	3.20	5.78	0.23	IAPAR-9
		qLWD3	3	217	RM15072-RM15164	5.77	10.73	− 0.31	Akihikari
	8.13	*qLWD1-2*	1	3	RM3740-RM220	3.37	8.23	0.26	IAPAR-9
(2) IAPAR-9/Liaoyan241 RIL population									
LRI		*qLRI4*	4	42	RM177-RM3367	4.30	8.57	0.34	IAPAR-9
	7.31	*qLRI9-2*	9	40	RM24240-RM409	7.61	16.12	0.48	IAPAR-9
	8.13	*qLRI1-3*	1	161	RM5646-RM6716	3.90	8.06	0.31	IAPAR-9
		qLRI10-2	10	61	RM216-RM8207	3.26	6.89	0.29	IAPAR-9
LWD	7.31	*qLWD9-1*	9	51	RM409-RM24424	2.73	8.21	0.28	IAPAR-9
	8.13	*qLWD9-2*	9	102	RM3600-RM553	2.52	8.73	0.33	IAPAR-9

LRI leaf rolling index, *LWD* leaf withering degree; 7.31, the QTLs attained as regard to the data investigated on July 31; 8.13, the QTLs attained as regard to the data investigated on August 13; *LOD* logarithm of odds, *PVE* percent variance explained

Akihikari, and Liaoyan241, and in the two RIL populations, and there were significant differences among the parents (Fig. 1; Supplementary Table S3). The GV, RN, RL, and SH traits in the two RIL populations contributed to the larger genetic variation and variation coefficient (Supplementary Table S3), and could be considered as indices to evaluate and analyze the drought difference between materials. Wang et al. (2008) examined the CL trait of 76 varieties under PEG-6000 conditions and considered that the uniformity between the CL trait and drought-tolerance required further study. In this paper, the CL and RCL traits showed little genetic variation and variation coefficient (Supplementary Table S3), indicating that they can only reflect the drought-tolerance from one aspect and cannot effectively evaluate and analyze drought tolerance among materials.

Leaf rolling can reduce water loss and light transmission by changing the plant leaf architecture (Richards et al. 2002; Zhang et al. 2015; Li et al. 2016). Leaf traits associated with drought stress are not easy to obtain and evaluate; there have been few studies of LRI and LWD. In our study, in the field stress experiment, we used the LRI and LWD traits at two time points to evaluate drought-tolerance (Fig. 1; Supplementary Table S3), The drought tolerance of IAPAR-9 was much stronger than that of Akihikari and Liaoyan241. With increasing time, the degree of drought stress became more serious and was related to the water transpiration rate from the rice leaves. The correlation coefficient (CC) for two RIL population-related traits between data in the field and in the simulated drought conditions was calculated and is displayed in the Supplemental Materials (Supplementary Table S5). A *t* test was carried out to determine the significance between the CCs. In general, $0.1 < CC < 0.3$, was defined as a weak correlation, and $0.5 < CC < 1$, was defined as a strong correlation. According to the value and significance in Supplementary Table S5, the CC for two RIL population-related traits between between data in the field and in the simulated drought conditions was a weak correlation ($0.1 < CC < 0.3$). In the IAPAR-9/Akihikari RIL population, the CC between root number (RN)

and leaf rolling index (LRI) from July 31 was 0.204; RN and leaf withering degree (LWD) from July 31 was 0.178; and root length (RL) and LWD from July 31 was 0.166. In the IAPAR-9/Liaoyan241 RIL population, the CC between RN and LRI from August 13 was 0.154, which suggested that LRI and LWD of leaf traits in the fields have some relevance to the RN and RL of root traits in the simulation condition; this result is consistent with Uga et al. (2011) and Yue et al. (2006). However, in the in the IAPAR-9/Liaoyan241 RIL population, the CCs between germination vigor (GV) and LRI and LWD from August 13 were 0.148 and 0.135, respectively, which suggested that seed germination capacity has some relationship with drought tolerance capacity in the seedling; the CC between seedling height (SH) and LRI from July 31 was 0.134 and LWD from August 13 was 0.149 which suggested that seedling height has some relationship with drought tolerance capacity in the seedling.

