QTL analysis of drought tolerance traits in rice during vegetative growth period

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Research Article

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Abstract

Drought affects the growth and development of rice from direct seeding or transplanting to maturity in north China. It is important to carry out QTL mapping for drought resistance to improve rice breeding. In this study, a rice RIL population consisting of 253 families was constructed by crossing Miyang 23 with Jileng 1, both of which exhibit strong drought resistance. The leaf rolling index (LRI), leaf withering degree (LWD) and leaf chlorophyll content (SPAD) were measured over three years, and QTLs for these traits were mapped. The results showed that LRI, LWD and SPAD were all quantitative traits controlled by multiple genes. A total of 28 QTLs related to three drought-resistant indices were detected; four loci controlling LRI and LWD were detected on Chromosome 1. *qLRI1-1* and *qLWD1-4* were located in the interval of AH01001843-RM302, explaining 10.30% and 4.90% of the phenotypic variation respectively; *qLWD1-2*^{B} and *qLRI-1J*^{C} were both located in RM315-S01167A, and *qLWD1-2*^{B} was detected three times in different growth periods in 2018, and explained 4.04%-11.15% of the phenotypic variation. *qLRI-1J*^{C} was detected four times in different growth periods in 2019, which explaining 4.45%-7.96% of the phenotypic variation. The three QTLs located on Chromosome 5, *qLRI5-1*, *qLWD5-1*^{B} and *qLRI5-2*^{B}, were all located in CMB0526.3486-RM87. *qLWD5-1*^{B} and *qLRI5-2*^{B} were detected three times in 2019 at different growth stages, and their contribution rates to phenotypic variation ranged from 6.81% to 6.93% and 5.46% to 9.13%, respectively. The four QTLs detected on Chromosome 12, *qLWD12-1*, *qLWD12-3*, *qLRI12-1*^{A} and *qLRI12-3*^{A}, were all located in the S12099-CMB1226.0 interval and were repeatedly detected in 2018-2019, explaining the 4.59%-7.25% of the phenotypic variation. The above QTLs, including *qLWD1-2*^{B}, *qLRI1-1J*^{C}, *qLWD5-1*^{B} and *qLRI5-2*^{B}, have been detected many times. In the future, it would be interesting to implement in-depth fine mapping of the QTLs and use the identified markers in the drought resistance molecular marker selection breeding of rice.

Introduction

Drought is one of the main adverse factors that affect plant growth and crop yield, and it leads to crop reduction and economic losses in China every year (Zhu et al. 2010). Rice is one of the three major grain crops in China. A high, stable yield of rice is important for ensuring China's food security. However, rice production in northern China is limited by water resources, and the growth and development of rice from direct seeding or transplanting to maturity are affected by drought. As the soil water deficit intensifies, rice plants become nitrogen-free, their leaf area decreases, their leaf color becomes significantly lighter, and plants stop developing or die under heavy drought conditions (Kuang et al.2007). Therefore, QTL mapping for drought resistance during vegetative growth period it is of great practical importance for rice breeding.

In the study of crop drought resistance, most scholars identify drought resistance by investigating the phenotypic symptoms of root, stem and leaf, the relative water content, osmotic adjustment and abscisic acid. Plant leaf is the most obvious morphological index, which determines photosynthesis intensity and transpiration efficiency, and facilitates rapid response to environmental changes such as drought, salt

and cold. Rolled leaves are considered a defense against adverse conditions by reducing plant water loss (Zhang et al. 2015; Kadioglu et al. 2012; Kadioglu and Terzi 2007). Therefore, leaf rolling index (LRI) and leaf withering degree (LWD) were considered as the most important drought tolerance indexes in the study of drought tolerance (Yang et al. 2016; Li et al. 2016; Zhang et al. 2015; Richards et al. 2002). In Xiao et al. (2012), the results showed that leaf curl and leaf mortality reflected drought tolerance and could be used as drought tolerance evaluation indices in drought stress research.

QTLs for root traits (Zheng et al. 2006; Zheng et al. 2000; Ray et al. 1996), leaf morphological traits (Champoux et al. 1995; Price et al. 2002; Wang et al. 2008) and physiological traits (Robin et al. 2003; Zhang et al. 2001; Babu et al. 2003; Price et al. 2002) have been found. In Ali et al. (2000), Hemamalinia et al. (2000), Li et al. (2005) and Zheng et al. (2000; 2003) QTLs for root number, maximum root length, root diameter, root penetration index and root dry weight were localized. Eight QTLs for root traits in rice (Oryza Sativa L.) were mapped by using a DH line of Jinyeqing 4/jing17 by Xu Jichen et al.(2002). Xu et al. (2005) used 254 backcross introgression lines as materials and evaluated plant height, heading date and grain yield in plentiful water and drought conditions for two consecutive years. Champoux et al. (1995), for the first time, identified 18 QTLs controlling LRI in C039/moroberekan RIL population for drought avoidance at the seedling stage, vegetative growth stage and mature stage. Babu et al. (2003) located 6 QTLs for drought resistance using a DH population, among which 3 QTLs controlled LRI and 3 QTLs controlled LWD. Courtois et al. (2000) studied 105 dihaploid lines in drought conditions; 11 QTLs related to LRI, 1 QTL related to LWD and 11 QTLs related to relative water content were located at 1,3,4,5 and Chromosome 9, respectively. Price et al. (2002) identified 15 QTLs associated with LRI and 11 QTLs associated with LDW using an F2 population of Bala/azucena.

As mentioned above, much work has been done to implement QTL mapping for drought resistance in rice in China and abroad. However, the results of QTL mapping have differed due to different populations, different growth periods and different environments, and few QTLs can be applied to drought resistance breeding (Luo et al. 2005). In addition, in the study of drought resistance in rice, most scholars used artificial drought stress identification methods such as dry shed, greenhouse, and high osmotic solution (PEG). In this study, we used an RIL population constructed by crossing Milyang 23 with Jileng 1, which had strong drought resistance. Under natural drought stress in the field, LRI, LWD and chlorophyll content (SPAD) were measured during the vegetative growth period. QTLs for these traits were analyzed in order to a provide scientific basis for the fine mapping and cloning of QTLs related to drought resistance in rice and for breeding drought resistant rice.

Materials And Methods

Materials

An RIL population containing 253 families was constructed by crossing the drought-tolerant Jileng 1 (JL1) with the drought-tolerant Miyang 23 (MY23). The parent, MY23, is a Korean indica rice variety and

the parent, JL1, is a japonica rice variety from the Institute of Rice Research, Jilin Academy of Agricultural Sciences.

Methods

From 2017 to 2019, the experiment was carried out in the Wanghong experimental base of the Crop Research Institute of the Ningxia Academy of Agriculture and Forestry in Yinchuan, Ningxia. The area is located in the northwest of China, which experiences drought and little rain all year. The average annual precipitation in Yinchuan is about 200 mm. Before transplanting, the land was leveled with a laser leveler, a 1-meter deep trench was dug around the field, and the plants were wrapped with plastic film to prevent water penetration. The transplanting standard was 26.7 cm × 9.9 cm, the path was 0.5 cm, and the 2 rows were 1 m long. Each row was planted with 10 holes in a single transplanting with 2 repetitions. After 10 days of transplanting, the water supply was cut off and the plants were treated with natural drought stress. The LRI, LWD and chlorophyll content (SPAD) of rice plants were measured, along with the degree of field drought stress and the leaf withering status. The date of sowing, transplanting seedlings and investigation in each year are detailed in Table 1.

