J Genet Resour2019;5(1): 22-30 *Homepage: http://sc.journals.umz.ac.ir/*

RESEARCH ARTICLE *DOI: 10.22080/jgr.2019.16063.1128*

Genetic Structure of Salinity Tolerance in Rice at Seedling Stage

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ARTICLE INFO

Article history:
Received 14 January 2019
Accepted 17 February 2019
Available online 29 February 2019

Keywords:
ISSR
QTL mapping
Quantitative Traits Loci Salinity
SSR

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p-ISSN 2423-4257
e-ISSN 2588-2589

ABSTRACT

The *Oryza sativa* L. F_8 population derived from a cross between salt tolerance cv. Ahlemi Tarom and salt sensitive cv. Neda was used in the study. Germinated seeds floated on water for 3 d, and after were transferred to float on Yoshida's nutrient solution for 11 d. two weeks after sowing, the seedling was transferred to nutrient solution containing 51.19 mM NaCl (electrical conductivity 6 dSm^{-1}) for 7 d, then NaCl concentration was increased to 163.8 mM (12 dSm^{-1}) for further 7 d. After this period, the traits were measured. The linkage map was performed using F_8 populations, 40 SSR markers, 16 ISSR markers (76 alleles), 2 IRAP markers (7 alleles) and iPBS marker (3 alleles). The map length was 1419 cM with an average distance of 13.07 cM between the 2 adjacent markers. The QTL analysis showed that a total of 73 QTLs were identified that controlled 20 traits under normal and stress conditions. Among the QTLs, qCHLN-8, qSLN-8, qWLN-3, qWLN-9, qLAN-3, qLAN-8 and qLAN-9, qRFWN-1, qRFWN-3b and qRFWN-8, qFBN-7, qRDN-1a and qRDN-3 and qNaKSHN-5 under normal conditions and qSL-8, qLL-1a, qNaR-3, qKSH-1 and qKSH-4 and qNaKSH-4 under salinity stress conditions were identified. There are more than 20% explanations for phenotypic changes in the traits. These QTLs, due to the high percentage of justification after validation, could be a good candidate for selection programs with the help of markers in the population of recombinant lines of rice.

Please cite this paper as: Sanchouli S, Ghorbanzade Neghab M, Sabouri H, Zare Mehrjerdi M. 2019. Genetic structure of salinity tolerance in rice at seedling stage. *J Genet Resour* 5(1): 22-30. doi: *10.22080/jgr.2019.16063.1128*

Introduction

Rice (*Oryza sativa* L.) is a major source of food and energy for more than 2.7 billion people on a daily basis and is planted on approximately one-tenth of the earth's arable land (Bizimana *et al.,* 2017). Rice is one of the most important cereal crops and serves as the staple food for over one-third of the world's population (Mohammadi-Nejad *et al*., 2010). However, the productivity of rice is greatly affected by soil salinity which is the second most widespread soil problem after drought, in rice growing areas of the world (Sabouri and Sabouri, 2008; Islam *et al*., 2011). Soil salinity is key abiotic stress for crop productivity worldwide and it is a major abiotic stress for rice (Ren *et al*. 2005; Jing *et al*. 2017). Salinity is an increasing concern for the productivity of staple food crop. Crops with improved salt tolerance are highly needed to cultivate saline lands (Srivastava et al., 2018).

Since salt tolerance related traits in plants have complex inheritance, it will be required to understand the genetic control mechanisms of salt tolerance in order to facilitate the development of new varieties with a high level of salinity tolerance. This can be done using molecular markers technology and mapping Quantitative traits loci (QTLs), controlling the salt tolerance-related traits (Sabouri and Sabouri, 2008). In this case, Koyama *et al*. (2001) detected 6 QTLs for sodium, potassium and sodium/potassium (for each 2 QTL traits) in rice stems. Shahid-Masood *et al*. (2004) identified QTLs for traits related to salinity stress on 1, 3, 4, 6, 8, 9, 10, 11 and 12 chromosomes. Haq *et al*. (2008) identified 2 QTLs for fresh stem weight on the chromosome 1 and 2, and 1 QTL for dry stem weight on chromosome 2 at the seedling stage. 34 QTLs were identified on 10 chromosomes for 5 traits, under salinity stress conditions by Rahman *et al.* (2017). Khan *et al.*

(2016) identified 6 QTLs for different agronomic traits under salt stress conditions. These QTLs were 52.1% and 65.81% of phenotypic variance. The results reported by Gimhani *et al.* (2016) showed QTL 83 for 11 traits in response to salinity stress, which explained 5.39 to 49.9% of the phenotypic variance. Although there have been extensive studies on QTL mapping for salinity tolerance in rice, little or no information has been reported on the mapping of salinity tolerance in local rice populations. The aim of the present study was to identify QTLs related to salt tolerance by using the Iranian rice population.

