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RESEARCH ARTICLE

Genetic structure of Fe toxicity tolerance in Iranian rice (*Oryza sativa* L.) inbred lines population at seedling stage

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ABSTRACT: Rice is the world's most important staple food and will continue to be so in the coming decades. Ferrous iron is essential for rice growth. A mapping population of 96 rice inbred lines derived by Neda (NAD) and Ahlemitarom (ATM) cross, was used to detect quantitative trait loci (QTLs) for fresh biomass (FB), root length (RL), shoot length (SL), root number (RN), leaf width (LW), root fresh weight (RFW), root dry weight (RDW) and Fe content (FC) under Fe toxicity condition in rice. Two parents and 96 inbred lines were evaluated for the traits by growing them under normal and Fe toxicity nutrient solution. Under stress condition, two QTLs were detected for FB on chromosome 10, with LOD of 2.859, and 2.465. Twelve QTLs were identified for RL on chromosomes 2, 4, 5, 6, 7, 8, 9, 10, and 12. Three QTLs were detected on chromosomes 6, 7, and 8 for RN, and two QTLs for RDW on chromosomes 2 and 9. One QTL controlling LW, RFW, and FC was located on chromosomes 10, 9, and 1, respectively. The other QTLs for FB, SL, and RN was located on chromosomes 12, 12, and 3 under normal condition, with respective contributions of 9.7, 10, and 9.9, respectively. qLWN-2, qLWN-7, and qLWN-12 were located for LW on chromosomes 2, 7, and 12. These QTLs, due to the high percentage of explanation after validation, are a good candidate for marker-assisted selection programs with the help of markers in the rice population.

KEYWORDS: Stress, QTL, Mapping, QTL, Marker-assisted selection.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important crops for human nutrition and trace elements. Improving production efficiency is a key approach to boosting rice grain output in the face of a fast-growing global population. Rice production capacity is limited by a variety of abiotic and biotic stressors [20].

Ferrous iron is an essential element for rice since it is involved in several physiological and metabolic processes. This element is a crucial cofactor for many enzymes and a significant structural motif for transcriptional regulatory proteins at trace levels. However, due to industrial and natural processes, excessive levels of ferrous iron induce heavy metal toxicity, which has a significant impact on rice growth and quality [18].

Rice production in tropic and subtropic regions is hampered by iron toxicity. In extremely aerobic conditions, acid sulfate soils, or acid soils, excess ferrous iron accumulates in the shoots, causing leaf discoloration and root loss. Resistant cultivars are a cost-effective and long-term option for increasing grain yields in Fe-stressed conditions. Several genes affect the genetic diversity of Fe toxicity tolerance in rice [5, 6]. Detecting stable QTLs with a large effect that influences complex characteristics

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under Fe toxicity circumstances is a difficult task nowadays. QTLs with a major effect, which control complex traits under Fe toxicity conditions, is a challenge. Wu *et al.* (1997) used a doubled haploid population formed from Azucena and IR64 under culture solution conditions to identify the first QTL for resistance to Fe toxicity [31]. Three QTLs for leaf bronzing symptom score and relative reduction in dry shoot weight were discovered, with phenotypic contributions ranging from 10% to 32%.

Various genetic populations, such as backcross inbred lines [27, 30, 4], recombinant inbred lines [4, 5, 27, 29, 30], chromosome segment substitution lines [9], doubled haploids [31, 32], wild rice accessions of *O. glaberrima* [4], and F3 and F2 introgression lines [8, 21, 22, 28] have been used to map a high number of QTLs for iron toxicity tolerance. Those 203 QTLs were found to be distributed mainly on seven chromosomes [1, 11, 13, 17, 21, 26, 35]. There were between 1 and 39 QTLs found, with phenotypic variation ranging from 4.2% (qCER) to 47.2% (qSDW) [19, 27]. DHs, RILs, and F3 and F2 introgression lines were used to determination of iron toxicity major QTLs in various genetic backgrounds, and they were mostly mapped on chromosomes 2, 4, 6 [11, 21, 35]. The QTLs on chromosomes 7, 5, 3, and 1 were identified using various genetic resources [6, 7, 29]. qLBI, qSR, qRDW, qTN, and qNPQ on chromosome 1, qSIC on chromosome 2, SFe and qBFe on chromosome 3, and qCCI on chromosome 7 showed a high phenotypic variation [7,31]. Wan et al. (2003a) discovered certain QTLs with large impacts on leaf bronzing, shoot and root biomass, and tiller number (from 20 to 48%) [27]. Wan et al., (2003b) also discovered fourteen QTLs with chromosomal CSSLs population that generated 11 to 28% phenotypic variation in leaf bronzing, plant height, stem dry weight, root dry weight, and root length in Asominori and IR24 crosses [28]. Ouyang *et al.* (2007) discovered numerous QTLs that impacted coleoptile elongation in the Zhenshan97B/Miyang46 population [19]. Under Fe toxicity, certain QTL for iron content of the shoot were reported on chromosomes 4 and 3 (Shimizu et al., 2005b), and it was discovered that this QTL on chromosome 3 co-located with a QTL for high iron content of the shoot [9]. A study found three and seven QTLs for leaf bronzing symptom score [30]. The QTLs on chromosomes 3 and 1 had additive effects on shoot tolerance and iron exclusion, respectively. In six regions of the rice genome, certain stable QTLs have been identified, which might represent important QTLs containing genes that influence iron toxicity tolerance [6]. Dufey *et al.,* (2015a) produced an integrated map that included all of the previously reported QTLs [4]. They highlighted the following four key genomic regions with a high-frequency QTL. Dufey *et al.,* (2015b) discovered iron toxicity tolerance QTLs in an interspecific backcross population of Caiapo/MG12/Caiapo crossings for the first time on chromosomes 1, 2, 3, 5, 7, and 10. Eleven QTLs were related to leaf bronzing score, shoot and root dry weight with explanations ranging from 5 to 18%, and seventeen QTLs were related to chlorophyll content, shoot water content, stomata conductance, non-photochemical quenching, the efficiency of photosystem II, sheath, blade, and root-plaque iron concentration with explanations ranging from 17 to 40% [7]. The finding of iron toxicity tolerance QTLs in rice genomic areas, as well as QTLs with substantial phenotypic explanation (up to 48%), will greatly increase iron toxicity tolerance breeding efficiency [5].