Table1

Date of sowing, transplanting, water cut off, investigation

Years	Sowing date	Transplanting date	Water cutoff date	Date of investigation					
2017	28-Apr	30-May	10-Jun	11-Jul	25-Jul				
2018	25-Apr	21-May	1-Jun	21-Jun	29-Jun	12-Jul	18-Jul	27-Jul	
2019	23-Apr	25-May	5-Jun	11-Jun	7-Jul	23-Jul	31-Jul	6-Aug	15-Aug

Measure of phenotypes

Under field drought stress, the LRI and LWD of the 2 parents and 253 populations were investigated and recorded by visual methods. The investigation time was between 11:00 am and 14:00 pm, during which the sensitivity of leaves to water stress was the strongest, and the leaf curling and withering were the most serious. The grading standards of LRI and LWD were adjusted based on the Turner (1997) method and assigned one of 5 grades according to the following criteria (Table 2, Fig. 1).

Table 2

Visual evaluation standard of LRI and LWD

LRI under drought stress		LWD under drought stress	
Level	Plant performance	Level	Plant performance
1	Leaves Healthy	1	without symptoms or sharp leaves slightly dead
3	beginning to appear curly, fold (V SHAPE)	3	Most of the dead leaves reached 1/4 of the length of the leaf surface
5	The dead leaves were cup-shaped (U-SHAPED)	5	1/4-1/2 of the dead leaves
7	the leaves edge-bound (0-shaped)	7	More than 2/3 of leaves are dead
9	the leaves are completely curled	9	the whole plant is obviously dead

The chlorophyll content of the fully opened leaves of the 2 parents and 253 RILs was measured by SPAD-502 from 9:00 am to 11:00 am under field drought stress conditions. Ten plants of each line were selected and the average SPAD reading value at 1/3 of the distance to the leaf tip was used as the chlorophyll content determination value.

Construction of molecular marker linkage map and QTL detection

DNA was extracted using the hexadecyl trimethyl ammonium bromide (CTAB) method. A total of 291 polymorphic markers, evenly distributed throughout the entire genome of the rice plant, were used to genotype the RIL population. The genetic linkage map was drawn by the Mapchart software (Voorrips 2002). The map covered the whole rice genome of 2619.06 cM, and 291 polymorphic markers were distributed evenly on 12 chromosomes, the average distance between the markers was 9.0 cM (Supplemental Fig. 1). The inclusive composite interval mapping (ICIM) method was used to determine the QTL by QTL IciMapping 4.2 (Li et al. 2007) with the scanning step of 1 cM, and the probabilities of adding and removing variables in stepwise regression set at 0.001 and 0.002, respectively. The minimum logarithm of the LOD score was 2.50. The QTL naming convention was implemented as described by McCouch et al. (1997).

Results

Phenotypic variation of drought tolerance traits of parents and RIL population

The two parents and 253 RIL populations were evaluated for drought-resistant traits over three years, and LRI and LWD were investigated on July 11 and July 25,2017, respectively. The results showed that the variation range of LRI and LWD was larger than 40.00% (Supplementary Table 1). In 2018, LRI and LWD were investigated on June 21, June 29, July 12, July 18 and July 27, when the symptoms of drought stress were more serious (Table 3). In the survey conducted on June 21, the LRI and LWD of JL1 were Grade 1 and Grade 3, respectively; the LRI and LWD of MY23 were Grade 1 and Grade 2, respectively. In the survey conducted on July 27, the LRI and LWD of JL1 were Grade 7 and Grade 4, respectively, while those of MY23 were Grade 2. Therefore, the drought resistance of MY23 was obviously stronger than that of JL1. On June 21, the ranges of LRI and LWD were 1-3 and 1-6, respectively, while on July 27, the ranges of LRI and LWD were 1-8 and 1-7, respectively. With increases in drought stress time, the degree of LRI and LWD also increased. From June 21 to July 27, the range of LRI was 1.17-5.37, the range of LWD was 2.52-3.52 and the coefficient of variation of both indices was over 27.00%.

Table 3

Variation of LRI and LWD in the vegetative stage for RILs under drought stress in 2018

Trait	Date of investigation	Parents		RILs population			
		JL1	MY23	Range of variance	Mean	SD	CV (%)
LRI	21-Jun	1	1	1 3	1.17	0.42	36.15
	29-Jun	5	1	1 8	4.19	1.31	31.31
	12-Jul	6	1	1 9	5.02	1.69	33.63
	18-Jul	7	2	1 9	5.39	1.53	28.44
	27-Jul	7	2	1 8	5.37	1.54	28.62
LWD	21-Jun	3	2	1 6	2.52	1.14	45.08
	29-Jun	4	1	1 5	2.55	1.00	39.25
	12-Jul	3	1	1 6	2.59	1.05	40.50
	18-Jul	3	1	1 7	2.96	1.17	39.39
	27-Jul	4	2	1 7	3.52	0.97	27.67

In 2019, the LRI and LWD of rice were investigated on June 11th, July 7th, July 23rd, July 31st, August 6th and August 15th. The chlorophyll content (SPAD) was investigated once on August 15 when the symptoms of drought stress were serious (Table 4). From June 11 to August 15, the variation ranges of LRI and LWD of JL1 were 2-4 and 3-8 respectively, and those of MY23 were 1-2 and 2-3 respectively. the results showed that the drought resistance of MY23 was stronger than that of JL1. The variation range of LRI was 1-6 and 1-9 in RILs, and the variation range of LWD was 1-8 and 1-9, respectively. With increases in drought stress time, the degree of LRI and LWD also increased; the variation coefficient of LRI and LWD of the RILs population

also increased; the variation coefficient of LRI increased from 23.68% to 46.22%; and the variation coefficient of LWD increased from 31.75% to 41.12%. The coefficients of variation of LRI and LWD of the RILs population were 46.22% and 41.12%, respectively. The LRI, LWD and SPAD values from August 15 were compared (Table 4), the coefficient of variation of LRI was the greatest (44.60%); the coefficient of variation of LWD was second (40.82%), and the coefficient of variation of SPAD was the smallest (7.06%).

Table 4

Variation of LRI and LWD in the vegetative stage for RILs under drought stress in 2019

Trait	Date of investigation	Parents		RILs population			
		JL1	MY23	Range of variance	Mean	SD	CV (%)
LRI	11-Jun	2	1	1 6	2.69	0.64	23.68
	7-Jul	2	1	1 6	2.72	1.05	38.57
	23-Jul	3	2	1 6	2.26	0.85	37.43
	31-Jul	4	2	1 9	2.83	1.31	46.11
	6-Aug	4	2	1 9	2.86	1.32	46.22
	15-Aug	4	2	1 9	3.26	1.45	44.60
LWD	11-Jun	3	2	1 8	3.94	1.25	31.75
	7-Jul	3	3	1 7	3.30	1.11	33.58
	23-Jul	6	2	1 8	3.65	1.42	38.88
	31-Jul	8	3	1 9	5.26	2.15	40.95
	6-Aug	8	3	1 9	5.27	2.17	41.12
	15-Aug	8	3	1 9	5.36	2.19	40.82
CC	15-Aug	39.88	43.63	33.27 48.37	40.38	2.85	7.06

According to the family distribution map of the population phenotypes (Fig. 2), the LRI, LWD and SPAD values of the RIL population were similar to the normal distribution. Most of the family traits were distributed in the middle of the range, and a minority of values were distributed at both ends, indicating quantitative traits controlled by multiple genes.