Materials and Methods

Oryza sativa L. F_8 population derived from a cross between the salt tolerance Ahlemi Tarom (ATM) and the salt sensitive Neda (NAD) were used in this study. The genetic material which involved 96 lines was used to evaluate salt tolerance. The seeds placed at 50 ˚C for 3 d to break dormancy, then germinated at 35 ˚C for 48 h. Finally, the germinated seeds were sown in holes of the *Styrofoam* board with a nylon net bottom, which floated on water for 3 d, and then were transferred to float on Yoshida's nutrient solution (Yoshida *et al.* 1976) for 11 d. Two weeks after sowing, the seedling was transferred to a nutrient solution containing 51.19 mM of NaCl (electrical conductivity 6 dSm^{-1}) for 7 d, then NaCl concentration was increased to 163.8 mM (12 dSm^{-1}) for further 7 d. This experiment was conducted in the controlled conditions with 16-h photoperiod, the irradiance of 1500 µmol $m^{-2}s^{-1}$, day/night temperature of 29/21˚C and minimum relative humidity of 70%. The culture solution was renewed weekly and the pH was adjusted daily to 5.5 by addition of either NaOH or HCl. After salt stress exposure for 2 weeks, plants were harvested and the first standard tolerance ranking (STR) test was recorded according to Gregorio *et al*. (1997). Then, root length (RL), shoot length (SHL), fresh weight of root (FWRO) and fresh weight of shoot (FWSH) were recorded. F_8 lines were washed with deionized water and dried in a forced-air oven (70 ˚C). Followed by measuring root dry weight (DRWRO) and shoot dry weight (DWSH). Sodium and potassium of shoot and root were measured according to Thomas *et al*. (1967) method. DNA was extracted from the main culm at seedling stage according to Saghi Maroof *et al* (1994) method.

Forty SSR primer pairs, 16 ISSR markers (76 alleles), 2 IRAP markers (7 alleles) and 1 iPBS marker (3 alleles) which were appropriately distributed on 12 rice chromosomes were chosen according to Chen *et al* (1997), Temnykh *et al.* (2000) and Kalendar *et al*. (2010). The SSR marker Saltol was used on chromosome 1. In addition, ISSR, iPBS, IRAP markers 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 dNTP, 156.8 mmol dm^{-3} of $MgCl_2$, 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 a 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 fast silver staining as described by An *et al.* (2009). Using Mapmanager QtbX17 program, 12 linkage groups were constructed with a minimum LOD score of 2. Map distances between markers were presented in centiMorgan (cM) using the Kosambi function (Kosambi, 1944) of the program.

Results

Under normal conditions, 4 QTLs for stem length (qSLN) were detected on chromosomes of 3, 8, 9 and 10. Among the identified QTLs, qSLN-8 had an explanation of more than 23% for a phenotypic variance. In terms of salt stress for stem length (qSL), 5 QTLs were detected on chromosomes of 3, 8, 9 and 10. Among the identified QTLs, qSL-8 had an explanation of more than 26% for a phenotypic variance. This QTL was common in both normal conditions and salinity stress (Table 1 and 2).

No locus was detected for root length under normal conditions, while under salt stress conditions, 1 QTL was detected on chromosome 5. The additive effect was 2.662 cm explaining the phenotypic variation of 15.4% (Table 1). Under normal conditions, 6 QTLs for leaf area (qLAN) were detected on chromosomes of 1, 2, 3, 8 and 9. The qLAN-3, qLAN-8, and qLAN-9 were very effective and were located near the ISSR11-2, ISSR4-6 and ISSR8-7 markers, respectively. Their additive effects were 20, 9.20 and 21 cm^2, respectively. Under salinity stress, the location of the leaf area controller gene was not detected (Table 1 and 2). Under normal conditions, 1 QTL was detected for root surface density (RDN); explaining 14.2% of phenotypic changes of the trait.

Table 1. Putative QTLs for salt tolerance in the seedling stage the F8 population derived from Ahlemi Tarom*.

