



## **Assessment of Salinity Tolerance and Analysis of SSR Markers Linked with *SaltoI* QTL in Sri Lankan Rice (*Oryza sativa*) Genotypes**

**B. A. Dahanayaka<sup>1</sup>, D. R. Gimhani<sup>1</sup>, N. S. Kottearachchi<sup>1\*</sup>  
and W. L. G. Samarasinghe<sup>2</sup>**

<sup>1</sup>*Department of Biotechnology, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila (60170) (NWP), Sri Lanka.*

<sup>2</sup>*Rice Research and Development Institute, Batalagoda, Ibbagamuwa, Sri Lanka.*

### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author BAD conducted the experiment, involved in data collection, analysis, and interpretation and wrote the first draft of the manuscript. Author DRG established the experimental design, involved in data collection, analysis and revision of the first draft of the manuscript. Author NSK guided and reviewed the experimental design and all drafts of the manuscript. Author WLGS guided and facilitated the collecting of required planting materials. All authors read and approved the final manuscript.*

### **Article Information**

DOI: 10.9734/AJEA/2015/20255

#### Editor(s):

(1) Sławomir Borek, Department of Plant Physiology, Adam Mickiewicz University, Poland.

#### Reviewers:

(1) Klara Kosova, Crop Research Institute, Prague, Czech Republic.

(2) Anonymous, University of Dhaka, Bangladesh.

(3) Shilpi Srivastava, Gorakhpur University, India.

Complete Peer review History: <http://sciencedomain.org/review-history/11232>

**Original Research Article**

**Received 18<sup>th</sup> July 2015**  
**Accepted 16<sup>th</sup> August 2015**  
**Published 2<sup>nd</sup> September 2015**

### **ABSTRACT**

**Aim:** This research was aimed at assessing Sri Lankan rice varieties for the salinity tolerance using morphological traits and to analyze the SSR markers closer to *SaltoI* QTL of the chromosome 1 to be used in rice breeding and gene mapping studies.

**Place and Duration of Study:** The research was conducted at the Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, from April 2013 to October 2013.

**Study Design:** Morphological traits were analyzed by ANOVA and polymorphic bands obtained from SSR markers were analyzed by Jaccard's similarity coefficient following the unweighted pair group method with arithmetic mean (UPGMA).

\*Corresponding author: E-mail: [kottearachchins@yahoo.com](mailto:kottearachchins@yahoo.com);

**Methodology:** Morphological traits of twenty rice germplasm of Sri Lankan origin, including traditional and improved varieties were assessed under 12 dS/m saline stress and five SSR markers located between 10 -15 Mb to *Saltol* QTL were analyzed in relation to salinity tolerance.

**Results:** A novel weighted indicator, Salinity Survival Index, revealed that Goda Wee, At354 and Al Wee varieties were highly salinity tolerant compared to the tested varieties including an accession of Pokkali and the morphological traits also showed the same validation. Diversity analysis of SSR marker alleles linked with *Saltol* QTL clustered the salinity tolerant and salinity susceptible germplasms as compatible with the distribution pattern of salinity survival index and the marker, RM1287 was more informative for the screening of rice germplasm for salinity tolerance.

**Conclusion:** Information derived on morphological traits under salinity stress and the polymorphic SSR marker patterns obtained from tolerant and susceptible varieties near *Saltol* region would be useful in selecting parental lines from the tested varieties for rice breeding and gene mapping programs designed for salt tolerance.

**Keywords:** Rice germplasm; salinity tolerance; *Saltol*; SSR markers.

## 1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important grain crops in Asian countries and it is consumed by two thirds of the world population as their staple food. With the escalating growth rate of world population, the demand for the rice is increasing year by year. Therefore, marginal lands like salt affected lands also need to be utilized to gain maximum production.

Among abiotic stresses, salinity has been recognized as the second most wide spread problem for reduction in growth and productivity of rice in all over the world [1]. Millions of hectares in coastal regions face salinity due to marine/brackish water intrusion to the ground while the inland salinity can occur due to poor irrigation, digestion of ores, human activities etc. In Sri Lanka, approximately 13% of the irrigated lands are affected by salinity stress [2] and this percentage increases gradually in both coastal regions and inlands [3].