Correlation analysis of drought resistance traits

The correlation analysis of drought resistance characteristics showed that there was significant correlation among LRI, LWD and SPAD under natural drought conditions. The correlation coefficient between LRI and LWD was 0.572, which was significant (r = 0.572), while the correlation coefficients between LRI and LWD and SPAD were -0.217 and -0.233, respectively. The results showed that the SPAD value was closely related to LRI and LWD under natural drought conditions, and the SPAD value could be used as an evaluation index for the drought resistance of rice. The content of chlorophyll in rice plants was related to adversity.The higher the content of chlorophyll was, the more drought resistance the plant exhibited.

QTL identification for all traits

Under field drought stress (Supplementary Table 2, Fig.3), in the JL1/MY23 population, 7 QTLs for traits associated with drought tolerance in the vegetative growth stage were detected on Chromosomes 1, 4, and 5 and 4 QTLs for LRI were identified: *qLRI1-1*, *qLRI4-1*^{A}, *qLRI5-1*, *qLRI5-2*; the LOD values were 6.6, 7.06, 8.20 and 4.66, respectively, and their phenotypic contribution rates were 10.30%, 7.69%, 9.13%, and 7.18%, respectively. *qLRI1-1* and *qLRI4-1*^{A} were favorable alleles derived from parent MY23; the beneficial alleles of *qLRI5-1* and *qLRI5-2* were derived from female JL1. *QLRI4-1*^{A} (S04085-RM451) on Chromosome 4 was detected twice in succession, and *qLRI1-1*(AH01001843-RM302) on Chromosome 1 was the major QTL with the highest phenotypic contribution. Three QTLs, *qLWD1-1*, *qLWD4-1*, *qLWD4-2*, were found and their LOD values were 2.62, 2.66 and 3.27, respectively. The phenotypic contribution rates were 5.19%, 3.91% and 5.44%, respectively. Except *qLWD1-1,* the favorable alleles was derived from female parent, JL1, the other QTLs were derived from male parent, MY23.

In 2018, five QTL loci (*qLRI2-2*^{A}, *qLRI4-1*^{A}, *qLRI7-1*^{A}, *qLRI7-2*^{A} and *qLRI12-1*^{A}) for

LRI were detected on 2,4,7 and Chromosome 12. The LOD scores were 9.67%, 3.34%, 3.35%, 4.99%, and 4.01%; the phenotypic contribution rates were 7.25%, 13.78%, 4.04%, 6.12% and 7.67%, respectively (Fig.4 and Table5). *qLRI12-1*^{A}, *qLRI2-2*^{A}, *qLRI4-1*^{A}, *qLRI7-1*^{A} and *qLRI7-2*^{A} were repeatedly detected in two consecutive assessments, and *qLRI2-2*^{A} (RM6-RM240) was the major QTL for LRI with a phenotypic contribution of 13.78%.*qLWD1-2*^{B}, controlling LWD,was detected three times on Chromosome 1. *qLWD1-2*^{B} (RM315-S01167A) explained 11.15% of the phenotypic variation and was the major QTL controlling LWD. All the allelic variations of the above loci were from female JL1.

Table 5

Summary of QTL detection and genetic effects in JL1/MY23RILs in 2018

Traits	QTL	Chr	Range	LOD	PVE(%)	Add	Source of allele
LRI	*qLRI12-1*^{A}	12	S12099-CMB1226.0	4.01	7.25	0.45	JL1
	qLRI2-2^{A}	2	RM6-RM240	9.67	13.78	0.53	JL1
	qLRI4-1^{A}	4	RM127-RM280	3.34	4.04	0.28	JL1
	qLRI7-1^{A}	7	RM21810-CMB0723.0	3.35	6.12	0.36	JL1
	qLRI7-2^{A}	7	RM21657-RM21725	4.99	7.67	0.43	JL1
LWD	*qLWD1-2*^{B}	1	RM315-S01167A	6.18	11.15	0.46	JL1

The superscript A stands for 2 detected, B stands for 3 detected, and C stands for 4 detected

In 2019, 15 QTLs were detected on 1,3,5,6,10,11 and Chromosome 12 in the RIL population; among them 6 were for LRI, 5 for LWD, and 4 for chlorophyll content (SPAD).

Six QTLs associated with LRI, *qLRI1-1J*^{C}, *qLRI3-2*^{B}, *qLRI5-2*^{B}, *qLRI5-3*, *qLRI12-1*^{A} and *qLRI12-3*^{A}, contributed 7.96%, 4.01%, 8.73%, 5.25%, 5.18% and 5.40%, respectively. *qLRI12-1*^{A} and *qLRI12-3*^{A} were detected twice, *qLRI3-2*^{B} and *qLRI5-2*^{B} were detected three times, *qLRI1-1J*^{C} was detected four times, and *qLRI5-2*^{B} (ID5010886-CMB0526.3) and *qLRI1-1J*^{C} (RM315-S01167A) had higher phenotypic contributions. With the exception of the qLRI3-2^{B} allele which was derived from the male parent, MY23, all other QTL alleles were derived from the female parent, JL1.

Table 6

Summary of QTL detection and genetic effects in JL1/MY23RILs in 2019

Traits	QTL	Chr	Range	LOD	PVE(%)	Add	Source of allele
LRI	*qLRI1-1J*^{C}	1	RM315-S01167A	4.26	7.96	0.54	JL1
	qLRI3-2^{B}	3	RM16-ID3010700	2.77	4.01	-0.28	MY23
	qLRI5-2^{B}	5	ID5010886-CMB0526.3	5.50	8.73	0.41	JL1
	qLRI5-3	5	CMB0526.3-RM87	4.25	5.25	0.37	JL1
	qLRI12-1^{A}	12	RM270-S12099	2.94	5.18	0.27	JL1
	qLRI12-3^{A}	12	S12099-CMB1226.0	3.47	5.40	0.40	JL1
LWD	*qLWD3-1*^{B}	3	ID3010700-AD03013905	2.87	4.20	-0.46	MY23
	qLWD5	5	CMB0526.3-RM87	3.96	5.85	0.55	JL1
	qLWD5-1^{B}	5	ID5010886-CMB0526.3	4.24	6.93	0.38	JL1
	qLWD6-2^{B}	6	RM217-RM253	3.47	5.90	0.57	JL1
	qLWD10-2^{B}	10	ID10003706-CMB1016.4	2.99	4.69	0.32	JL1
CC	qCC-1	1	RM522-RM259	3.25	6.45	-0.79	MY23
	qCC-3	3	RM130-RM570	5.29	6.73	0.81	JL1
	qCC-5	5	S05062-S05064	2.81	3.59	-0.61	MY23
	qCC-11	11	CMB1107.1-RM536	4.17	5.22	-0.73	MY23

The superscript A stands for 2 detected, B stands for 3 detected, and C stands for 4 detected The letter J is different from qLRI1-1 in 2017

qLWD3-1^{B}, *qLWD5*, *qLWD5-1*^{B}, *qLWD6-2*^{B} and *qLWD10-2*^{B} were the five QTLs associated with LWD, with LOD values of 2.87, 3.96, 4.24, 3.47 and 2.99, respectively. The phenotypic contribution rates were 4.20%, 5.85%, 6.93%, 5.90%, and 4.69%, respectively. *qLWD3-1*^{B}, *qLWD5-1*^{B}, *qLWD6-2*^{B} and *qLWD10-2*^{B} were detected repeatedly three times, and the phenotypic contribution of *qLWD5-1*^{B} (ID5010886-CMB0526.3) was the largest. With the exception of *qLWD3-1*^{B}, the alleles of the QTLs were from the parent MY23. Among the four QTLs related to chlorophyll content (SPAD), *qCC-3* had the largest phenotypic contribution, explaining 6.73% of the genetic variation, and the beneficial alleles, *qCC-1*, *qCC-5* and *qCC-11,* were derived from the male parent, MY23.