Traits	QTL	Chr.	Flanking markers	LOD	Position	Additive effect	R2	Direction of allele
Shoot length	qSL-3	3	ISSR20-7-RM301	3.506	90	-2.793	19.6	NAD
	qSL-8	8	ISSR4-6-ISSR13-3	5.009	16	7.129	26.8	ATM
	qSL-9	9	RM205-ISSR8-7	2.929	96	4.155	16.7	ATM
	qSL-10a	10	ISSR13-4-IRAP17-2	2.306	34	6.278	13.4	ATM
	qSL-10b	10	RM2948A-RM591	2.013	98	2.625	11.8	ATM
Root length	qRL-5	5	ISSR1-3-ISSR2-1	2.682	30	2.562	15.4	ATM
Shoot fresh weight	qSFW-7	7	ISSR4-6-RM500	2.026	50	0.006	11.8	ATM
Fresh biomass	qFB-7	7	ISSR4-6-RM500	2.148	50	0.007	12.5	ATM
Na+ shoot content	qNaSH-2	2	ISSR12-2-ISSR8-1	2.536	66	7.342	15.2	NAD
	qNaSH-5a	5	ISSR2-1-RM39	2.216	44	5.853	13.4	NAD
	qNaSH-5b	5	RM39-RM194	2.262	48	4.908	13.6	NAD
	qNaSH-6	6	ISSR9-1-ISSR6-2	2.344	90	8.349	14.1	NAD
Na+ root content	qNaR-1	1	RM10748-RM10773	2.909	102	5.539	16.6	NAD
	qNaR -2	2	ISSR8-2-ISSR5-3	3.081	4	9.857	17.4	NAD
	qNaR -3	3	RM143-ISSR11-2	3.777	88	-6.305	20.9	ATM
	qNaR -9a	9	ISSR20-5-ISSR14-1	2.179	18	-8.267	12.7	ATM
	qNaR -9b	9	RM205-ISSR8-7	2.427	96	7.517	14	NAD
	qNaR -10	10	ISSR13-4-IRAP17-2	2.343	40	15.211	13.6	NAD
K+ shoot content	qKSH-1	1	RM10748-RM10773	3.689	102	3.558	21.8	ATM
	qKSH-2a	2	ISSR8-2-ISSR5-3	3.264	2	4.249	19.6	ATM
	qKSH-2b	2	RM300	2.593	62	2.664	15.9	ATM
	qKSH-3	3	ISSR11-2-RM504	2.72	92	-3.495	16.6	NAD
	qKSH-4	4	ISSR8-3-RM252	3.655	48	4.807	21.6	ATM
	qKSH-8	8	ISSR4-6-ISSR13-3	3.139	20	6.225	18.9	ATM
K+ root content	qKR-2a	2	ISSR8-2-ISSR5-3	3.364	6	7.13	18.9	ATM
	qKR-2b	2	RM300-ISSR12-2	2.126	64	3.875	12.4	ATM
	qKR-7	7	ISSR5-4-ISSR4-7	2.343	108	5.099	13.6	ATM
Na+/K+ shoot	qNaKSH-1	1	RM10748-RM10773	2.372	102	0.488	13.7	ATM
	qNaKSH-4	4	ISSR8-3-RM252	3.806	48	0.833	21.1	ATM

* Ahlemi Tarom= ATM, a salt tolerance variety; Neda= NAD, a salt sensitive variety.

It additive effect was 7.591 and its LOD was 396.2. No QTL was detected under stress conditions for this attribute (Table 1 and 2).

In normal conditions, 1 QTL close to the RM39 marker was detected on chromosome 5. Parent NAD alleles have increased this trait. In terms of salt stress, QTL was detected in chromosome 7 for this trait (Table 1 and 2).

In normal conditions, 5 QTLs were identified for Root fresh weight (RFW) on chromosomes 1, 2, 3 and 8, which explaining 18-25% of the phenotypic variance of the trait. The qRFWN-1, qRFWN-3b, and qRFWN-8 had an explanation of more than 20% for phenotypic variance and were close to the RM10773, ISSR11-2 and ISSR 13-3 markers. In the conditions of salinity, there was no genetic location controlling this trait (Table 1 and 2).

In normal conditions, 5 QTLs were identified for chromosomes 5, 6 and 7 for fresh biomass (FBN). The qFBN-7 on chromosome 7 justified 21.6% of the phenotypic changes of the trait and had a LOD of 9.13 and an additive effect of 0.011. In terms of salinity stress, 1 QTL was detected on chromosome 7, which had an incremental effect, and its LOD was 0.007 and 148.2, respectively (Table 1 and 2).

In non-stress conditions, 1 QTL for root dry weight (RDN) was detected on chromosome 1 and closed to the ISSR13-2 marker, explaining 14.3% of the variance of the phenotype. In the salinity stress conditions, there was no genetic location controlling this trait (Table 1 and 2).

Under normal conditions, 1 QTL on chromosome 1 was identified for dry biomass (DBN). The qDBN-1 was closed to the ISSR13-2 marker and had the additive effect of -0.002. The alleles of Neda parent increased this trait in QTLs. This QTL allele from NAD increased DB. However, under dry conditions, no biomass controller gene was detected (Table 1 and 2). The seven genetic locations for root diameter (RDN) were detected in normal conditions on chromosomes of 1, 3, 4, 8 and 12. The qRDN-1a and qRDN-3 showed 1.24 and 4.24% of the variance of adjunctive

phenotype, and they were called "major effect QTL". Their additive effects were 174.1 and -0.19 respectively. In the salinity stress conditions, the location of the root diameter controller gene was not detected.