Therefore, in the IAPAR-9/Akihikari population, RN had a significant positive correlation with LRI and LWD among the strains, and GV was positively associated with LRI on July 31. In the IAPAR-9/Liaoyan241 population, GV and RN had significant positive correlations with LRI, while GV and SH were positively associated with LWD on August 13 (Supplementary Table S5). These results suggested the drought-tolerance traits of PEG-simulated stress and field drought stress are strongly correlated: the LRI and LWD traits among the strains increased, accompanied by increased GV, RN, and SH under drought stress (Supplementary Table S5).

In addition, in simulated drought stress conditions, we obtained not only the absolute phenotype data of GV, CL, RN, RL, and SH, but also the relative phenotype data of RGV, RCL, RN, RL, and RSH for the drought tolerance traits (Supplementary Table S3). They showed a consistent change trend, which could be regarded as replicates to some extent. Using this method, steady and reliable QTLs could be identified. In our study, the QTLs detected using the absolute phenotype data and the relative phenotype data for the same traits were mostly consistent in the two RIL populations.

QTLs for drought tolerance traits

Hundreds of rice drought QTLs have been reported (Sandhu et al. 2016), and QTLs for morphological traits such as SH, RL, and RN were identified and have different genetic characteristic. Jiang et al. (2016) used RILs to examine four QTLs controlling SH in marker intervals RM163-RM459 and RM459-RM161 respectively, on chromosome 5 and one QTL on chromosomes 3 in interval RM16-RM426, which explained 9.87–17.74% of observed phenotype variation. Liu et al. (2013) used an $F_{2:3}$ family to identify one QTL for SH on chromosome 5 located in interval RM509-RM405 and two QTLs in intervals RM545-RM231 and RM426-RM1334, respectively, on chromosome 3, which contributed 5.40–24.48% of the phenotypic variation. In our research, one QTL, *qSH5* for SH, was detected on chromosome 5 in RM2020-RM267 in the IAPAR-9/Akihikari RILs population, which explained 15.61% of the phenotypic variation, and one QTL, *qSH3* for SH, in interval RM15072-RM15164 was found on chromosome 3 in the IAPAR-9/Liaoyan241 population, which contributed 10.71% the phenotypic variation. According to our results, the chromosomal locations of the QTLs for SH and phenotypic interpretation rate are consistent with those of Jiang et al. (2016) and Liu et al. (2013). Although the marker intervals are different because of the use of different markers in our study, the positions on the chromosomes in our experiment were consistent with those of Jiang et al. (2016) and Liu et al. (2013), which suggested that our results are authentic and reliable. Jiang et al. (2016) found three QTLs for RL in intervals RM302-RM476B, RM476B-RM315, and RM472-RM104 on chromosome 1, while Liu et al. (2013) identified one QTL for RN in RM1247-RM1254 on chromosome 1. We identified one QTL, *qRL1* for RL, in interval RM220-RM490 on chromosome 1 in the IAPAR-9/Akihikari RILs population, and one QTL, *qRRL1* for RRL, in interval RM12137-RM6840 on chromosome 1 in the IAPAR-9/Liaoyan241 population. According to the results, comparing the position of RM302-RM476B, RM476B-RM315, RM472-RM104, and RM220-RM490 with the positions of RM1247-RM1254 and RM220-RM490 on chromosome 1, we found they were located at the same chromosome end region. Zhou et al. (2016a, b) used chromosome segment substitution lines to find two QTLs for RN on chromosomes 9 and 11, respectively, and Mu et al. (2003) used a doubled haploid population (DH) and found one QTL for RN on chromosome 11. In our study, in the IAPAR-9/Akihikari RILs population, three QTLs, *qRN9*,