Co-mapping QTLs for drought resistance-related traits

A total of 28 QTLs were detected at 1,2,3,4,5,6,7,10,11 and Chromosome 12, among which 15 controlled LRI, 9 controlled LWD, and 4 controlled the chlorophyll content (SPAD). QTLs associated with LRI and LWD were repeatedly detected on 1,5 and Chromosome 12 in different years (Fig. 6 and Table 7).

Table 7

QTLs found repeatedly in different years

QTL	Chr	Range	LOD	PVE(%)	Add	Source of allele
qLRI1-1	1	AH01001843-RM302	6.63	10.30	-0.69	MY23
qLWD1-4	1	AH01001843-RM302	2.52	4. 90	0.30	JL1
qLRI5-1	5	ID5010886-CMB0526.3	8.20	9.13	0.64	JL1
qLWD5-1^{B}	5	ID5010886-CMB0526.3	4.24	6.93	0.38	JL1
qLRI5-2^{B}	5	ID5010886-CMB0526.3	5.50	8.73	0.41	JL1
qLWD1-2^{B}	1	RM315-S01167A	6.18	11.15	0.46	JL1
qLRI1-1J^{C}	1	RM315-S01167A	4.26	7.96	0.54	JL1
qLWD12-1	12	S12099-CMB1226.0	4.34	5.36	0.29	JL1
qLWD12-3	12	S12099-CMB1226.0	2.63	4.59	0.33	JL1
qLRI12-1^{A}	12	S12099-CMB1226.0	4.01	7.25	0.45	JL1
qLRI12-3^{A}	12	S12099-CMB1226.0	3.47	5.40	0. 40	JL1

The superscript A stands for 2 detected, B stands for 3 detected, and C stands for 4 detected The letter J is different from qLRI1-1 in 2017

Four common sites associated with LRI and LWD were detected on Chromosome 1; both *qLRI1-1* and *qLWD1-4* were located in the marker region AH01001843-RM302. The distance between the two loci was 30.19 cm, and the phenotypic contributions were 10.30% and 4.90%, respectively. The allelic variation of *qLRI1-1* was derived from the parent MY23, and that of *qLWD1-4* was derived from the parent JL1. *qLWD1-2*^{B} and *qLRI1-1J*^{C} were located in the RM315-S01167A marker interval and the distance between the was 15.94 cm. *qLWD1-2*^{B} was detected three times in 2018 and its phenotypic contribution was 4.04%-11.15%. *qLRI1-1J*^{C} was detected four times in 2019, its phenotypic contribution was 4.45%-7.96%, the additive effect was positive, and the allelic variation was derived from the parent JL1.

qLRI5-1, *qLWD5-1*^{B} and *qLRI5-2*^{B} were co-located within the range of CMB0526.3486-RM87 on Chromosome 5, and the distance of the range was 8.61 cm. *qLWD5-1*^{B} and *qLRI5-2*^{B} were detected three times in 2019, and their synergistic alleles originated from the parent JL1.

Multiple QTLs (*qLWD12-1*, *qLWD12-3*, *qLRI12-1*^{A}, *qLRI12-3*^{A}) were detected in the Chromosome 12 region of S12099-CMB1226.0 in 2018-2019. The marker interval was 14.07 cm and the phenotypic contribution was 4.59%-7.25%. The synergistic allele was derived from the parent JL1.

It was concluded that the AH01001843-RM302 loci on Chromosome 1, CMB0526.3486-RM87 loci on Chromosome 5 and S12099-CMB1226.0 loci on Chromosome 12 were not only closely related to LRI and LWD, but also to drought resistance. Moreover, these 3 loci were detected repeatedly, which indicated that they could express stably and were key loci for the control drought resistance.

Discussion

Phenotype variation for drought resistance traits

Drought resistance in plants is a complex life process, which is controlled by quantitative trait loci and environmental factors (Zhu et al.2010). In view of the drought resistance of rice at the seedling stage, tillering stage and heading and flowering stage, many scholars have identified the drought resistance of rice by artificial simulation (Nie et al. 2021). In order to obtain stable QTLs that can be directly applied in rice breeding for drought resistance, the method of direct identification of the natural drought environment in the field was used. Compared with the artificial indirect identification method, the direct identification method of the natural drought environment in the field is relatively simple, repeatable and suitable for large-scale identification. The method is generally applicable to the northwest region which receives less natural precipitation, including Gansu, Ningxia and Xinjiang. In addition, the drought-resistant germplasm and QTLs discovered by field drought stress identification are objective and reliable and can be effectively used in rice breeding.

In this study, LRI, LWD and chlorophyll content (SPAD) of the RILs were continuously distributed under natural drought stress conditions and were controlled by multiple genes, which was consistent with the results of previous studies (Bing et al. 2018). With increases in drought stress, the LRI and LWD values of the RILs increased. The correlation analysis showed that there was a significant negative correlation between the SPAD value and LRI and LWD, indicating that the higher the chlorophyll content was, the stronger the drought resistance. This demonstrated that chlorophyll content could be used as another important index for measuring drought resistance, which is consistent with the results of Song et al (2007).

QTLs for drought tolerance traits

Champoux et al. (1995) detected 5 QTLs controlling LRI on 3,4 and Chromosome 8 using the C039/Moroberekan RI population: RZ394-RZ576H, RG482-RG910, RG214-RG476C, RG190-RZ69, and

RG190-RZ649. RZ394-RZ576H and RG482-RG910 were located on Chromosome 3, RG214-RG476C and RG190-RZ649 were located on Chromosome 4, and RG1-RZ649 was located on Chromosome 8.

Courtois et al. (2000) detected two QTLs controlling LWD at RG172-RG563 and RG1094-RG167 on Chromosome 10 using a DH population of IR64/Azucena. Six QTLs controlling LRI were detected in the RM16-ID3010700 interval of Chromosome 3, the RM127-RM280 and the S04085-RM451 intervals of Chromosome 4, the ID8003584-RM331, the CMB0802.8-RM22420 and the S08003A-RM22321 intervals of Chromosome 8. Nine QTLs controlling LWD were detected in the ID3010700-AD03013905 interval of Chromosome 3, the RM307-ID4003524, RM252-AD04009559, S04097A-RM255 and S04070C-RM273 interval of Chromosome 4, RM253-RM276 and RM217-RM253 interval of Chromosome 6, RM447-GW8-AG and S08105-RM447 interval of Chromosome 8, RM311-ID10002842 and ID10003706-CMB1016.4 interval of Chromosome 10 (Supplementary Table 3 and Supplementary Table 4). The location of QTLs for LRI and LWD on specific chromosomes was consistent with the findings of Champoux et al. (1995) and Courtois et al. (2000). Price et al. (2002) detected four QTLs controlling LRI in the Chromosome 5 C624-C43 marker using Bala/Azucena F6 population and one QTL controlling LWD near chromosome 12 RG543. Courtois et al. (2000) detected one QTL controlling LRI in the RZ67-RZ70 marker range in the Chromosome 5 and one QTL controlling LWD in the SDH1-RG463 marker Range of Chromosome 12. QTLs for LRI and LWD were repeatedly detected in RM6-RM240 of Chromosome 2, ID5010886-CMB0526.3 of Chromosome 5, and S12099-CMB1226.0 of Chromosome 12 (Table 7). Compared with Price et al. (2002) and Courtois et al. (2000), the results showed that C624-C43, RZ67-RZ70 and ID5010886-CMB0526.3 were in the same region, and RG543, SDH1-RG463 and S12099-CMB1226.0 were in the same region at the end of the Chromosome 12. QTLs controlling LWD were also repeatedly located at RM6-RM240 of Chromosome 2, which was consistent with the finding of Ray et al. (1996) that there was a QTL at RG73-RG322 controlling root morphology detected on Chromosome 2 using the C039/Moroberekan RI population.

