Research article

Assessment of Some Promising Lines of Rice (*Oryza sativa* L.) for Salt Tolerance using Microsatellite Markers Associated with the *saltol* QTL

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Received 30 December 2020 Accepted 19 July 2021 (*Corresponding Author)

Abstract Rice is one of the most important staple food crops in the world that grown extensively under irrigation. Salinity is an important physical factor influencing rice (*Oryza sativa* L.) production. To assess this limiting factor, YAU developed 100 breeding lines of rice. After screening at seedling and vegetative stages at different salinity levels (0.2, 6.0, and 8.0 dS m^{-1}), seven rice lines were selected as moderately saline-tolerant genotypes. These seven lines (V1: YAU- 1211-14-1-1; V2: YAU1201-90-2-4; V3: YAU-1211-18-1-1; V4: YAU1211-195-1-1; V5: YAU-1201-26-1-1; V6: YAU1201-26-1-3; and V7: YAU-1211-82-1-1) along with three local control varieties (Yatanatoe, Superhnankaut and Theehtatyin), one salt tolerance check (Pokkali) and one susceptible check (IR 29) were used in this study. Seven *Saltol* QTL associated SSR markers (RM5, RM9, RM140, RM472, RM493, RM1287 and RM3412) were used to check the usefulness of microsatellite (SSR) markers associated with *Saltol* QTL. The number of alleles on the SSR markers ranged from 2 for RM140 to 4 for RM3412. Polymorphic information content (PIC) value varied from 0.00 for RM140 to 0.62 for RM3412, with an average of 0.36. The SSR marker, RM3412, was found to be superior for analysis as an indicator of genetic diversity in this study. Cluster analysis of the rice genotypes based on SSR data divided the genotypes into three groups, each of which include Yatanatoe, Theehtatyn, Superhnankaut and susceptible check IR29 (cluster 1), V1, V2 and V3 (cluster 2), V4, V5, V6, V7 including salt tolerance genotypes Pokkali (cluster 3), respectively. Of the seven lines, four SSR markers (RM5, RM493, RM1287 and RM3412) could discriminate Pokkali (*saltol*) from the IR29 (susceptible) genotype. Two specific alleles were found by RM5 (170) and RM493 (220) for Pokkali. At locus RM140, almost all genotypes possessed the same allele as Pokkali (260) except Theehtatyin and IR29 (null allele). RM1285 indicated four YAU rice lines (V4, V5, V6 and V7) as salt tolerance lines. The study revealed V4: YAU1211-195-1-1 as a tolerance genotype. The RM5, RM493, RM1287 and RM3412 markers were able to discriminate the tolerant genotypes and hence could be useful for marker-assisted selection of *Saltol* QTL.

Keyword promising rice lines, *Saltol* QTL, SSR marker

INTRODUCTION

Rice (Oryza sativa) is the staple food of half of the population of the world. Identifying a rice crop that resists saline incursions due to climate change is prevalent among farmer's requirements. Soil salinization has become a serious problem all over the world and around 20% of the world's cultivated land is affected (Sumner, 2000). In Myanmar, soil salinization is found in both coastal and inland regions, and about 3% of the total rice sown area is affected by salinity (DOA, 2012). Coastal salinity is often due to seawater intrusion/ infiltration during floods, resulting in salt accumulation in the topsoil in the summer season. It occurs commonly in Ayeyarwady, Yangon, Yakhaing and Taninthari regions. Inland salinity is common in dry zone areas of central Myanmar such as the Mandalay, Magway and Sagaing regions. Rice productivity is quite low due to this salinization, with limited water resources, and low soil fertility in these inland areas of Myanmar (Oo et al., 2017). Swe and Ando (2017) have pointed out that salinity is becoming a prominent abiotic problem, with declining rice production in the central dry zone and which has had little attention paid to it. An improvement in rice breeding programs which offers rice varieties suitable for these changing conditions is important.

There are many salt tolerant rice varieties providing an opportunity to improve crop salt-stress tolerance through genetic means. Some attempts based on highly tolerant traditional rice cultivars i.e. Pokkali and Nona-Bokra have been made to these develop salt-tolerant genotypes (Akbar et al., 1985; Gregorio and Senadhira, 1993). DNA based molecular markers have been used extensively to assess the genetic diversity in most crop species (Mohammadi-Nejad et al., 2008). Currently, simple sequence repeats (SSRs) or microsatellite markers have been used to study genetic diversity, phylogenetic relationships, classification, evolutionary processes and quantitative trait loci in many crops. This technique has been used effectively to map QTLs associated with salt tolerance (Lang et al., 2000 and 2001; Singh et al., 2007). A major QTL located on chromosome 1 has been identified for salt tolerance using F8 recombinant inbred lines (RILs) of Pokkali/ IR29 cross (Gregorio et al., 1997). Therefore, it could be stated that Pokkali represents the most widely used salt tolerant parent by rice breeders. Our group have developed hundreds of rice breeding lines to transfer salt tolerance gene of Pokkali to local height yielding varieties.