Most of the rice varieties are extremely sensitive to salinity during young seedling stage and early development stage [4]. Even though there are agronomical practices like improved field drainage, maintenance of adequate amount of water around 2 to 3 cm height until early reproductive stage [5], establishment of crop by transplanting [3] and use of organic manure instead of inorganic fertilizer are used to address salinity, these practices are not cost effective, efficient and favorable for a long-term solution. Hence, cultivation of durable resistant rice varieties appears more appropriate as a long lasting solution. Therefore, identification of salt

tolerant varieties and their transformation into high yielding cultivars is necessary in order to utilize salt affected lands for sustainable rice cultivation.

Salinity tolerance is considered as a quantitative trait as it is governed by a collection of many genes that are called quantitative trait loci (QTL). *Saltol* is one such previously reported major QTL which is reported to be responsible for the salinity tolerance for many tolerant rice cultivars [6]. DNA based molecular markers which are used in marker assisted selection (MAS) are extensively used to tag QTL with phenotypic characters to accelerate breeding processes. Hence, this study was conducted to evaluate the growth performance of the seedlings of twenty rice cultivars under salt stress and to detect their genotype at *Saltol* region aiming at finding the relationship with salt tolerance in order to use them in marker assisted selection and gene mapping studies.

## 2. MATERIALS AND METHODS

### 2.1 Planting Material

The phenotypic evaluation of the germplasm was conducted in a rain sheltered plant house at Wayamba University of Sri Lanka in mid 2013. Twenty rice germplasm from diverse genomic background representing traditional and improved varieties were collected from Plant Genetic Resources Centre, Gannoruwa, Sri Lanka, and Rice Research and Development Institute (RRDI), Batalagoda, Sri Lanka (Table 1).

**Table 1. Varietal information and level of salinity tolerance by standard evaluation score (SES) and salinity survival index (SSI)**

Variety	Accession No <sup>#</sup>	Cultivation background	Average of SES	Salinity tolerance	SSI
Kaluheenati 1(KH1)	003989	Traditional	4	T	0.435
Kaluheenati 2(KH2)	011041	Traditional	5	M	0.410
Lanka Samurdhi (LS)	008921	Improved	8.5	S	0.152
Kuruluthuda (KT)	004759	Traditional	6	M	0.354
Suwadel (Suw)	010729	Traditional	9	HS	0.108
Pokkali (Pok)	005556	Traditional	3.5	T	0.525
Maa Wee (Maa)	008551	Traditional	3.5	T	0.585
Al Wee (Al)	004023	Traditional	3	T	0.738
Goda Wee (Goda)	006182	Traditional	2	HT	0.776
Moraberakan (Mor)	006897	Traditional	6	M	0.379
Muhudu Ralla (MR)	004773	Traditional	6	M	0.321
Kivul Handiran (Kivul)	004106	Traditional	4	T	0.454
Rathu Heenati (RH)	004992	Traditional	6	M	0.351
Basmathi (Bas)	006820	Traditional	7	S	0.268
Bw 400 <sup>*</sup>	005311	Improved	3.5	T	0.488
At 401 <sup>*</sup>		Improved	3.5	T	0.638
Bg 357 <sup>*</sup>		Improved	8	S	0.240
Ld 356 <sup>*</sup>		Improved	6	M	0.371
At354 <sup>*</sup>		Improved	2	HT	0.763
Bg352 <sup>*</sup>		Improved	8	S	0.223

<sup>#</sup> Varieties with accession numbers were obtained from Plant Genetic Resources Centre, Gannoruwa, Sri Lanka.