QTLs Controlling LRI and LWD were repeatedly detected in the RM315-S01167A and AH01001843-RM302 marker intervals, *qLRI1-1J*^{C} and *qLWD1-2*^{B} were located in the RM315-S01167A marker intervals and explained 7.96%-11.15% of the genetic variation. This region was in the same location as RM472-RM104 and RG109-ME10 of Hemamalini et al. (2000) and Babu et al. (2003). *qLRI1-1* and *qLWD1-4* were located within the marker interval AH01001843-RM302, contributed 4.90%-10.30% to the phenotypic variation and was the main QTL. However, the distance of this marker is large and it requires further study in the future.

Meta-QTLs analysis on Chromosome 1

In recent years, many studies have reported the detection of QTLs associated with various drought tolerance traits on Chromosome 1 (Fig. 7). Singh et al. (2016) found that *qDTY1.1* affected grain yield and Dixit et al. (2014) located QTLs associated with flowering days (*qDTF1.1*) and plant height (*qDTH1.1*) under drought stress conditions. *qRL1* was related to root length under drought stress conditions and was identified by Jiang et al. (2016) on RM302-RM476B, RM476B-RM315 and RM472-

RM104. Liu et al. (2013) mapped a QTL related to root length under drought stress conditions using RM220-RM490. Han (2018) mapped qSH1 and qRL1 related to seedling height and root length under drought stress in RM265-RM3482 and RM220-RM490. Zou et al. (2014) found that *QTLqGV1* was related to seed germination under drought stress at RM1350-RM570. According to Fig. 7, the *qLRI1-1* and *qLWD1-4* found in this study can be co-located with *qDTY1.1*, *qDTF1.1*, *qDTH1.1*, *qSH1*, *qRL1* and *qGV1* in the same region on Chromosome 1, indicating that LRI and LWD are related to SH, RL and GV. QTLs for SH, RL and GV have been shown to affect drought resistance in rice, indicating that *qLRI1-1* and *qLWD1-4* are closely related to drought resistance. However, the marker interval was relatively large (30.19 cm) and requires further study.

Declarations

Acknowledgements

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Author contributions

LC conducted field work, generated the phenotypic data, performed the data analysis and wrote the manuscript; JM generated the phenotypic and genotypic data; XM performed the genotyping of the mapping population; DC assisted with field work; BH assisted with field work; JS designed the research and implemented manuscript revisions; LH designed the experiment and guided the experiments and manuscript revisions. All authors read and approved the final version of the manuscript.

Data availability

All data included in this study are available upon request. Please contact with the corresponding author.

Conflict of interest

The authors declare that they have no competing interests.

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Figures

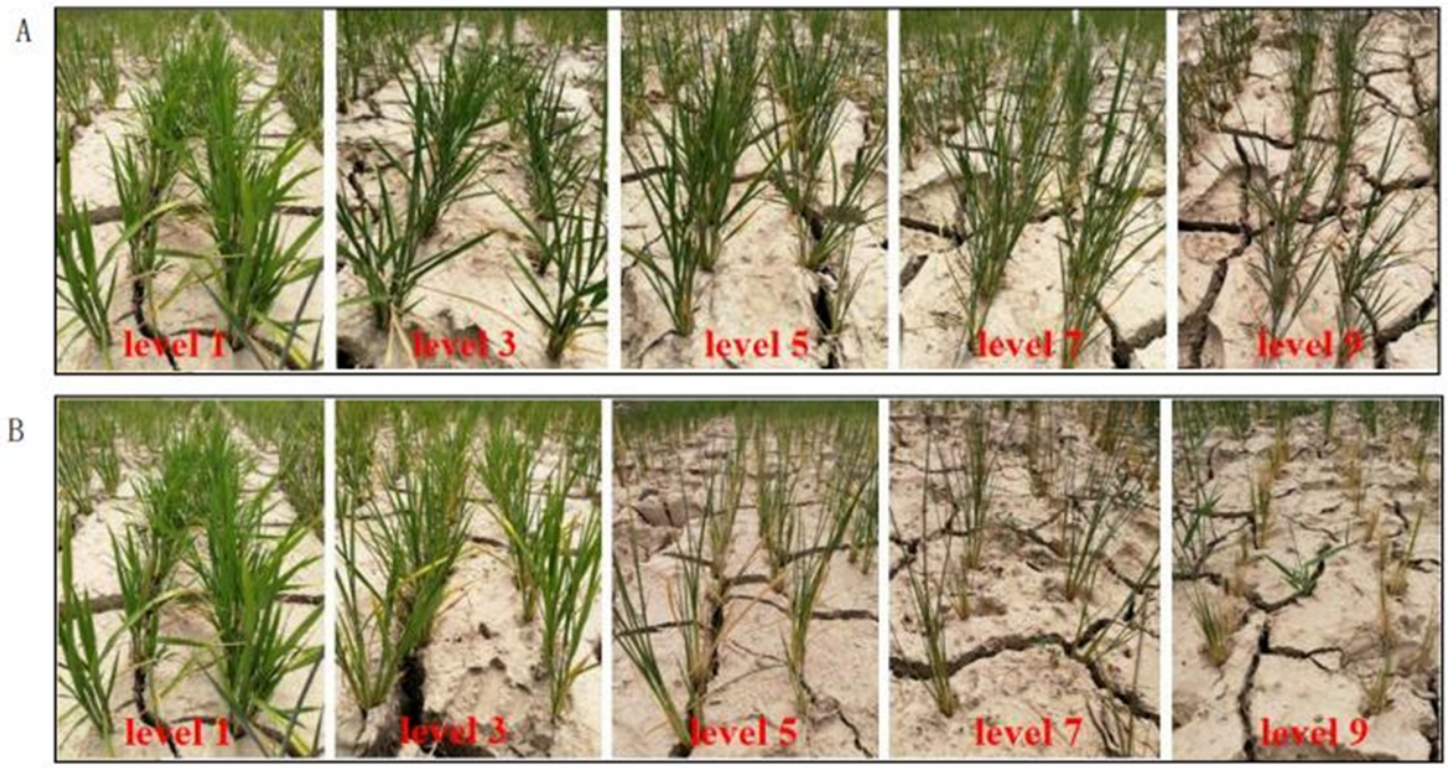

Figure 1

Field grading reference standard (A for LRI and B for LWD)