Different groups are exploiting the rice diversity panels' availability, some of which have previously been genotyped using thousands of SNPs [36], to do association mapping for iron toxicity tolerance. Currently, the use of molecular marker-assisted breeding for iron toxicity tolerance (by MSA) is severely limited since most QTLs reported are for minor impacts, and even for the few major ones, lack of validation in other genetic environments and backgrounds (actual field testing) or large confidence intervals are significant drawbacks [1].

Many populations have reported QTL mapping for several Iranian rice agronomic characteristics; however, genes affecting iron toxicity tolerance have yet to be found in the Iranian rice population. We used an Iranian RIL population derived from rice cultivars Ahlamitaroum × Neda to identify the QTLs associated with iron toxicity tolerance by analyzing seven agronomic traits: shoot length, fresh biomass, root number, root length, root fresh weight, leaf width, root dry weight, and iron content.

MATERIALS AND METHODS

Evaluations of iron toxicity tolerances at the seedlings stage were performed in turn in the greenhouse in the College of Agriculture Science and Natural Resource of Gonbad Kavous University.

Two cultivars (*Oryza sativa* L.) Neda (NAD) and Ahlemitarom (ATM) were chosen as parental varieties. Since "NAD" is susceptible to iron toxicity and "ATM" is tolerant [16]. From a cross between NAD and ATM, 94

lines F8 generation were derived, and were used in this study.

Completely randomized design (CRD) consisting of 96 lines with 2 replicates were applied for both control and stress conditions. For iron toxicity experiment, the temperature was around 32/25 ℃ (day/night) and the relative humidity was ~75%.

The seeds were placed at 50 ˚C for three days to break dormancy, then sterilized with 5% sodium hypochlorite solution for 20 min and rinsed well with distilled water. Then seeds were soaked in distilled water in the dark at 30 ℃ for 48 h. Finally, 10 uniformly germinated seeds of each accession were directly sown in holes of perforated styrofoam sheets (10 lines × 13 rows) with a nylon net bottom in a plastic container according to Gregorio et al., 1997. The styrofoam sheets were allowed to float on the water for up to five days and then transferred to Yoshida solution [33] for five days.

Its macronutrients were composed of 50 mg/L Si, 40 mg/L N, K, Mg and Ca and 10 mg/L P. Its micronutrients were provided with 2.0 mg/L Fe, 0.5 mg/L Mn, 0.2 mg/L B, 0.05 mg/L Mo, 0.01 mg/L Zn, and 0.01 mg/L Cu. The culture solution was renewed weekly and pH of the solution was adjusted to 5.0 with 1-N NaOH/HCl every day.

At the three-leaf stage, the Fe in the form of FeSO4.7H2O at the concentration of 300 mg/ L (5.36 mM) (2.0 mg/L for control) was applied. The pH of the solution was adjusted to 5.0 at the alternative day by 1 M NaOH/HCl. The solution was renewed every five days.

After plants were harvested, shoot length (SL) and root length (RL), fresh weight of root (FWR) and fresh weight of shoot (FWS), and root number (RN) were measured.

The concentration of Fe in shoot samples under stress conditions was determined by atomic absorption spectrometry (AAS, Series2, Thermo Electron Corporation) with the wet digestion method (GB/T 14609–2008). About 1 g of dried shoot samples from each line was digested with 5 ml mix acid (HNO3:HClO4 = 4:1, V/V) using a graphite liquation furnace. The heating process was as follows: 80 ℃ for 15 min, 120 ℃ for 20 min, 150 ℃ for 30 min and 180 ℃ for 60 min. Finally, the colorless or slightly yellow transparent liquid was diluted in a 100 ml volumetric flask with distilled water. For Fe determinations, calibration standard solutions were prepared by diluting 1000 μg/ml standard solution (NCS, China).

Forty SSR primer pairs, 16 ISSR markers (76 alleles), two IRAP markers (7 alleles), and one iPBS marker (3 alleles) were appropriately distributed on 12 rice chromosomes were chosen according to Chen et al (1997), Temnykh et al. (2000) and McCouch et al. (2002) [2, 15, 25]. ISSR, iPBS, and IRAP were used to check the rate of polymorphism from previous articles.