Under normal conditions, 2 QTLs for stomatal width (SWN) were detected on QTLs 1 and 4. These QTLs justified less than 10% of ariance of adjunctive phenotype. The alleles of the parent NAD reduced this trait. In salt stress conditions, the location of gene controller was not detected (Table 1 and 2). In normal conditions, no QTL was detected for sodium stem concentration while in salinity stress, 4 QTLs were detected on sodium stem concentrations (NaSH) on chromosomes 2, 5 and 6. The alleles of Neda parent increased this trait in all 4 QTLs. The qNaSH-2, had the highest R^2 among these QTLs, with a justification of 15.2% of the phenotype variation (Table 1 and 2). Under normal conditions, 1 QTL was identified on chromosome 4 for sodium root concentrations (NaRN). This QTL explained 12.3% of phenotypic variation and was located at an interval of RM280-ISSR11-4. In terms of salt stress, 6 QTLs were located on chromosomes 1, 2, 3, 9 and 10 for sodium root concentrations (NaR) (Fig. 1). The qNaR-3 had a justification of more than 20% for a phenotypic variance. This QTL was located at the distance between the markers of RM143-ISSR11-2. The effect of this QTL was -6.305, and the alleles of parents of ATM reduced this trait (Table 1 and 2). Under normal conditions, 5 QTLs for potassium stem (KSHN) were detected on chromosomes of 2, 3, 5, 6 and 8. The qKSHN-2 was able to justify 18.2% of the phenotypic variance of the trait. This QTL was closed to the RM300 marker and had an additive effect of 35.3. In salinity stress conditions, 6 QTLs were identified on chromosomes of 1, 2, 3, 4 and 8. The qKSH-1 and qKSH-4 indicated more than 20% of the phenotypic variance of the trait. These QTLs had a positive additive effect of 3.558 and 4.807mg/g, respectively (Table 1 and 2).

In salt stress conditions, 3 QTLs for root potassium (KR) were identified on chromosomes of 2 and 7. qKR-2a, qKR-2b and qKR-7 were close to the ISSR5-3, ISSR12-2, and ISSR5-4 markers, respectively, and justified 9.9%, 12.4%, and 13.6% of the phenotypic variance of the attribute. Normally, this QTL attribute was not detected (Table 1 and 2). In normal conditions, 3 QTLs were identified for Na^+ to K^+ ratio on chromosomes of 2, 5 and 10. The qNaKSH-5 with an effect of 0.319 could justify 30.1% of phenotypic variation of the trait. This QTL was closed to the ISSR2-1 marker. In salinity stress conditions, 2 QTLs were detected for the Na^+/K^+ ratio on chromosomes of 1 and 4. The qNaKSH-4 was able to explain 21.1% of the phenotypic changes of the trait and had an additive effect of 0.833 mg/g. The alleles ATM have increased this trait (Table 1 and 2). In normal conditions, a QTL was detected for Na^+/K^+ ratio (NaKRN) on the chromosome of 5. This QTL was located between the RM39-RM194 markers and justified 19.1% of the variance of adjective phenotype. In salt stress conditions, the QTL of Na^+/K^+ ratio root was not detected (Table 1 and 2).

Discussion

Rice is a sensitive crop under salt stress. due to high Na accumulation, high Na accumulation, low K concentration, and K/Na imbalance. The molecular analyses of Na^+ and K^+ concentration and Na^+/K^+ ratio under salt stress showed that they are often controlled by polygene (Jing *et al* 2017). In this study, the linkage map covered a total of 1419 cM with an average 2 loci interval of 13.07 cM. Bizimana *et al*. (2017) have identified some QTLs associated with shoot length on chromosomes 1, 6 and 12. Pascual *et al*. (2017) have identified 3 QTLs associated with shoot length on chromosomes 1, 9 and 12, which was dissimilar with our data except for the identification of chromosome 9 for shoot length. Sabouri (2010) have reported 1 QTL on chromosome 12 for root length. while 1 QTL, 1 QTL on chromosome 5 for root length was identified in the present study. Pascual *et al*. (2017) have reported 2 QTL associated with shoot fresh weight on chromosomes 4 and 12.

Bizimana *et al.* (2017) have identified some QTLs with shoot fresh weight on chromosomes 4, 9 and 12. In this study, 1 QTL associated with shoot fresh weight was detected on chromosome 7. Sabouri and Sabouri (2008) have reported some QTLs for Na^+ uptake of rice seedling on chromosomes 1 (qNAUP-1a, qNAUP-1b), 3 (qNAUP-3), 9 (qNAUP-9a, qNAUP-9b) and 10 (qNAUP-10). Pascual *et al.* (2017) have identified 1 QTL associated with Na^+ uptake on chromosome 1.