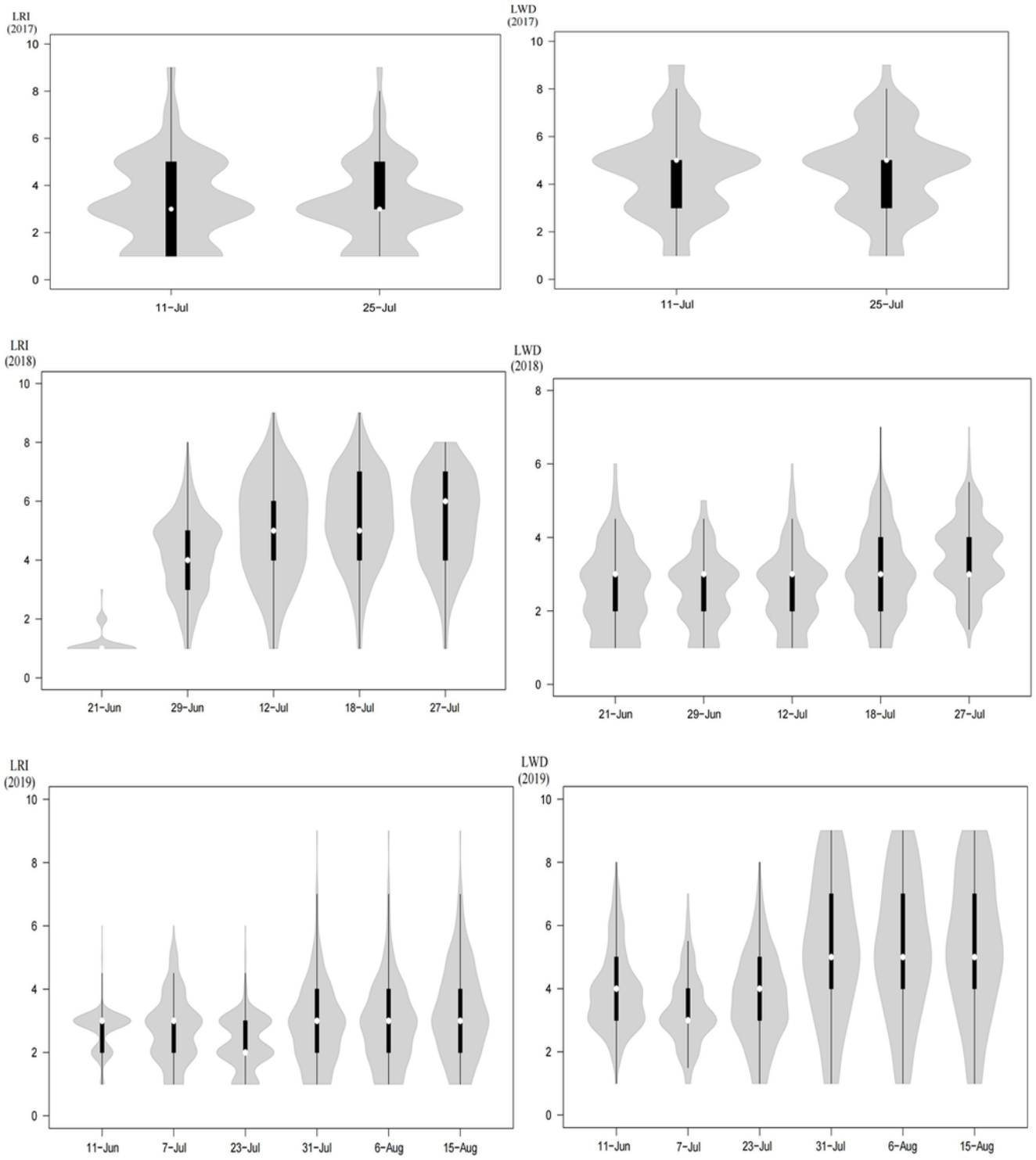

Figure 2

Violin plots of frequency distributions for each trait in the recombinant inbred line (RIL) populations (Spitzer et al. 2014).

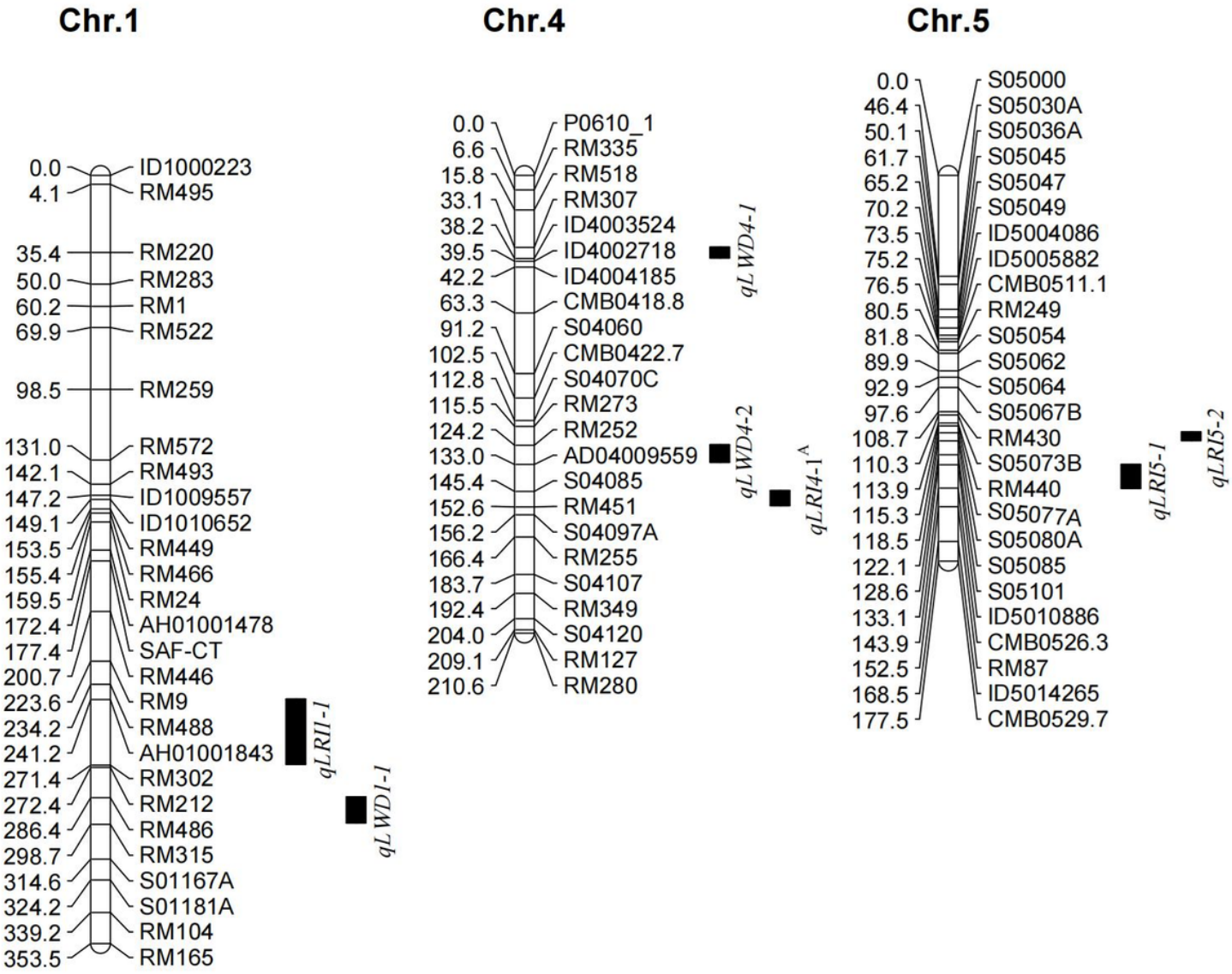

Figure 3

Quantitative trait locus (QTL) analysis of vegetative growth stage traits and leaf rolling index (LRI) and leaf withering degree (LWD) for JL1/MY23 RIL populations under field drought stress in 2017. 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. The superscript A stands for twice detected.