Polymerase chain reaction (PCR) was carried out in a total volume of 0.01 cm^{-3} containing 2 ng of template DNA, 39.2 μmol dm^{-3} of each primer, 117.6 mmol dm^{-3} of each dNTP, 156.8 mmol dm^{-3} $MgCl_2$, 19.6 unit of Taq polymerase, and 0.098 cm^3 of 10× PCR buffer. PCR amplification was performed on a thermal cycler (BIORAD, America) in the genetic laboratory of Gonbad University of Iran. PCR products were separated on 6% (m/v) polyacrylamide gels (38:2 acrylamide:bis-acrylamide) and detected by the fast silver staining method [12]. Using Mapmanager *QtbX17*, 12 linkage groups were constructed with a minimum LOD score of 2. Map distances between were presented in centi Morgan (cM) derived using the Kosambi function [14] of the program.

RESULTS

Frequency distribution

The frequency distributions of RILs and the values of both parents for average values of the eleven traits viz., SL, RL, FWR, FWS, FWR, FWS, RV, FB, DB, LA, and RN on seedling in rice showed in Figure 1. All of traits in two conditions were segregated continuously and approximately fit normal distributions that had absolute values of both skewness and kurtosis less than 1.0, indicating that studied attributes were suitable for mapping of QTLs analysis. These results demonstrate that all the eleven traits are quantitative traits controlled by polygenes and no major genes were contained. For all of the traits measured there is a clear difference between Ahlamitaroum and Neda. All the traits showed transgressive segregation, thereby implying the positive and negative genes for the traits scattered throughout the entire genome of rice. Correlation coefficients among the eleven traits were presented in Figures 1 and 2. Under both growth conditions, Fe was positively correlated with all attributes except RL under normal condition, but Fe

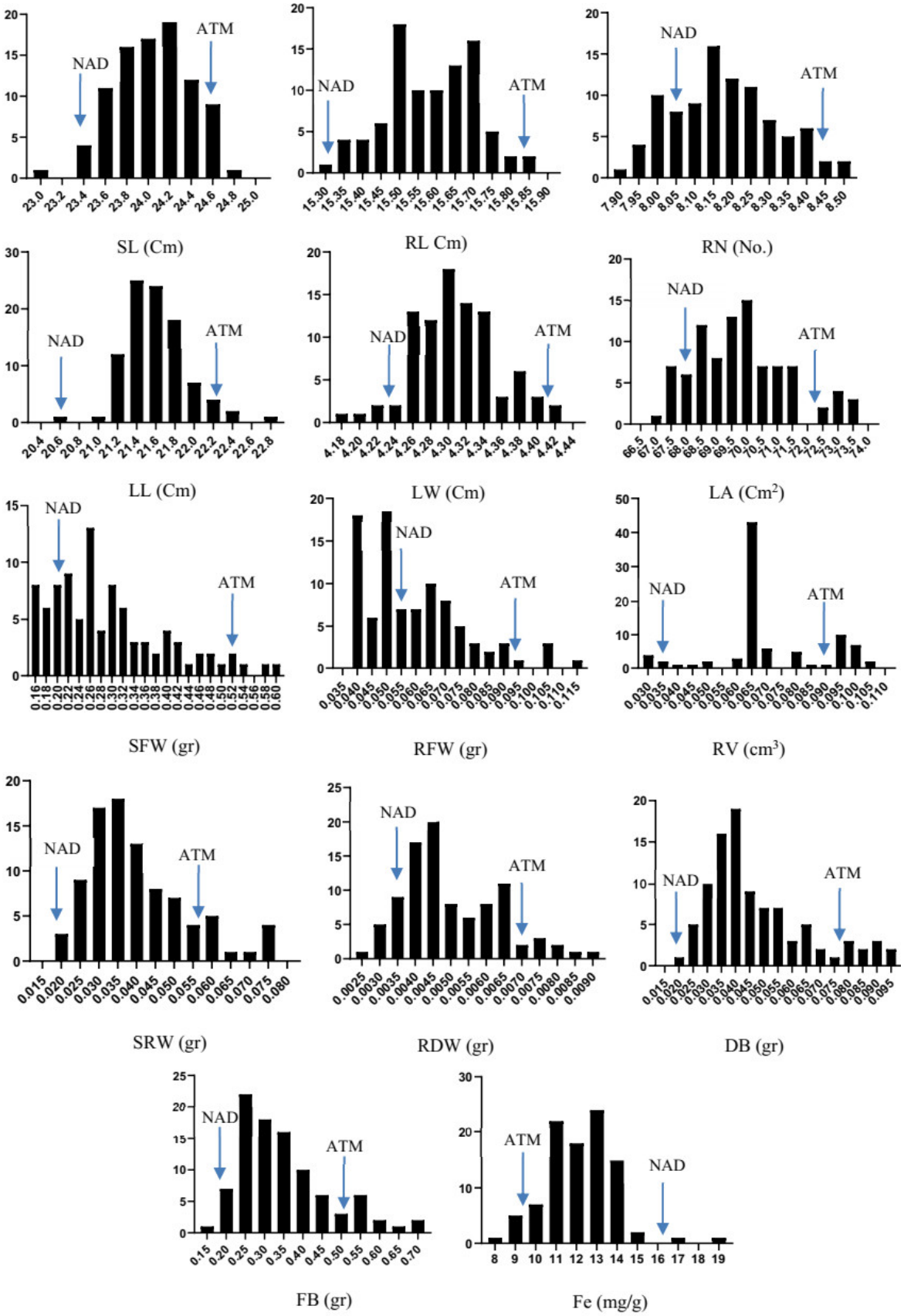

Figure 1. Frequency distribution of studied attributes in 96 F_8 rice recombinant inbred lines derived from ATM × NAD crosses under normal condition.