OBJECTIBES

The present study intended to (i) evaluate salt tolerant rice breeding lines at different growth stages; seedling and vegetative, and (ii) test the usefulness of microsatellite (SSR) markers associated with *Saltol* QTL to identify promising YAU rice lines.

MATERIALS AND METHODS

Evaluation of Selected Improved Rice Lines at Vegetative Stage

This experiment was conducted as a two-factor factorial in a randomized complete block design, with three replications at vegetative stage. Seven promising lines out of YAU 100 improved line were formerly selected by conducting farmer participatory variety selection based on their preference of eating quality and visual assessment in the field. The seven rice selected lines with Pokkali (tolerant check) and IR29 (susceptible check), were grown at three different levels of salinity (0.2, 6.0, and 8.0 dS m^{-1}). Salinity tolerance was rated using a modified standard evaluation system (SES) rating the visual symptoms of salt toxicity (Gregorio et al., 1997) as shown in Table 1. Following Gregorio et al. (1997), who modified the method for the screening of rice genotypes at vegetative stage, the wall of plastic pot was drilled with 3-4 mm diameter holes 2 cm apart, with the topmost circle of holes at least 3 cm below the rim of the plastic pot. Cotton cloth was used to line the plastic pots. The pots were filled with fertilized soil, and then placed in the plastic tray filled with ordinary tap water, which served as a water bath. Four to five pre-germinated seeds of the tested varieties were placed on the soil surface of each pot. Two weeks after seeding, seedlings were thinned to one per pot and the water level was raised to a level about 1 cm above soil. When the seedlings were 25 days old, all water was siphoned out. Then salinized water, at the different EC saturations, was introduced.

The water level was maintained on a daily basis and the plants protected from any pests and diseases. Scoring was started at two weeks after salinization by using a modified standard evaluation system to rate the visual symptoms of salt toxicity (Gregorio et al., 1997) with results presented in Table 1. Plant height (cm) and the number of tillers per hill were recorded at 6 weeks after salinization.

Table 1 Modified Standard Evaluation Score (SES) of visual salt injury at seedling and vegetative stages

Score	Observation	Tolerance
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips of few leaves whitish and rolled	Tolerant
5	Growth severely retarded, most leave rolled	Moderately tolerant
7	Complete cessation of growth, most leaves dry, some plant dying	Susceptible
9	Almost all plant dead or dying	Highly susceptible

Source; Gregorio et al., 1997

Microsatellite (SSR) Analysis

A total of twelve genotypes including seven YAU promising rice line (V1: YAU- 1211-14-1-1; V2: YAU1201-90-2-4; V3: YAU-1211-18-1-1; V4: YAU1211-195-1-1; V5: YAU-1201-26-1-1; V6: YAU1201-26-1-3; and V7: YAU-1211-82-1-1) and three local control varieties (Yatanatoe, Superhnankaut and Theehtatyin), one susceptible check (IR29) and one tolerance check (Pokkali) were used to extract total genomic DNA. A modified CTAB method was used to extract DNA from the plants' young leaves. The relative purity and concentration of extracted DNA was estimated with NanoDrop ND-1000 (NanoDrop Technologies, Inc., Wilmington, DE, USA). The final concentration of each DNA sample was adjusted to 50 ng/µl.

Seven *Saltol* QTL associated SSR markers (RM5, RM9, RM140, RM472, RM493, RM1287 and RM3412) were used to check the usefulness of microsatellite (SSR) markers associated with *Saltol* QTL in the identification of promising YAU rice lines (Mohammadi-Nejad et al., 2008). GoTaq®Colorless Master Mix M713 was used according to the manufacturer's procedure for PCR amplification. The PCR reaction was a 10 µl volume using a Boeco Thermal Cycler TC-SQ (Boeco Germany). The PCR profile; 5 min of denaturation at 94°C, 35 cycles were performed for 1 min at 94°C, 45 s at 55°C, 1 min at 72°C, and a final extension step of 5 min at 72°C. PCR amplified products were separated in 2% agarose gel at 100 V for 1 h in 1 x TBE buffe. RedSafe™ Nucleic Acid Staining Solution (20,000x) was used to stain DNA in the agarose gel. The resulting DNA bands were scored as base pairs using grid lines in photoshop, comparing 100 bp DNA ladder bands.