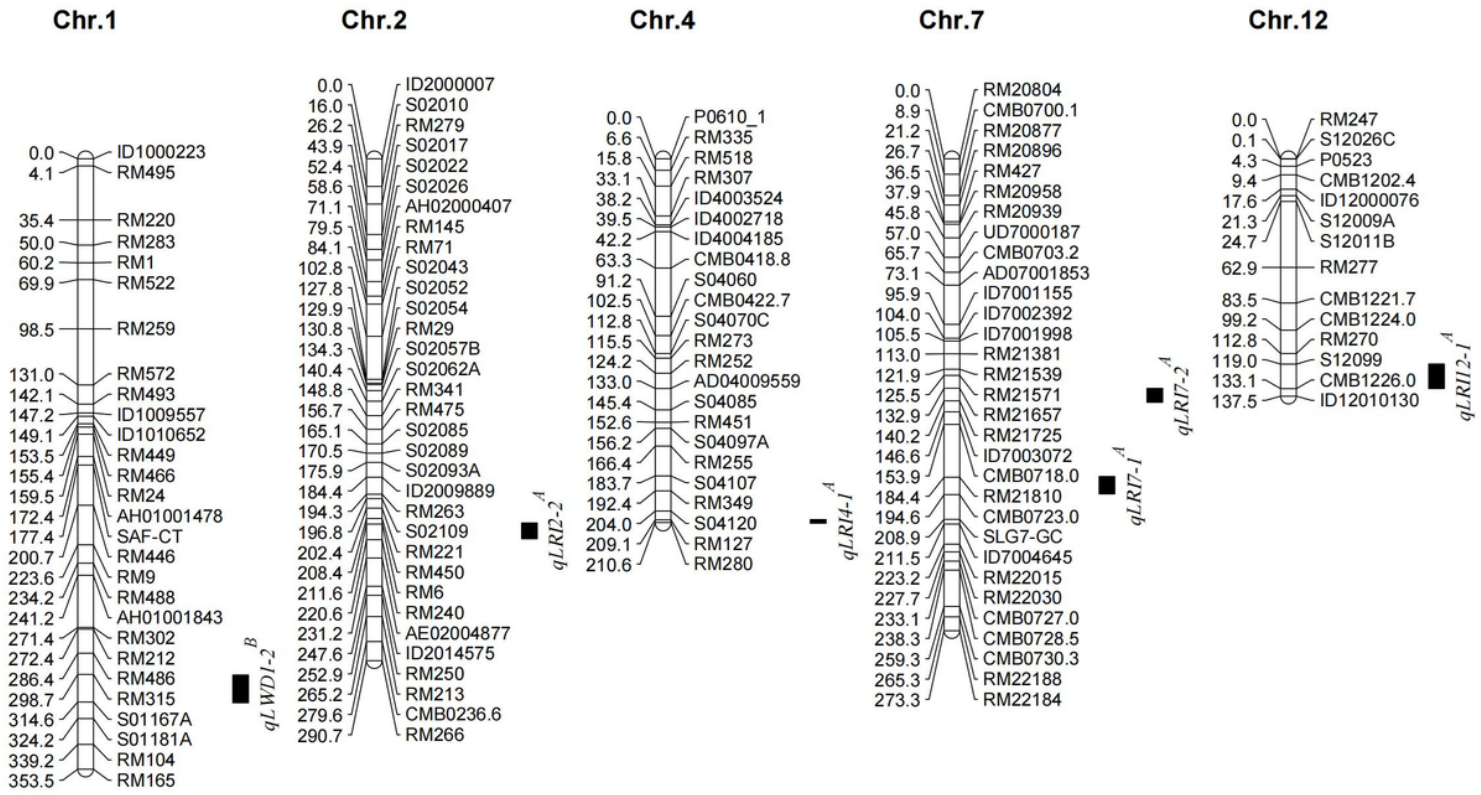

Figure 4

Quantitative trait locus (QTL) analysis of vegetative growth stage traits and leaf rolling index (LRI) and leaf withering degree (LWD) for JL1/MY23 RIL populations under field drought stress conditions in 2018. 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. The superscript A stands for twice detected, B stands for 3 detected, and C stands for 4 detected.

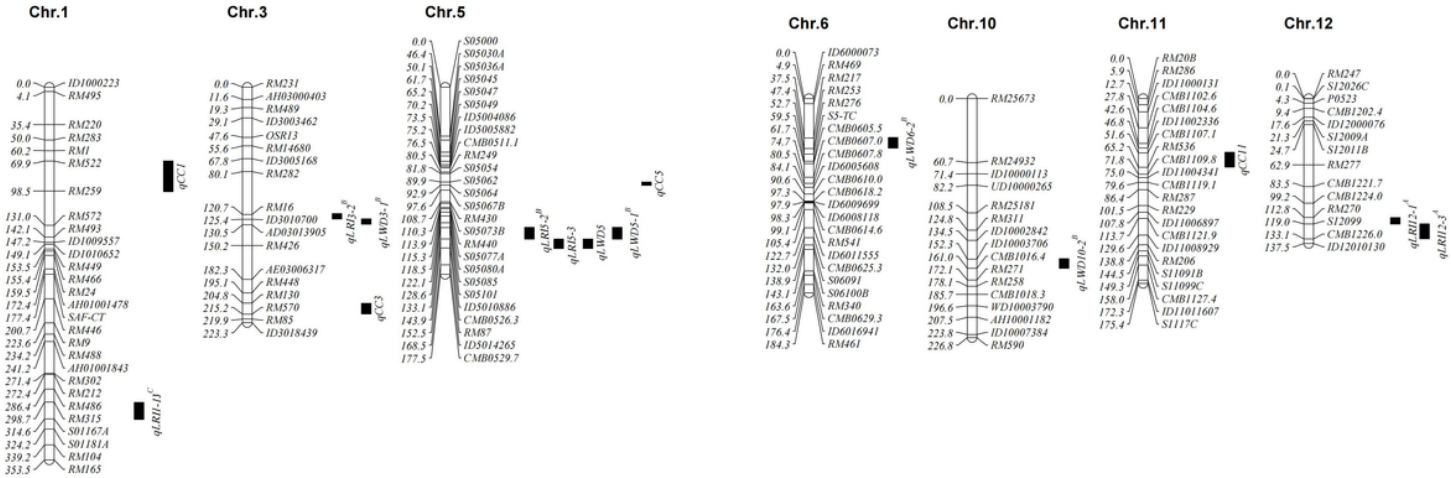

Figure 5

Quantitative trait locus (QTL) analysis of vegetative growth stage traits and leaf rolling index (LRI) and leaf withering degree (LWD) for JL1/MY23 RIL populations under field drought stress conditions in 2019. 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. The superscript A stands for twice detected, B stands for 3 detected, and C stands for 4 detected.