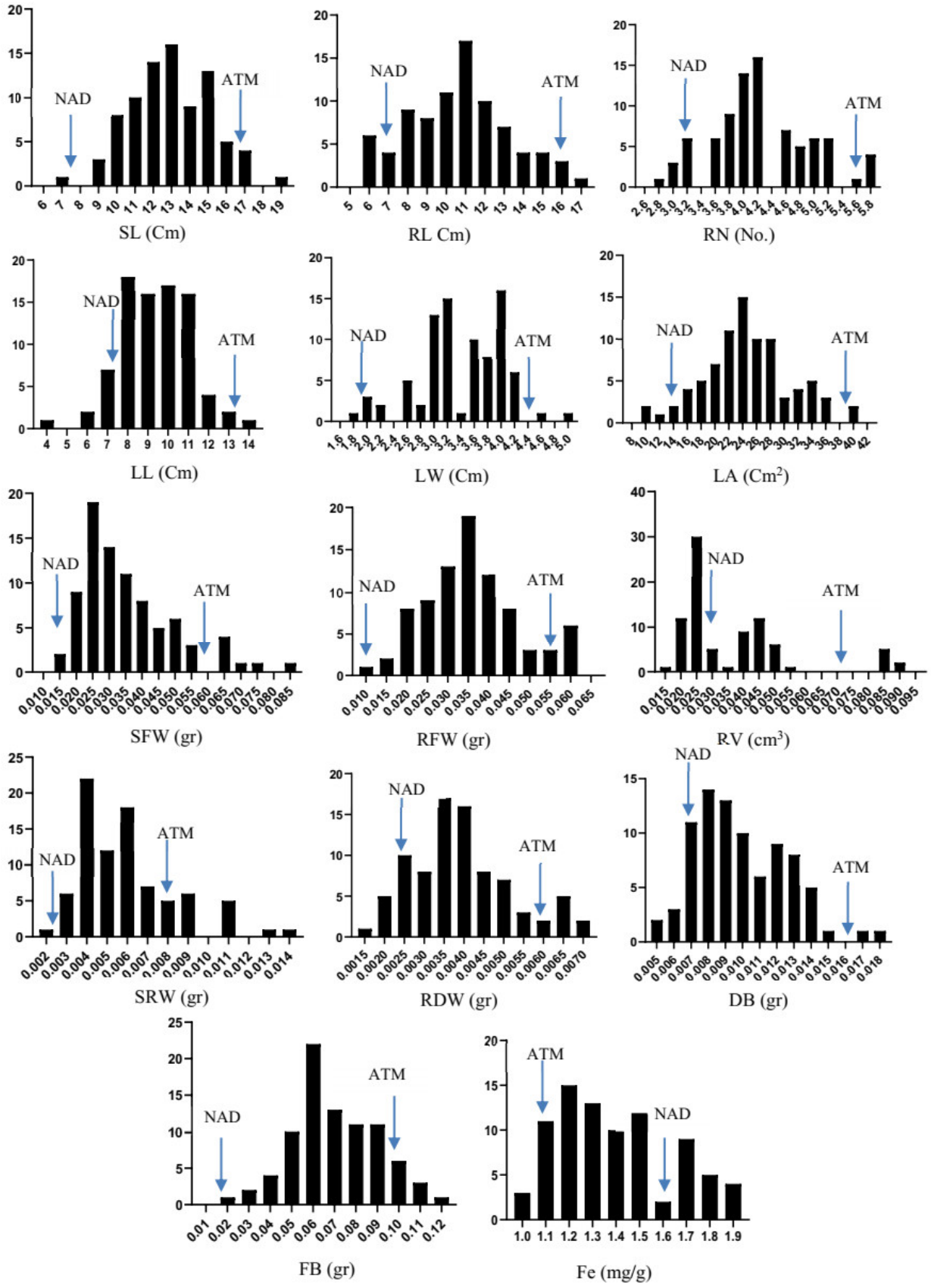

Figure 2. Frequency distribution of studied attributes in 96 F_8 rice recombinant inbred lines derived from ATM × NAD crosses under Fe toxicity condition.

was negatively correlated with all traits except LW and RN (Figure 3).

Mapping QTLs under normal and Fe toxicity conditions

Fresh biomass: Under normal condition, one QTL was detected for FB on chromosome 12. qFBN-12 explaining 9.7 % of the phenotypic variance. In terms of Fe stress, two QTLs were detected for fresh biomass on chromosome 10. Their additive effect was -0.015 and -0.037 gr, respectively (Figures 4 and 5).