Table 2. Putative QTLs for the normal condition in the seedling stage the F8 population derived from Ahlemi Tarom*

Traits	QTL	Chr.	Flanking markers	LOD	Position	Additive effect	R^2	Direction of allele
Shoot length	qSLN-3	3	ISSR11-2-RM504	2.674	90	-2.777	15.3	NAD
	qSLN-8	8	ISSR4-6-ISSR13-3	4.303	16	7.508	23.5	ATM
	qSLN-9	9	RM205-ISSR8-7	2.078	96	3.986	12.1	ATM
	qSLN-10	10	ISSR13-4-IRAP17-2	2.766	34	7.678	15.8	ATM
Leaf area	qLAN-1	1	RM10748-RM10773	2.65	102	0.376	15.2	ATM
	qLAN-2a	2	ISSR8-2	2.546	0	0.341	14.7	ATM
	qLAN-2b	2	RM300	2.236	62	0.305	13	ATM
	qLAN-3	3	ISSR11-2-RM504	3.586	92	-0.481	20	NAD
	qLAN-8	8	ISSR4-6-ISSR13-3	3.76	12	0.837	20.9	ATM
	qLAN-9	9	RM205-ISSR8-7	3.795	96	0.653	21	ATM
Root fresh weight	qRFWN-1	1	RM10748-RM10773	4.086	102	-0.017	22.5	NAD
	qRFWN-2	2	ISSR8-2-ISSR5-3	3.29	4	-0.027	18.5	NAD
	qRFWN-3a	3	RM143-ISSR11-2	3.498	88	0.016	19.6	ATM
	qRFWN-3b	3	ISSR11-2-RM504	3.878	92	0.019	21.4	ATM
	qRFWN-8	8	ISSR4-6-ISSR13-3	4.589	18	-0.036	24.8	NAD
Shoot fresh weight	qSFWN-5	5	RM39-RM194	2.396	48	-0.008	13.9	NAD
Fresh biomass	qFBN-5a	5	IRAP17-4-RM538	2.007	132	0.007	11.7	ATM
	qFBN-5b	5	RM538-ISSR2-4	2.147	138	0.009	12.5	ATM
	qFBN-6a	6	ISSR4-5-IRAP17-1	2.303	34	0.016	13.4	ATM
	qFBN-6b	6	ISSR9-1-ISSR6-2	2.255	116	-0.032	13.1	NAD
	qFBN-7	7	iPBS2078-3-ISSR4-6	3.918	44	0.011	21.6	ATM
Root dry weight	qRDWN-1	1	RM10864-ISSR13-2	2.489	88	-0.001	14.3	NAD
Dry biomass	qDBN-1	1	ISSR13-2-ISSR20-6	2.92	90	-0.002	16.6	NAD
Root diameter	qRDN-1a	1	RM10748-RM10773	4.421	102	0.174	24.1	ATM
	qRDN-1b	1	RM10825-RM10843	2.189	106	0.142	12.7	ATM
	qRDN-3	3	ISSR11-2-RM504	4.491	92	-0.196	24.4	NAD
	qRDN-4	4	ISSR1-4-RM280	2.512	124	-0.155	14.5	NAD
	qRDN-8	8	ISSR4-6-ISSR13-3	3.06	14	0.292	17.3	ATM
	qRDN-9	9	ISSR4-6-ISSR13-3	2.562	96	0.201	14.7	ATM
	qRDN-12	12	ISSR16-1	2.096	78	4.203	12.2	ATM
Root density	qRDN-8	8	RM331-ISSR2-5	2.47	64	7.591	14.2	ATM
Width stomata	qWSN-1	1	ISSR1-5-RM165	2.186	94	-0.68	9.4	NAD
	qWSN-4	4	IRAP18-2-ISSR1-4	2.213	122	-0.611	4	NAD
Na^+ root content	qNaRN-4	4	RM280-ISSR11-4	2.118	136	-0.966	12.3	ATM
K^+ shoot content	qKSHN-2	2	iPBS2078-2-RM300	3.236	58	3.35	18.2	ATM
	qKSHN-3	3	RM143-ISSR11-2	2.224	86	-2.711	12.9	NAD
	qKSHN-5	5	ISSR9-4-IRAP17-4	2.693	122	3.286	15.4	ATM
	qKSHN-6	6	ISSR9-1-ISSR6-2	2.695	90	4.787	15.4	ATM
	qKSHN-8	8	ISSR4-6-ISSR13-3	2.196	16	4.607	12.8	ATM
K^+ root content	qKRN-8	8	ISSR4-6-ISSR13-3	2.253	22	-0.237	13.1	NAD
Na^+/K^+ shoot	qNaKSHN-2	2	ISSR12-2-ISSR8-1	3.111	66	0.194	17.6	ATM
	qNaKSHN-5	5	ISSR2-1-RM39	5.757	38	0.319	30.1	ATM
	qNaKSHN-10	10	ISSR13-4-IRAP17-2	2.074	42	0.296	12.1	ATM
Na^+/K^+ root	qNaKRN-5	5	RM39-RM194	3.416	48	2.112	19.1	ATM

* Ahlemi Tarom= ATM, a salt tolerance variety; Neda= NAD, a salt sensitive variety