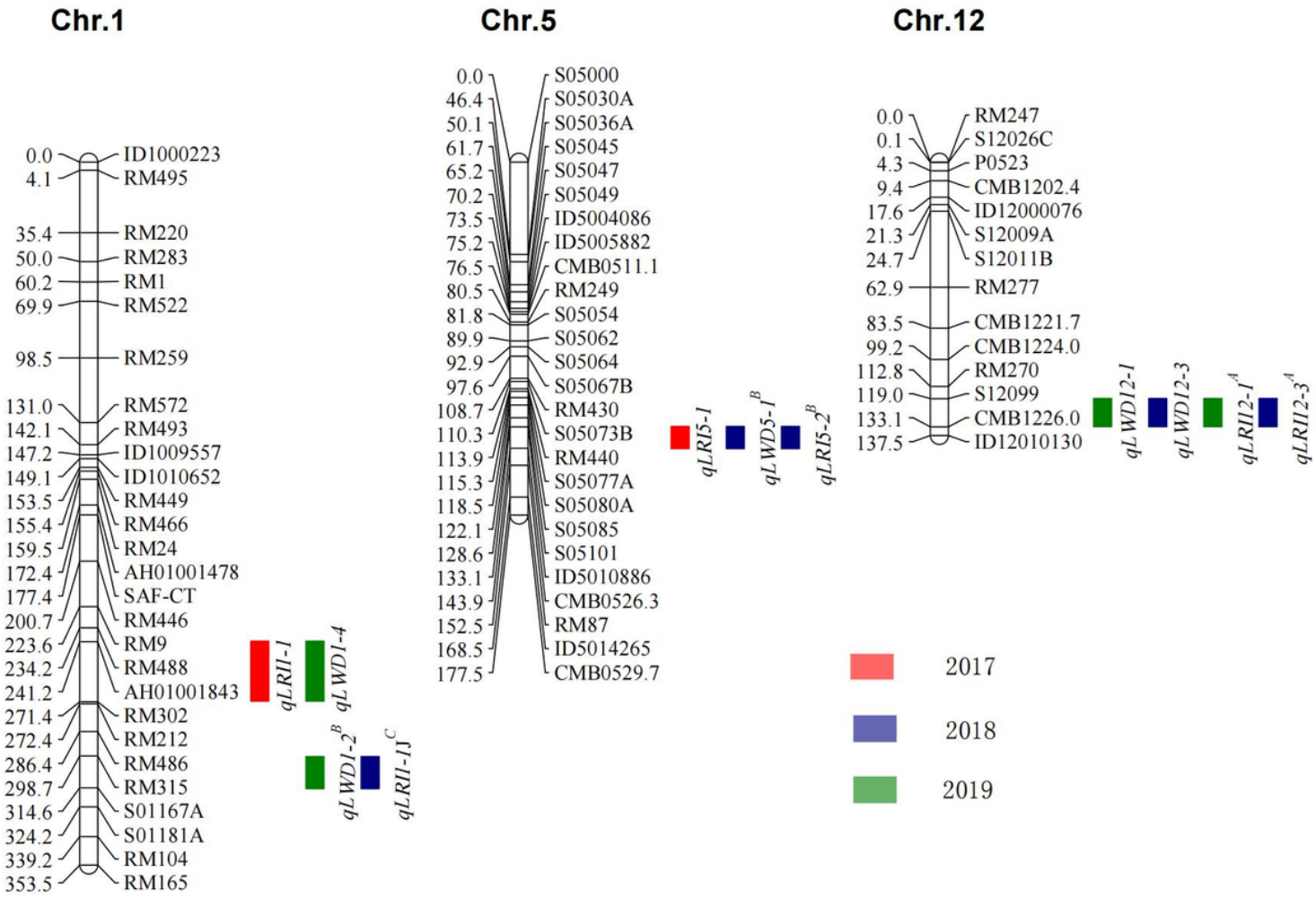

Figure 6

Genetic linkage map of RIL, with map positions of QTLs for the LRI and LWD under drought stress conditions. Red, blue and green represent 2017, 2018 and 2019, respectively.

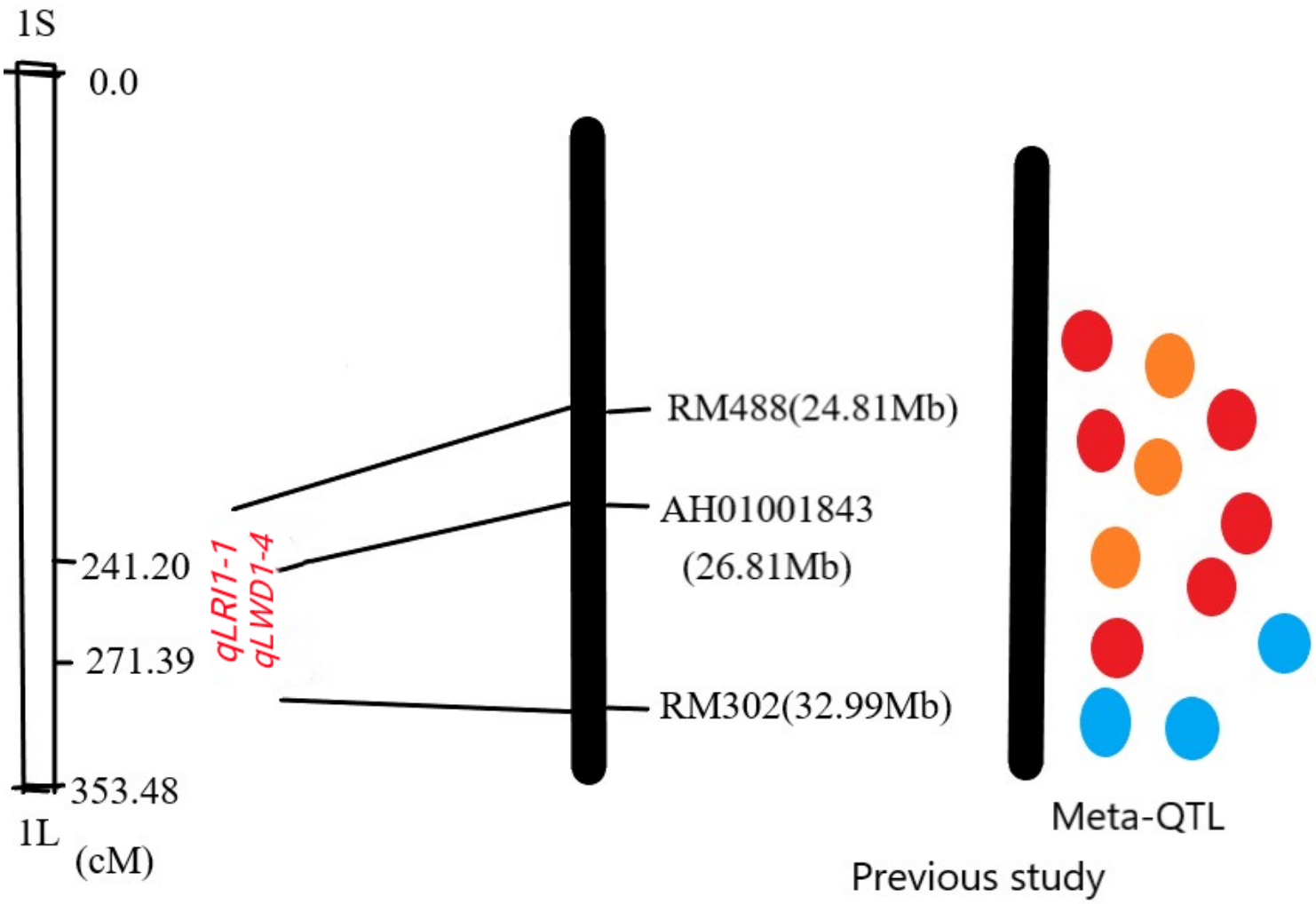

Figure 7

The meta-analysis of all QTLs on Chromosome 1. Gray circles: QTLs for variety-harnessing reported by Singh et al. (2016) and Dixit et al. (2014). Red circles: QTLs for drought tolerance in the seedling stage reported by Jiang et al. (2016)Liu et al. (2013) and Zou et al. (2014). Blue circles denote the QTLs for drought tolerance in the booting stage reported by Han et al. (2018) (Color figure online).