Shoot length: Under normal condition, a QTL was detected for SL on chromosome 12. Parent NAD alleles have decreased this trait. QTL was not detected in stress conditions for this trait.

Root length: Under stress condition, 12 QTLs were identified for RL on chromosomes 2, 4, 5, 6, 7, 8, 9, 10 and 12. qRL-10a that explained of more than 20% for phenotypic variance and was close to the ISSR14-2 marker. QTL was not detected under normal condition for this trait.

Root number: Under normal condition, a QTL was detected for RN on chromosome 3. Its additive effect and LOD were 0.145 and 2.167 respectively. Under Fe stress condition, three QTLs were identified for root number on chromosomes 6, 7, and 8. qRN-6, qRN7 and qRN8 were close to the IRAP17-1, ISSR5-4 and RM281, respectively.

Leaf width: Under normal condition, three QTLs were identified on chromosomes 2, 7, and 12 for LW. Their additive effect was 0.024, 0.328, and -0.42, respectively. qLW-10 was located on chromosome 10 for leaf width under Fe stress conditions. It explained 16.1% of phenotypic variation. The parent AHT alleles increased this trait.

Root fresh weight: Under Fe stress condition, a QTL was found on chromosome 9 for RFW. This QTL was located between RM205 and ISSR 8-7 markers and justifies 10.9% of phenotypic variation. Under normal condition, no QTL was detected RFW under QTL control condition.

Root dry weight: Under Fe stress condition, two QTLs were located for RDW on chromosomes 2 and 9. Their additive effect was 0.001 and -0.001gr, respectively. qRDW-2 and qRDW-9 were able to explain 13.6 and 11.3% of the phenotypic variation of the trait.

Fe content: qFC-1 was detected on chromosome 1 for Fe content, in Fe stress. It was located between the RM10864 and ISSR13-2 markers and justifies 11.9% of phenotypic variation. Under normal condition, no QTL was detected Fe content under QTL control condition.

Fe is a necessary element for some biological processes such as photosynthesis, respiration and nitrogen assimilation [13]. In this study, Linkage map covered a total of 1419 cM with an average two locus interval of 13.07 cM.

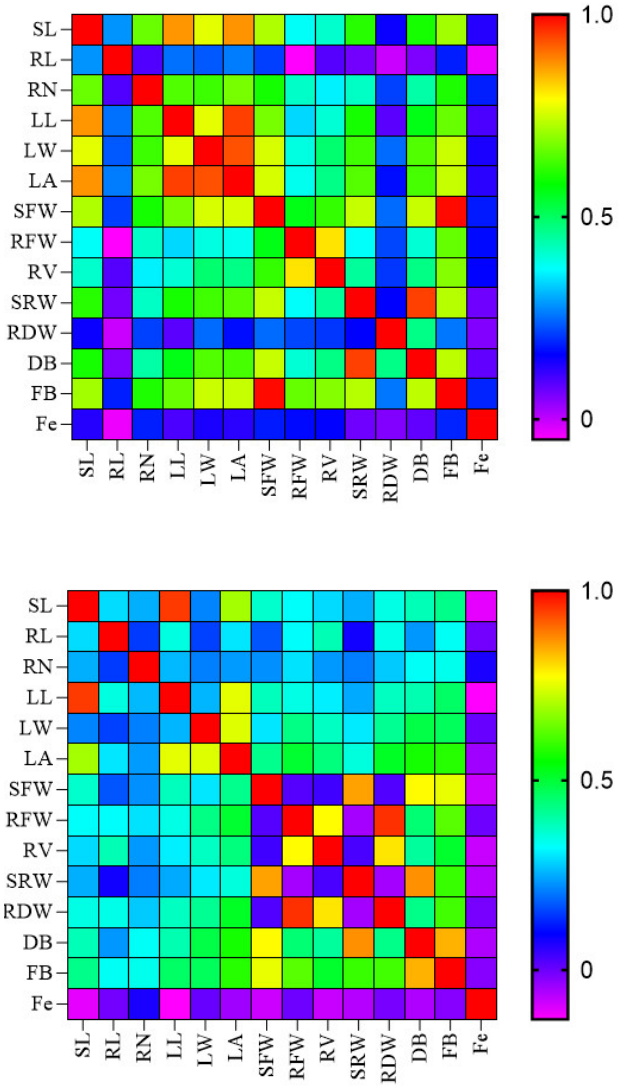

Figure 3. Graphical presentation of correlation coefficients among studied traits under normal (above) and Fe toxicity (down) conditions in seedling stage and 96 F_8 rice recombinant inbred lines derived from ATM × NAD crosses.