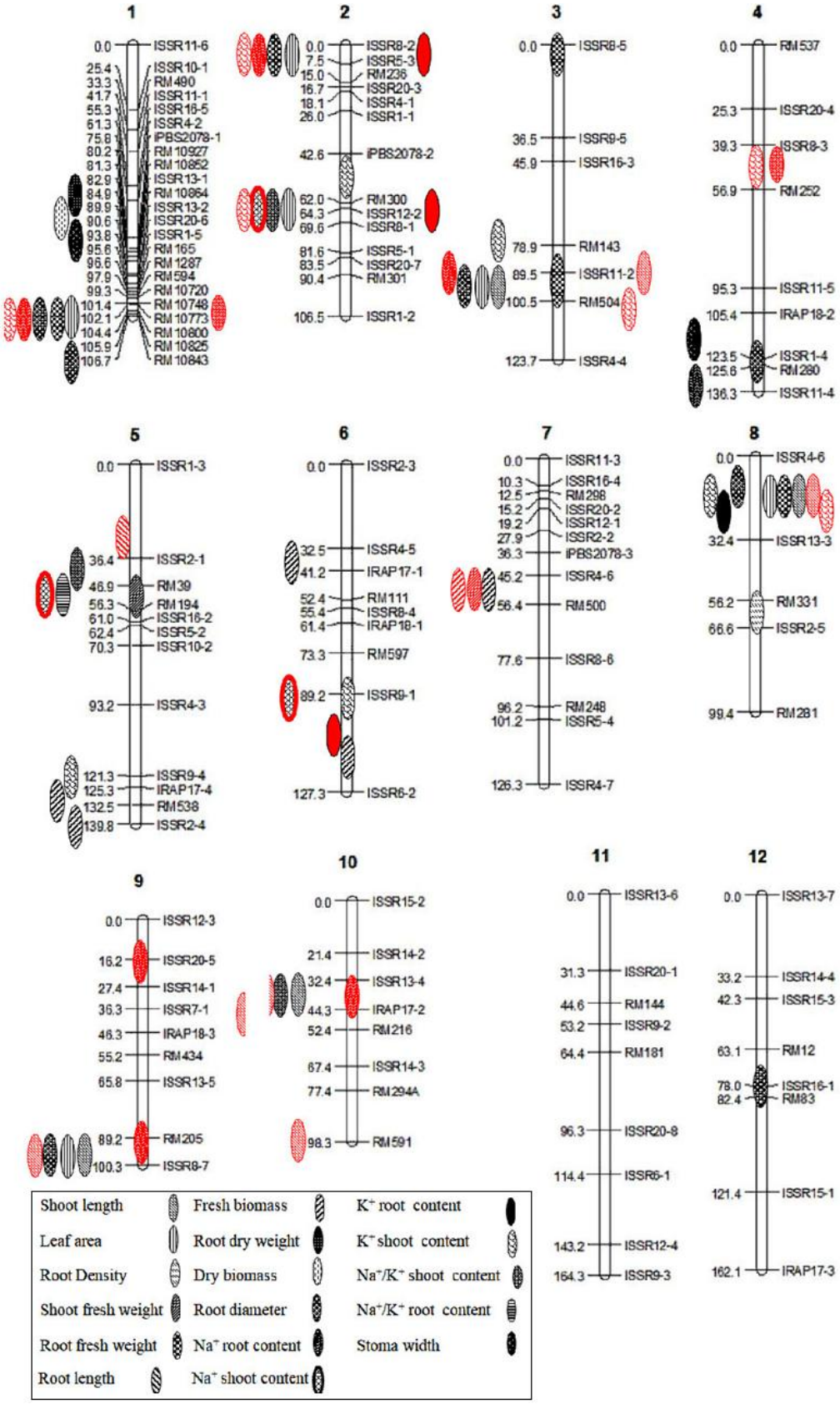

Fig. 1. Genetic linkage maps QTLs identified under normal and salt stress conditions of the in the seedling stage the F_8 population derived from ATM × NAD: Red are the same under salt stress conditions.

Here we explored these QTLs associated with Na^+ uptake in shoot and root located on chromosomes 1, 2, 3, 5, 6, 9 and 10, which was in compatible with the previous researches.

Jing *et al* (2017) have identified some QTLs associated with K^+ concentrate on chromosome 1. Pandit *et al*. (2010) have reported 1 QTL associated with K^+ concentrate on chromosome 1. In this study, we detected QTLs related to K^+ content of shoot and root at the seedling stage. These QTLs were mapped on chromosomes 1, 2, 3, 4, 7 and 8. The qKR-2a, qKR-2b, qKR-7, qKSH-1, qKSH-2a, qKSH-2b, qKSH-3, qKSH-4, and qKSH-8 explained most of the total phenotypic variation of 18.9, 12.4, 13.6, 21.8, 19.6, 15.9, 16.6, 21.6 and 18.9, respectively.

