



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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ABSTRACT

Rice is one of the most important staple food crops in the world that grown under extensive irrigation environment. Salinity is an important physical factor influencing rice (*Oryza sativa* L.) production. To combat this limiting factor, YAU developed 100 breeding lines of rice. After screening at seedling and vegetative stages at different salinity level (0.2, 6.0, and 8.0 dS m⁻¹), seven rice lines were selected as moderately tolerance genotypes. These seven promising salt tolerance 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) and three local check varieties (Yatanatoo, Superhankaut and Theehtatyn), 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. Number of alleles of 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 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 having Yatanatoo, Theehtatyn, Superhankaut and susceptible check IR29 (cluster 1), V1, V2 and V3 (cluster 2), V4, V5, V6, V7 including salt tolerance genotypes Pokkali (cluster 3), respectively. Out of seven, four SSR markers (RM5, RM493, RM1287 and RM3412) could 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 Theehtatyn and IR29 (null allele). RM1287 pointed out that four YAU rice lines (V4, V5, V6 and V7) as salt tolerance lines. The present result collectively revealed that V4: YAU1211-195-1-1 as a tolerance genotype. The RM5, RM493, RM1287 and RM3412 markers could discriminate the tolerant genotypes and hence could be useful for marker-assisted selection of *Saltol* QTL.

INTRODUCTION

Rice (*Oryza sativa*), the staple food of half of the population of the world. Rice crop that resists 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 are affected (Sumner, 2000). In Myanmar, soil salinization was found in coastal and inland regions. Coastal salinity was affected by seawater intrusion/ infiltration during flood resulting salt accumulation in the topsoil in the summer season. It was commonly happened in Ayeyarwady, Yangon, Yakhaing and Taninthari regions. Inland salinity is commonly seen in dry zone areas of the central Myanmar such as Mandalay, Magway and Sagaing regions. Sween and Ando (2017) observed that salinity is becoming a prominent abiotic problem declining rice production in central dry zone which little attention was paid in the past. According to further improvement of rice breeding program in national level, it offers doing research and providing rice varieties for farmer needs are recently lined out. A major quantitative trait locus (QTL) for salt tolerance named *Saltol* was mapped on chromosome 1 (Mohammadi-Nejad et al.2008). This work intended to test the usefulness of microsatellite (SSR) markers associated with *Saltol* QTL for YAU promising rice lines.

MATERIAL AND METHODS

YAU developed 100 breeding lines of rice were screened at seedling and vegetative stages at different salinity level (0.2, 6.0, and 8.0 dS m⁻¹) (Table 1). Seven rice lines were moderately tolerant to 8.0 dS m⁻¹ during six weeks of application in term of leaf mortality (Table 2). A total of twelve genotypes including seven YAU promising rice line, three local check, one susceptible check (IR29) and one tolerance check (Pokkali) were used to extract total genomic DNA. Modified CTAB method was used to extract DNA from the young leaves of rice. 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 (Table 3). Gold taq PCR mastermix[®] was used as manufacturer's procedure for PCR amplification. The PCR profile: 5 min of denaturation at 94°C, 35 cycles were performed with 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 at 2% agarose gel at 100 V for 1 h in 1 x TBE buffer. The resulted bands were scored as base pairs using grid lines in photoshop comparing 100 bp ladder bands. Power Marker (Version 3.25) was used for genetic diversity analysis. The dendrogram was constructed with the help of Mega 6 software program (Figure 1).

RESULTS

Table 1 Modified 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 leaves rolled	Moderately tolerant
7	Complete cessation of growth, most leaves dry, some plant dying	Susceptible
9	Almost all plant dead or dying	Highly susceptible

Table 2. Salinity reactions of improved rice lines in terms of Standard Evaluation Score (SES) under three different salinity levels at vegetative stage in the net house

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. Number of alleles and polymorphism information content (PIC) value 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

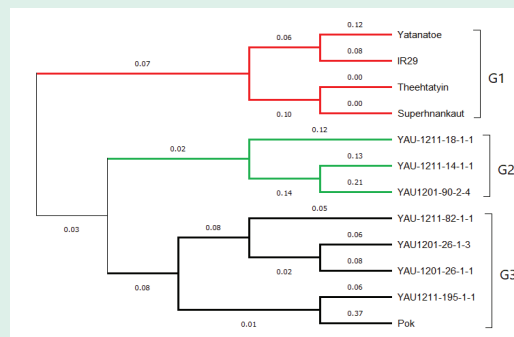


Fig 1. Dendrogram of 12 rice genotypes based on 7 polymorphic SSR markers according to the Shared allele distance with neighbour joint method

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