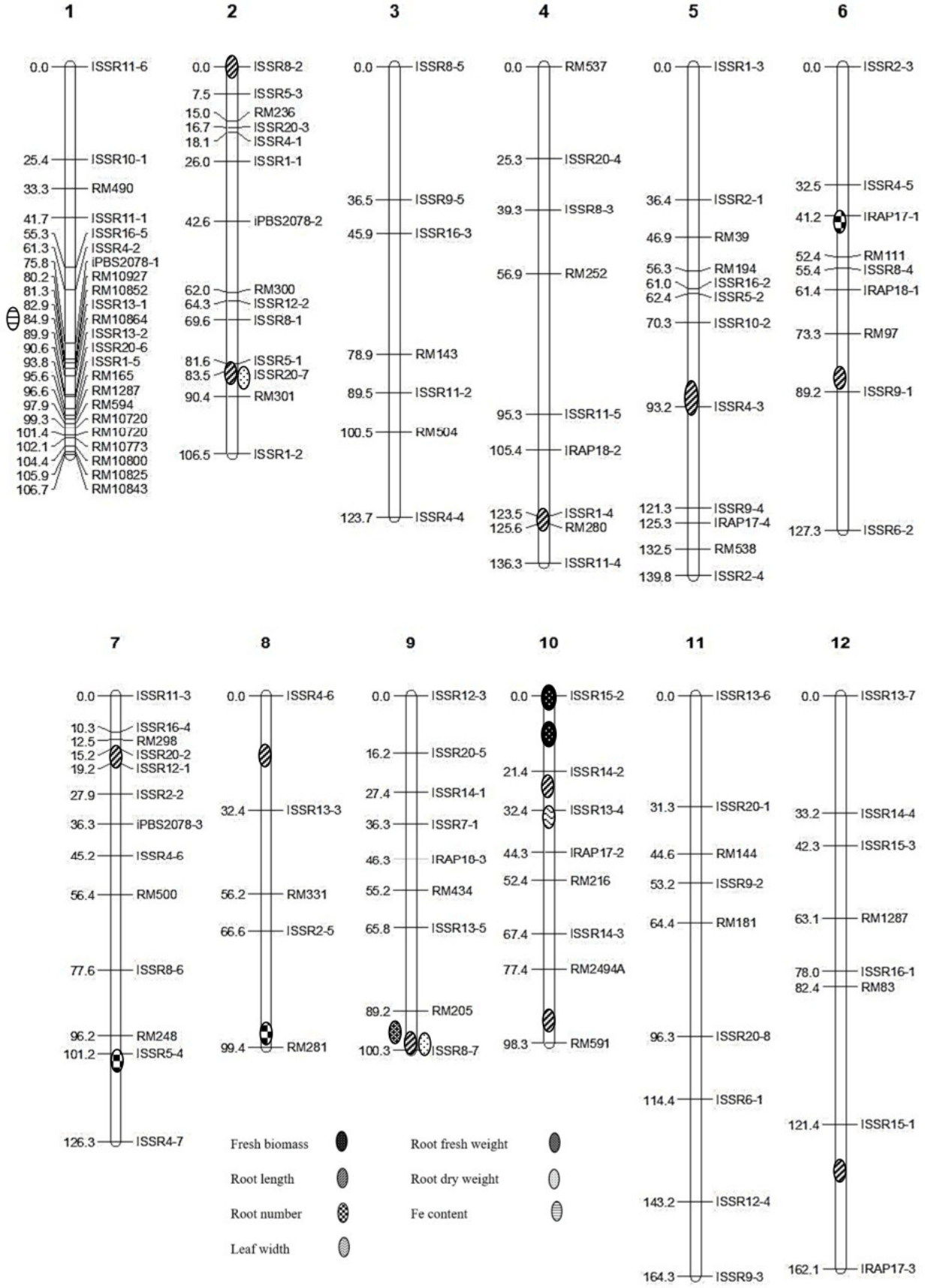

Figure 4. Genetic linkage maps and QTLs identified under normal conditions in seedling stage the F8 population derived from ATM × NAD.