Data Analysis

The data collected were analyzed statistically using Analysis of Variance (ANOVA) techniques, and rice lines means were compared by least significant different (LSD) method at a 5% probability level. All statistical analyses were done using Statistix 8.0 software and Excel program (2010).

The variability at each locus was measured in terms of the number of alleles, major allele frequency (MAF), and polymorphic information content (PIC) using PowerMarker 3.25 (Liu and Muse, 2005). The UPGMA algorithm of MEGA6 software embedded in PowerMarker was used to construct an unrooted neighbour-joining tree of each accession based on the shared allele distances (Tamura et al., 2007).

RESULTS AND DISCUSSION

Evaluation of Selected Improved Rice Lines at the Vegetative Stage

The sight evaluation scores (SES), showed a variation in response to salt stress among the rice lines. SES increased with an increase in stress level, indicating greater susceptibility at higher stress level

(Table 2). All seven improved rice lines at vegetative stage grew strongly and showed uniform green colour in non-salinized conditions (0.2 dS m^{-1}). In salinized conditions (6.0 and 8.0 dS m^{-1}), the rice lines showed significant differences for salt tolerance at the vegetative stage, with scores ranging from score 1 (highly tolerant) to score 9 (highly susceptible) (Table 2). The seven rice lines selected showed a high degree of tolerance and moderate tolerance, respectively, under increasing salinity levels (6.0 and 8.0 dS m^{-1}).

The reactions of the selected improved rice lines in terms of plant height and number of tillers per hill, under three different salinity levels at the vegetative stage, are showed in Table 3. The plant heights are significantly different among the rice lines in the non-salinized condition (0.2 dS m^{-1}). In contrast, these parameters were not significantly different among the rice lines in the two salinized conditions. Similarly, the number of tillers per hill was not significantly different among the rice lines in all conditions.

Table 2 Reactions of improved rice lines at vegetative stage to salinity at three different levels measured by Standard Evaluation Score (SES)

Improved rice lines	Reaction to salinity at 6 weeks after salinization		
	0.2 dS m^{-1}	6.0 dS m^{-1}	8.0 dS m^{-1}
YAU-1211-14-1-1	Highly tolerant	Tolerant	Moderately tolerant
YAU-1201-90-2-4	Highly tolerant	Tolerant	Moderately tolerant
YAU-1211-118-1-1	Highly tolerant	Tolerant	Moderately tolerant
YAU-1211-195-1-1	Highly tolerant	Tolerant	Moderately tolerant
YAU-1201-26-1-1	Highly tolerant	Tolerant	Moderately tolerant
YAU-1201-26-1-3	Highly tolerant	Tolerant	Moderately tolerant
YAU-1211-82-1-1	Highly tolerant	Tolerant	Moderately tolerant
Pokkali	Highly tolerant	Tolerant	Tolerant
IR29	Highly tolerant	Highly susceptible	Highly susceptible

Table 3 Reactions of selected improved rice lines at the vegetative stage to salinity at three different levels measured by plant height and number of tillers per hill

Improved rice lines	Plant height (cm)			Number of tillers per hill		
	0.2 dS m^{-1}	6.0 dS m^{-1}	8.0 dS m^{-1}	0.2 dS m^{-1}	6.0 dS m^{-1}	8.0 dS m^{-1}
YAU-1211-14-1-1	39.0 bcd	32.7	26.3	4.7	4.0	3.0
YAU-1201-90-2-4	37.3 de	32.3	25.7	4.3	4.0	3.3
YAU-1211-118-1-1	42.0 ab	32.7	23.7	4.3	3.7	3.3
YAU-1211-195-1-1	39.7 bcd	33.3	24.0	4.0	3.7	3.3
YAU-1201-26-1-1	44.7 a	32.0	26.7	5.0	4.3	4.0
YAU-1201-26-1-3	38.0 cde	35.7	22.7	4.0	3.3	3.3
YAU-1211-82-1-1	40.7 bc	34.0	27.3	4.3	3.7	4.0
Pokkali	35.3 ef	36.3	25.0	4.3	3.3	3.3
IR29	33.0 f	31.7	24.0	3.7	3.7	3.3
F-test	**	ns	ns	ns	ns	ns
C.V %	4.6	5.5	9.2	15.2	18.6	19.4

Values in the same column followed by the same letter are not significantly different at the 5% level by the LSD test, (**) significantly different at P≤0.01, ns − not significant

Microsatellite (SSR) Analysis

The number of alleles of the SSR markers ranged from 2 for RM140 to 4 for RM3412. Polymorphic information content (PIC) values varied from 0.00 for RM140 to 0.62 for RM3412, with an average of 0.36 (Table 4). The measure, or value of the PIC, is determined by the ability of a marker to establish polymorphism in the population depends on the number of alleles detected and on their distribution frequency (Botstein et al., 1980). The PIC value of the marker is defined as the expected fraction of informative offspring from the type of pedigree (Hildebrand, et al. 1992). In this regard, SSR marker, RM3412, was found to be superior for discrimination of potential breeding lines in this study.