We found QTLs related to Na^+/K^+ content ratio on chromosomes 1 and 4. QTLs associated with Na^+/K^+ content ratio in other reports were found on chromosomes 1 (Dahanayaka *et al*., 2017; Koyama *et al*., 2001), 2 (Ming zhe *et al*., 2005), 4 (Koyama *et al*., 2001, Dahanayaka *et al*., 2017) 10 and 12 (Grigorio, 1997). It is probably due to the low density of SSR linkage map.

We found QTL related for dry biomass on chromosome 1 on normal condition. Cui *et al* (2002) have reported some QTLs for dry biomass on chromosome 1, 3, 5, 6 and 9. In this study, we detected QTLs related shoot fresh weight and root dry weight. which were mapped on chromosomes 5 and 1, respectively. Most of the total phenotypic variation was belonged to qSFWN-5 and qRDWN-1 as 13.9 and 14.3, respectively. Lian et al (2005) identified QTLs for shoot fresh weight on chromosomes 1, 5, 6 and 11. Cui *et al* (2002) found some QTLs for root dry weight on chromosomes 1, 5 and 10.

Veldboom *et al*. (1994) showed that correlated traits often have QTLs that map to the same chromosomal region. In the present study, the QTLs associated with LA, RFW and RD in the region of ISSR11-2–RM504 chromosome 3 was overlapped, in normal conditions. The QTLs of qKSHN-8 and qSLN-8 on chromosome 8 and the QTLs of qSLN-9, qLAN-9 and qRDN-9 on chromosome 9 were found the same map locations, in the distance between RM205-ISSR8-7 markers.

The qLAN-1, qRFWN-1, and qRDN-1 were located on chromosome 1 and at the distance of RM10748-RM10773 marker and had a positive and significant correlation (0.027^*). The LA had a high correlation (0.186) with the RD, and this was QTLs in the various traits detected for these traits. In conditions of salt stress, although many QTLs were detected in similar regions, but it had low correlations. For example, qNaR-1 and qKSH-1 were located on chromosome 3 (r=-0.139) and qSL-9 and qNaR-9a were located on chromosome 9 (r=0.027).

According to the results in normal and saline conditions, it seems that there is a polyotropy effect of genes controlling the traits. Sabouri *et al* (2010) found qNA-3, qSTR-3, and qNAK-3 in chromosome 3 and qNAK-6, qK-6, qNA-6 and qSTR-6 were found at approximately the same map locations in chromosome 6, which indicated the high correlation.

In the present study, QTLs were identified for SHL on chromosomes 8, 9 and 10, which were common in normal and normal stress conditions. Also, in the distance between the ISSR4-6 and ISSR13-3 markers on chromosome 8, the location of the genotype controlling the KSH was detected, which was common in both normal conditions and salinity stress. In this study, genetic locations with more than 20% genes were identified for some traits, including qCHLN-8, qSLN-8, qWLN-3, qWLN-9, qLAN-3, qLAN-8 and qLAN-9, qRFWN-1, qRFWN-3b and qRFWN-8, qFBN-7, qRDN-1a and qRDN-3 and qNaKSHN-5 under normal conditions and qSL-8, qLL-1a, qNaR-3, qKSH-1 and qKSH-4 and qNaKSH-4 under salinity stress condition. These QTLs, due to the high percentage of justification after validation, could be a good candidate for selection programs with the help of markers in the population of recombinant lines of rice.

References

An ZW, Xie LL, Cheng H, Zhou Y, Zhang Q, He XG. 2009. A silver staining procedure for nucleic acids in polyacrylamide gels without fixation and pretreatment. *Anal Biochem* 391(1):77-9.

Bizimana JB, Luzi-kihupi A, Murori RW, Singh RK. 2017. Identification of quantitative trait loci for salinity tolerance in rice (*Oryza sativa* L.*)* using IR29/Hasawi mapping population. *J Genet* 96(4):571-582.

Chen X, Temnykh S, Xu Y, Cho YG, McCouch SR. 1997. Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). *Theor Appl Genet* 95: 553-567.

Cui KH, Peng SB, Xing YZ, Xu CG, Yu SB. 2002. Molecular dissection of seedling-vigor and associated physiological traits in rice. *Theor Appl Genet* 105: 745-753.

Dahanayaka BA, Gimhani DR, Kottearachchi DR, Samarasighe WLG. 2017. QTL mapping for salinity tolerance using elite rice (*Oryza sativa*) breeding population. *SABRAO J Breed Genet* 49 (2): 123-134.

Gimhani DR, Gregorio GB, Kottearchchi NS, Samarasinghe WLG. 2016. SNP-based discovery of salinity- tolerant QTLs in a bi-parental population of rice (*Oryza sativa*). *Mol Genet Genomics* 291(6):2081-2099.

Gregorio GB. 1997. Tagging salinity tolerance genes in rice using amplified fragment length polymorphism (AFLP). doc. thesis. University of Philippines, Los Banos.

Haq TU, Akhtar J, Gorham J, Khalid M. 2008. Genetic mapping of QTLs, controlling shoot fresh and dry weight under salt stress in rice cross between CO39×Moroberekan. *Pak J Bot* 40(6):2369-2381.