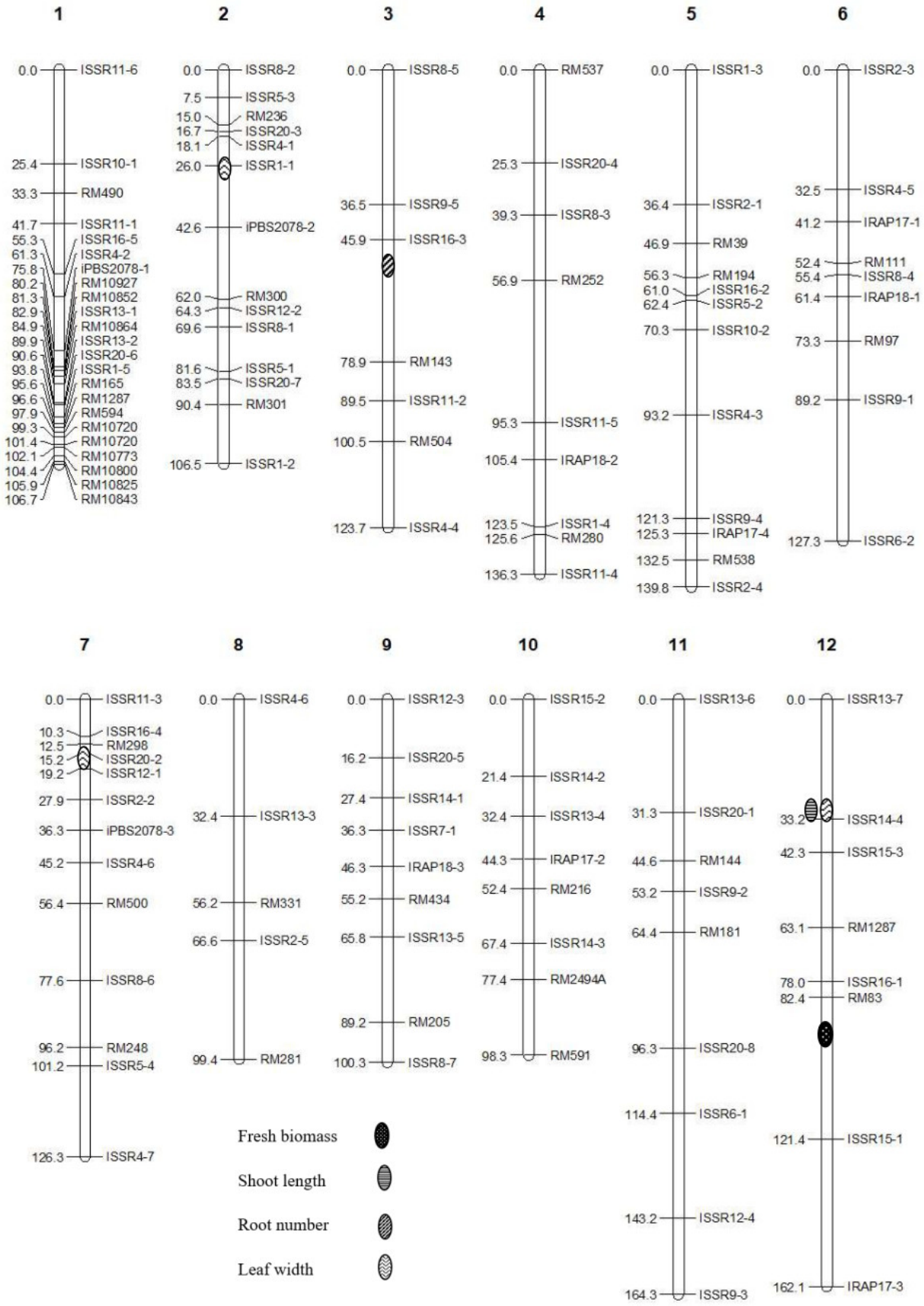

Figure 5. Genetic linkage maps QTLs identified under Fe toxicity conditions in seedling stage the F8 population derived from ATM × NAD.

Table 1. Putative QTLs for iron toxicity in seedling stage the F_8 population derived from Ahlemitarom (ATM; a tolerant to iron toxicity variety) and Neda (NAD; a susceptible to iron toxicity variety).

Traits	QTL	Chr.	Flanking markers	LOD	Position	Additive effect	R^2	Direction of ph.
Fresh biomass	qFB-10a	10	ISSR15-2	2.859	0	-0.015	14.5	NAD
	qFB-10b	10	ISSR15-2-ISSR14-2	2.465	10	-0.037	12.6	NAD
Root length	qRL-2a	2	ISSR8-2	2.133	0	-0.992	11.0	NAD
	qRL-2b	2	ISSR20-7-RM301	2.015	84	1.145	10.5	ATM
	qRL-4	4	ISSR1-4-RM280	2.386	124	1.197	12.3	ATM
	qRL-5a	5	ISSR10-2-ISSR4-3	2.243	92	-1.004	11.6	NAD
	qRL-5b	5	ISSR4-3-ISSR9-4	2.243	94	-0.965	11.6	NAD
	qRL-6	6	RM597-ISSR9-1	3.006	88	-4.945	15.2	NAD
	qRL-7	7	ISSR20-2-ISSR12-1	3.198	16	-21.144	16.1	NAD
	qRL-8	8	ISSR4-6-ISSR13-3	2.384	18	-2.028	12.3	NAD
	qRL-9	9	RM205-ISSR8-7	2.667	100	-1.214	13.6	NAD
	qRL-10a	10	ISSR14-2-ISSR13-4	4.442	26	-4.239	21.5	NAD
	qRL-10b	10	RM294A-RM591	3.383	90	-2.15	16.9	NAD
	qRL-12	12	ISSR15-1-IRAP17-3	3.023	130	3.339	15.3	ATM
Root number	qRN-6	6	IRAP17-1-RM111	2.784	44	-0.472	14.2	NAD
	qRN-7	7	ISSR5-4-ISSR4-7	2.391	104	-0.538	12.3	NAD
	qRN-8	8	ISSR2-5-RM281	2.381	98	0.122	12.2	ATM
Leaf width	qLW-10	10	ISSR13-4-IRAP17-2	3.202	34	0.650	16.1	ATM
Root fresh weight	qRFW-9	9	RM205-ISSR8-7	2.112	98	-0.006	10.9	NAD
Root dry weight	qRDW-2	2	ISSR20-7-RM301	2.659	84	0.001	13.6	ATM
	qRDW-9	9	RM205-ISSR8-7	2.197	100	-0.001	11.3	NAD
Fe content	qFC-1	1	RM10864-ISSR13-2	2.317	86	1.432	11.9	NAD

Table 2. Putative QTLs for normal condition in seedling stage the F_8 population derived from Ahlemitarom (ATM; a tolerant to Fe toxicity variety) and Neda (NAD; a susceptible to Fe toxicity variety).