Cluster analysis of the rice genotypes based on SSR data, separated the genotypes into three groups, each of which had Yatanatoe, Theehtatyn, Superhnankaut and susceptible check IR29 (cluster 1), V1, V2 and V3 (cluster 2), V4, V5, V6, V7, including salt tolerance genotypes Pokkali (cluster 3), respectively (Figure 1). Out of the seven varieties, four SSR markers (RM5, RM493, RM1287 and RM3412) were able to discriminate Pokkali (*Saltol*) from IR29 (susceptible) genotype. Two specific alleles were found by RM5 (170) and RM493 (220) for Pokkali. At locus RM140, almost all genotypes possessed the same allele as Pokkali (260), except for Theehtatyin and IR29 (null allele). Null alleles were likely to be encountered in populations with a large size, with unusually high mutation rates in the flanking regions, and those that have diverged from the population from which the cloned allele state was drawn and the primers designed (Chapuis and Estoup, 2007). Theehtatyin and IR29 would exhibit mutations in this locus, RM140. RM1285 was an indicator that four YAU rice lines (V4, V5, V6 and V7) are salt tolerant lines. These results, in combination, revealed V4: YAU1211-195-1-1 as saline tolerant genotypes. The RM5, RM493, RM1287 and RM3412 markers were able to discriminate for tolerant genotypes and hence could be useful for marker-assisted selection of *Saltol* QTL.

Table 4 Number of alleles and polymorphism information content (PIC) values of SSR markers for 12 rice genotypes

Marker	Frequency of major allele	No. of allele	PIC	Amplicon size range (bp)
RM5	0.92	2	0.14	150-170
RM9	0.44	3	0.57	100-170
RM140	1.00	1	0.00	260
RM472	0.83	2	0.24	320-350
RM493	0.50	3	0.48	220-250
RM1287	0.56	3	0.49	125-160
RM3412	0.42	4	0.62	200-260

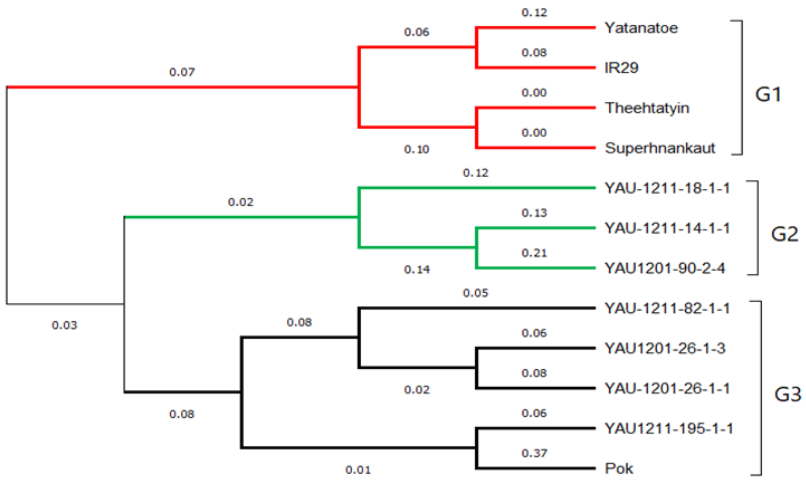

Fig. 1 Dendrogram of 12 rice genotypes based on 7 polymorphic SSR markers derived from the Shared allele distance with neighbor joint method

CONCLUSION

In conclusion, in this study seven YAU improved rice lines were observed for relative salt tolerance in terms of agronomic parameters such as tiller numbers, panicle numbers and grain yield. Results show that four rice breeding lines; V4, V5, V6, V7 have the potential to be developed for future improvement as salt tolerant rice varieties. Furthermore, the result highlights the usefulness of SSR markers associated with *saltol* QTL for the screening of rice breeding lines. The RM5, RM493, RM1287 and RM3412 markers may be useful for marker-assisted selection.

ACKNOWLEDGEMENTS

The authors would like to thank the JICA (Japan International Cooperation Agency) for their financial support for this project and the capacity development they have fostered at Yezin Agricultural University.

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