qRN11-1, and qRN11-2 for RN were identified on chromosomes 9, 11, and 11, respectively (Table 1). Studies on the GV trait have been carried out. Wang et al. (2008) studied drought stress and found drought-related QTLs for GV on chromosomes 3, 6, and 8. In our study, under PEG drought stress, we considered GV as a drought stress-identifying index. Two QTLs for GV were identified on chromosome 3 in the IAPAR-9/Akihikari RILs population, while the QTL for GV in the IAPAR-9/Liaoyan241 population was located on chromosome 4, which suggested the influence of the environment plays an important role in drought tolerance traits (Table 1). In addition, Zou et al. (2014) detected the QTL *qEW-3*, for flag leaf stretch angle, in interval RM1350-RM570 chromosome 3, the located position of *qEW-3* was close to *qGV3* and *qRGV3*, which were located in interval RM426-RM570 chromosome 3 in this study. For the CL trait, Hu et al. (2006) used RILs to find two QTLs, *qCL1a* and *qCL1b*, for CL on chromosome 1, which explained 4.40–8.16% of the phenotypic variation. In the present study, in the IAPAR-9/Akihikari population, one QTL, *qRCL1*, was identified on chromosome 1 that contributed 9.37% of the phenotypic variation (Table 1). These QTLs verified each other, which demonstrated the authenticity and reliability of the QTLs, and suggested that gene pleiotropism is involved in the phenotypic variation.

QTLs for LRI and LWD have been associated with drought tolerance. Yue et al. (2006) examined four QTLs for LRI on chromosomes 1, 4, and 9, which explained 7.11–26.36% of the phenotypic variation, and detected five QTLs for LWD on chromosomes 1, 3, and 9, which contributed 5.13–16.10% of the phenotypic variation. Among them, they found one major QTL in interval RM219-RM296 on chromosomes 9 that explained 26.36% of the phenotypic variation under two different environments in 2003 and 2004. Yang et al. (2016) detected QTLs on chromosome 1 for LRI and LWD through BSA of the extreme phenotypes in the RIL lines. In our study, in the IAPAR-9/Akihikari RIL population, five QTLs for LRI were identified on chromosomes 1, 4, and 9, which contributed 4.32–18.82% of the phenotypic variation, while in the IAPAR-9/Liaoyan241 population, three QTLs for LRI were identified on chromosomes 1, 4, and 9, which explained 8.06–16.12% of the phenotypic variation. For the LWD trait, in the IAPAR-9/Akihikari RIL population, three QTLs were

identified on chromosomes 1 and 3, which explained 5.78–10.73% of the phenotypic variation, while in the IAPAR-9/Liaoyan241 population, two QTLs for LWD were identified on chromosomes 9 that contributed 8.21–8.73% of the phenotypic variation. The major QTL, *qLRI9-1*, was located in interval RM3600-RM553 on chromosomes 9 and explained 13.66% of the phenotypic variation (Table 2). In our study, the major QTL *qLRI9-1* is located at one end position on chromosome 9, Further study showed that the *qLRI9-1* for LRI were detected in RM3600 (17108.844 kbp) and RM553 (19325.247 kbp) on chromosome 9 in the reference cultivar Nipponbare. In a previous study the *Dro1* gene was located in the interval RM24393 (16679.5 kbp) and RM7424 (17287.9 kbp) on 9 chromosome, which delimit a 608.4 kb interval. Nipponbare maintained high yield performance under drought conditions relative to the recipient cultivar (Uga et al. 2011, 2013). In a study by Yue et al. (2006), *QDlr9* was located at the other end of chromosome 9, which suggested that QTL *qLRI9-1* was a genuine QTL and significantly related to the rice yields under drought conditions.

In the two RIL populations, certain QTLs for LRI and LWD were consistent with previously identified QTLs (Table 2), which suggest that LRI and LWD are steady drought-tolerant indices in different populations, and that these QTLs will be important to identify new genes. In addition, Yang et al. (2013) detected *qGL10.1* for grain length and *qGLWR10.1* for grain length to width ratio in interval RM6100-RM3773, while Zhu et al. (2016) identified two QTLs, *qNGP10* for number of grains per panicle and *qGY10* for grain yield per plant, in interval RM6704-RM6100 on chromosome 10. In our study, *qLRI10-1* was located in interval RM6100-RM3773 on chromosome 10, which suggested that *qLRI10-1* for LRI was closely linked with yield QTLs (Comas et al. 2013). The two QTL, *qLRI9-1* and *qLRI10-1*, have be detected repeatedly at the two times point, suggesting that these QTLs are stable against the environmental impact.