Islam MR, Salam MA, Hassan L, Collard BCY, Singh RK, Gregorio GB. 2011. QTL mapping for salinity tolerance at seedling stage in rice. Emir *J Food Agric* 23(2):137-14.

Jing W, Deng P, Cao C, Zhang W. 2017. Fine mapping of qSKC-1, a major quantitative trait locus for shoot K^+ concentration, in rice seedlings grown under salt stress. *Breed Sci* 67:286-295.

Kalendar R, Antonius K, Smýkal P, Schulman AH. 2010. iPBS: a universal method for DNA fingerprinting and retrotransposon isolation. *Theor Appl Genet* 121(8):1419-30

Khan MSK, Saeed M, Iqbal J. 2016. Quantitative trait locus mapping for salt tolerance at maturity stage in indica rice using replicated F2 population. *Braz J Bot* 39(2):641-650.

Kosambi DD. 1944. The estimation of map distances from recombination values. *Ann Eugen* 12:172-175.

Koyama ML, Levesley A, Koebner RMD, Flowers TJ, Yeo AR. 2001. Quantitative trait loci for component physiological traits determining salt tolerance in rice. *Plant Physiol* 125:406-422.

Lian X, Xing Y, Yan H, Xu G, Li X, Zhang Q. 2005. QTLs for low nitrogen tolerance at seedling stage identified using a recombinant inbred line population derived from an elite rice hybrid. *Theor Appl Genet* 112:85-96.

Mohammadi-Nejad G, Singh RK, Arzani A, Rezaie AM, Sabouri H, Gregorio GB. 2010. Evaluation of salinity tolerance in rice genotypes. *Int J Plant Prod* 4:199-208.

Pandit A, Rai V, Bal S, Sinha S, Kumar V, Chauhan M, Gautam RK, Singh R, Sharma PC, Singh AK, Gaikwad K, Sharma TR, Mohapatra T, Singh NK. 2010. Combining QTL mapping and transcriptome profiling of bulked RILs for identification of functional polymorphism for salt tolerance genes in rice (*Oryza sativa* L.). *Mol Genet Genomics* 284:121-136.

Pascual ED, Dela-Vina CB, Mendioro MS, Hernandez JE, Amas JC, Sajise AGC, Gregorio CB. 2017. New QTL for salt tolerance at the seedling stage in rice var. Hasawi using recombinant inbred lines. *Philipp Agri Sci* 100(2):391-401.

Rahman MA, Bimpong IK, Bizmana JB, Pascual ED, Arceta M, Swamy BPM, Diaw F, Rahman MS, Singh RK. 2017. Mapping QTLs using a novel source of salinity tolerance from Hasawi and their interaction with environments in rice. *Rice* 1-17.

Ren HZ, Gao JP, Li LG, Cai XL, Huang W, Chao DY, Zhu MZ, Wang ZY, Luan S, Lin HX. 2005. A rice quantitative trait locus for salt tolerance encodes a sodium transporter. *Nature Genet* 37 (10):1141-1146.

Sabouri H, Sabouri A. 2008. New evidence of QTLs attributed to salinity tolerance in rice. *Afr J Biotechnol* 7:4376-4383.

Sabouri H. 2010. Mapping of QTLs of germination characteristics in rice using microsatellite markers under salin condition. *Iranain J Biol* 23:333-342.

Saghi Maroof MA, Biyaoshev RM, Yang GP, Zhang Q, Allard RW. 1994. Extra ordinarily polymorphic microsatellites DNA in barly species diversity,

chromosomal location, and population dynamics. *Proc Natl Acad Sci USA* 91:4566-5570.

Shahid-Masood, M, Seiji Y, Shinwari ZK, Anwar R. 2004. Mapping quantitative trait loci for salt tolerance in rice using RFLP. *Pak J Bot* 36(4): 825-834.

Srivastava N, Vadez V, Nigam SN; Upadhyaya HD, Narasu L. 2018. Screening groundnut (*Arachis hypogaea* L.) germplasm for salinity tolerance. *J Genet Res*. 4(2):130-140.

Temnykh S, Park WD, Ayres N, Cartinhour S, Hauck N, Lipovich L, Cho YG, Ishii T, McCouch SR. 2000. Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). *Theor Appl Genet* 100:697-712.

Thomas RL, Sheard RW, Moyer JR. 1967. Comparison of conventional and automated procedures for nitrogen, phosphorus, and potassium analysis of plant material using a single digestion. *Agron J* 59:240-243.

Veldboom LR, Lee M, Woodman WL. 1994. Molecular-marker facilitated studies in an elite maize population. 1. Linkage analysis and determination of QTL for morphological traits. *Theor Appl Genet* 88:7-16.

Yoshida S, Forno DA, Cock JH, Gomez KA. 1976. Laboratory manual for physiological studies of rice IRRI, Los Banos, Philippines.