Traits	QTL	Chr.	Flanking markers	LOD	Position	Additive effect	R^2	Direction of ph.
Fresh biomass	qFBN-12	12	RM83-ISSR15-1	2.134	104	-0.113	9.7	NAD
Shoot length	qSLN-12	12	ISSR13-7-ISSR14-4	2.190	32	-1.863	1.0	NAD
Root number	qRNN-3	3	ISSR16-3-RM143	2.167	56	0.145	9.9	ATM
Leaf width	qLWN-2	2	ISSR1-1	2.093	26	0.024	9.6	ATM
	qLWN-7	7	ISSR20-2-ISSR12-1	2.221	16	0.328	10.1	ATM
	qLWN-12	12	ISR13-7-ISSR14-2	2.085	32	-0.420	9.5	NAD

Dong et al. (2006) and Stein et al., (2009) were mapped three QTLs associated with Zn^{2+} toxicity tolerance on chromosomes 1, 3 and 10 [3, 24]. qZNT-1 explained 21.9% of the total phenotypic variation and showed the largest effect on the trait. In this study, a QTL was identified for the Fe content on chromosome 1 under Fe toxicity conditions. qFC-1 was located near the RM10864 markers with a LOD value of 2.317 and explained 11.9% of the total phenotypic variation (Table 1).

Dufey et al. (2015b) have reported some QTLs for SDW and RDW on chromosomes 1, 3, 5, and 12. In this study, we detected QTLs related to RDW in the seedling stage. These QTLs were mapped on chromosomes 2 and 9. qRDW-2 and qRDW-9 explained 13.6% and 11.3 % of the total phenotypic variation, respectively [7].

Zebeau and Vos (1993) showed that correlated traits are often controlled by QTLs that are located on chromosomes in similar regions [34]. In the present study, under normal conditions, the QTL associated with SH and LA in the region of ISSR13-7-ISSR14-4 chromosome 12 overlapped and had a positive and significant correlation (0.765^{**}). According to the results under normal conditions, it seems that there is a significant relationship owing to pleiotropy or the relationship between the genes controlling the traits. qRL-9 and qRDW-9 were found at the same map locations in chromosome 9, but they had low and negative significant correlations (Table 2).

CONCLUSION

QTL analysis helped to identify several major QTLs that are of potential value for the improvement of Fe toxicity tolerance in rice. Under stress condition, five QTLs were detected for root length on chromosome 6, 7, 10, and 12, and their contributions to whole variation were 15.2%, 16.1%, 21.5%, 16.9% and 15.3, respectively. One QTL detected on chromosomes 10 for leaf width. It explaining

16.1% of phenotypic variation. These QTLs, due to the high percentage of justification after validation, could be a good candidate for marker-assisted selection programs with the help of markers in the desirable genetic background of rice.

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ساختار ژنتیکی تحمل به سمیت آهن در جمعیت لاین‌های نوترکیب برنج ایرانی در مرحله گیاهچه‌ای

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چکیده

برنج مهمترین غذای اصلی جهان است و نیاز به آن در دهه‌های آینده نیز ادامه خواهد داشت. آهن برای رشد برنج ضروری است. در این پژوهش، یک جمعیت مکان‌یابی شامل ۹۶ لاین خالص نوترکیب برنج مشتق شده از تلاقی ندا (NAD) و اهلمی طارم (ATM) برای شناسایی مکان های ژنی کنترل کننده صفات کمی (QTLs) مانند زیست توده (FB)، طول ریشه (RL) ، طول ساقه (SL) ، تعداد ریشه (RN)، عرض برگ (LW) ، وزن تر ریشه (RFW) ، وزن خشک ریشه (RDW) و محتوای آهن (FC) تحت شرایط سمیت آهن استفاده شد. دو والد و ۹۶ لاین اینبرد در شرایط رشدی با محلول غذایی نرمال و سمیت آهن مورد ارزیابی قرار گرفتند. در شرایط استرس، دو QTL برای FB در کروموزوم ۱۰، با LOD برابر ۲/۵۸۹ و ۲/۴۶۵شناسایی شدند. دوازده QTL برای RL در کروموزوم‌های ۲، ۴، ۵، ۶، ۷، ۸، ۹، ۱۰ و ۱۲ شناسایی شدند. سه QTL در کروموزوم های ۶، ۷ و ۸ برایRN و دو QTL برای RDW در کروموزوم‌های ۲ و ۹ شناسایی شد. یک QTL برای کنترل LW ، RFW و FC به ترتیب بر روی کروموزوم های ۱۰، ۹ و ۱ ردیابی شدند. QTLهای دیگر برای FB، SL و RN روی کروموزوم های ۱۲، ۱۲ و ۳ در شرایط نرمال قرار داشتند و به ترتیب ۹/۷، ۱۰ و ۹/۹ تغییرات را کنترل کردند. qLWN-2، qLWN-7 و qLWN-12 برای LW روی کروموزوم های ۲، ۷ و ۱۲ قرار داشتند. این QTL ها به دلیل درصد بالای توجیه، پس از اعتبار سنجی، کاندیدای خوبی برای برنامه های انتخاب به کمک نشانگر در جمعیت برنج هستند.

کلمات کلیدی: تنش، QTL ، مکان یابی ژن های کمی، انتخاب به کمک نشانگر