According our results, we chose QTL *qLRI9-1* located at the marker interval RM3600-RM553 on chromosome 9 as the target QTL and constructed chromosome segment substitution lines (CSSLs). Using these CSSLs, drought-tolerance gene fine mapping is ongoing. In this study, most QTLs for SH and RSH, RL and RRL, RN and RRN, CL and

RCL, and LRI and LWD demonstrated the phenomenon of pleiotropism, which can be associated with the gene expression level. These results will be useful to mine drought tolerance genes and to enhance the water utilization efficiency by MAB for drought tolerance.

Verifying the two populations to ensure stable QTLs

The study of drought tolerance in rice is quite complicated; too many influencing factors play important roles in the stress-tolerance mechanism, and it has been recognized as the most complicated mechanism among all adverse stresses. Therefore, the study of drought stress mechanisms requires new ideas in terms of materials selection and evaluation methods to provide materials for the genetic improvement of drought tolerance. Classical QTL analysis, carried out using one genetic population has many disadvantages if the germplasm resources have extensive genetic variation. For example, representativeness is low, in which the same locus only contains two alleles that can vary, which leads to a lack of comparison of multi-allele variation and different genetic backgrounds. Using multiple genetic populations can increase the QTL detection efficiency, and can obtain stable QTLs under different backgrounds. Liu et al. (2007) used two RIL populations to detect plant height and heading period QTLs in rice, Li et al. (2013) used eleven RIL populations to find stable QTLs to provide a valuable reference for fine mapping and map-based cloning under different backgrounds in maize. Yang et al. (2012) used eleven RIL populations to detect maize QTLs for stamen traits under different backgrounds. In our study, two RIL populations were used to detect QTLs for drought tolerance under PEG stress and field drought stress in rice. In the IAPAR-9/Akihikari population, the detected 18 QTLs were located on chromosomes 1, 3, 4, 5, 9, 10, and 11, respectively; in the IAPAR-9/Liaoyan241 population, the 16 detected QTLs were found on chromosomes 1, 3, 4, 9, 10, and 11, respectively (Supplementary Tables S3 and S4). Eight co-located QTLs were found in the two populations under the two environments. These co-located QTLs verified each other and demonstrated the authenticity and reliability of our QTL analysis, and suggested that gene interactions affected the phenotypic variation. These results will be useful to mine

drought tolerance genes and enhance the water utilization efficiency by MAB for drought tolerance. The QTLs located at the same interval on the same chromosome can be identified as major effect and stable QTLs, which may be attributed to the pleiotropic effect of a gene, or closely linked or overlapping QTLs. Among them, qLRI9-1, controlling LRI in the IAPAR-9/Akihikari population, and qLWD9-2, controlling LWD in the IAPAR-9/Liaoyan241 population, were both located in interval RM3600-RM553 on chromosomes 9, which is a new major QTL. Only on chromosome 5 were there no QTLs in the IAPAR-9/Liaoyan241 population. There are a number of explanations for this phenomenon. The different backgrounds and the interaction effect between the gene and environment can result in consistent QTLs. The different density of SSR markers in the two genetic linkage maps could also explain these observations. Thus, we used two populations and the multi-group data verified each other, which ensured that the located QTLs are authentic and reliable. Our data will be useful and valuable for further studies aimed at identifying the underlying genes.

Meta-QTLs analysis on the 9 chromosome

In recent years, many QTLs for various drought-tolerant traits were located on chromosome 9 (Fig. 4), Yue et al. 2006 found three QTLs (QRy9, QRsf9, QRhi9) located at RM316-RM219 for RY (relative yield per plant), RSF (relative spikelet fertility), and RHI (Relative harvest index (grain yield/biomass). One QTL (QRgw9) was located at RM444-RM316 for RGW (relative grain weight); another QTL (QDrvd9) for for RGW was located at RM219-RM296; and a final QTL (QMrdd9) was located at RM160-RM215 for MRDD (maximum root depth under drought). Zhou et al. (2016a, b) found two QTLs (QMRL9.1, QMRL9.2) for MRL (maximum root length). Uga et al. (2011) identified the gene Dro1 at RM24393-RM7424 for RDR (the ratio of deep rooting RT/RY). Singh et al. (2016) found DTY9.1 for grain yield under drought stress. Li et al. (2015) located the qRT9 for RT or RL on RM410-RM7048. Swamy et al. (2013) located the QTL for day after sowing. Sandhu et al. (2013) found the QTL qPH9.1 and qPL9.1 at RM524-RM410 for plant height and panicle length. Li et al. (2011) found QTLs qBRT9.1, qBRT9.2, qBRT9.3, and
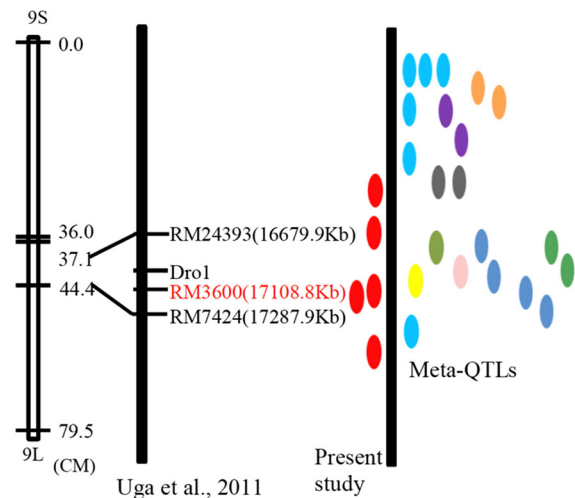
Fig. 4 The meta analysis of all QTLs on 9 chromosome. Sky blue circles: the QTLs for RY, RSF, RHI, RGW, DRVD, MRDD traits in Yue et al. 2006. Violet circles: the QTL for MRL traits in Zhou et al. 2006. Yellow circles: the QTL for RDR in Uga et al. 2011. Green circles: the QTL for grain yield in Singh et al. 2016. Pink circles: the QTL for RTor RY in Li et al. 2015. Grey circles: the QTL for DS in Swamy et al. 2013. Orange circles: the QTLs for PH and PL in Sandhu et al. 2013. Blue circles: the QTL for BRT in Li et al. 2011. Grey circles: the QTL for grain yield in Dixit et al. 2014. Red circles: the QTLs for RN, SH, LRI and LWD in present study. *RY* relative yield per plant, *RSF* relative spikelet fertility, *RHI* relative harvest index (grain yield/biomass), *RGW* relative grain weight, *DRVD* deep root rate in volume under drought conditions, *MRDD* maximum root depth under drought, *MRL* maximum root length, *RDR* the ratio of deep rooting, *RT/RY* root thickness and root length, *DS* daying after sowing, *PH* plant height, *PL* panicle length, *BRT* basal root thickness. (Color figure online)

qBRT9.4 for basal root thickness at RM566, RM7048, RM278, and RM215, respectively. Dixit et al. (2014) determined the QTLs at RM24350-RM24390 and RM321-RM566. Therefore, the drought-tolerance traits used to mine the QTLs in our study were root phenotype and agronomic characters. According to Fig. 4, on chromosome 9, the QTL *qLRI9-1* found in this paper can be co-located with *Dro1*, *qRT9*, *qBRT9.2*, *DTY9.1*, and *qDTY9.1* on the same region, suggesting that LRI has some relationship with the RDR, DTY, and BRT. The QTLs for RDR, DTY, and BRT have been demonstrated to influence rice yields, so the *qLRI9-1* can be a QTL for yield traits under drought-stress. In addition, other QTLs were distributed on the flanking region of the *Dro1* gene region as a bridge to compare different QTLs in different populations. The QTL *qLRI9-1*, located on chromosome 9, has great relevance to many yield traits under drought-tolerance capacity.

Compliance with ethical standards

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