<sup>\*</sup> Varieties were obtained from Rice Research and Development Institute, Bathalegoda, Sri Lanka. HT- highly tolerant T- tolerant M- moderately tolerant S- susceptible HS- highly susceptible

**Table 2. Sequences, map position and annealing temperatures of six SSR markers close to *Saltol* region**

SSR marker	Sequence information 5'-3'	Position in the consensus map (Mb) ( <a href="http://www.gramene.org">www.gramene.org</a> )	Annealing temperature °C
RM10772	GCACACCATGCAAATCAATGC CAGAAACCTCATCTCCACCTCC	12.16	55
RM1287	GTGAAGAAAGCATGGTAAATG CTCAGCTTGCTTGTGGTTAG	10.83	54
RM10694	TTTCCCTGGTTTCAAGCTTACG AGTACGGTACCTTGATGGTAGAAAGG	10.96	60
RM493	GAGGTGAGTGAGACTTGACAGTGC GCTCATCATCCAACCACAGTCC	12.28	60
RM10864	GAGGTGAGTGAGACTTGACAGTGC GCTCATCATCCAACCACAGTCC	14.25	60

## 2.2 Extraction of Genomic DNA and PCR Amplification

Genomic DNA was extracted from 14 day old tender plant leaves using a mini-prep method [7] DNA samples were amplified using six SSR markers located between 10-15 Mb, on the *Saltol* QTL region of the chromosome 1 [6,8,9] (above Table 2). Amplification profile consisted of initial denaturation at 95°C for 5 minutes followed by 35 cycles of denaturation for 1 minute at 95°C, annealing at marker specific temperature (Table 2) for 30 seconds, elongation at 72°C for 5

minutes and final extension at 72°C for 5 minutes. The amplified products were electrophoresed on 3% agarose gel and stained with ethidium bromide. SSR allelic composition at each of the defined loci was determined for each genotype.

## 2.3 Establishment of Hydroponic System with Saline Stress

Hydroponics system was established according to the procedure described by Gregorio et al. [1]. Germinated seeds (two replicates with 10

seedlings per each) from each germplasm with emerging roots around 1 to 2 cm were placed according to the Completely Randomized Design (CRD) on the styrofoam floats without damaging the roots. Transplanted seedlings were allowed to stand in water for 2 days to recover damages during transplantation. After 2 days, Yoshida's nutrient solution was added with an appropriate amount of NaCl (analytical grade) to give 6 dS/m. The salinity was increased up to 12 dS/m by adding NaCl after 24 hours. The pH was adjusted daily and the solutions were renewed every 8<sup>th</sup> day. Control experiment was maintained with four seedlings under Yoshida's nutrient solution without NaCl by providing the same conditions as that of NaCl treated seedlings.

#### 2.4 Estimation of Growth Performance

The growth parameters of the seedlings such as root length (RL), shoot length (SL) and root fresh weight (RFW) were measured at the 21<sup>st</sup> day of salinity treatment. Plant materials were oven dried for 3 days at 70°C and shoot dry weight (SDW) and root dry weight (RDW) were measured. Mean values of all parameters under salinity ( $V_{Msal}$ ) were converted to relative values (RV) [5] as indicated below,

$$RV = \frac{V_{Msal}}{V_{MCon}}$$

where V is the respective value of recorded parameters such as root length, shoot length etc.,  $V_{Msal}$  is the mean value under salinity and  $V_{MCon}$  is the mean value under control.

#### 2.5 Assessment of Germplasm by Modified Standard Evaluation Score of Visual Salt Injury (SES)

The degree of visual injuries showed by the seedlings on 16<sup>th</sup> and 21<sup>st</sup> day were evaluated using the standard evaluation score method described by Gregorio et al. [1]. According to the morphological appearance, SES was assigned to each variety from 1 to 9 which is represented by 1: Highly Tolerant, 3: Tolerant, 5: Moderately Tolerant, 7: Susceptible and 9: Highly Susceptible to the salinity and, the mean value of SES was calculated.

#### 2.6 Assessment of Germplasms by Salinity Survival Index (SSI)

The number of dead plants was counted on every 3<sup>rd</sup> day after salinization and the respective

seedling survival percentage of each day was calculated until 21 days after salinization (DAS). The SSI was calculated using the formula,

$$SI = \frac{\sum_{k=1}^n D_k S_k}{\left(\sum_{k=1}^n D_k\right)} 100$$

where, D is the Day After salinization (DAS), S is the survival percentage of that particular day, n is the total period in DAS (in this experiment n is 21 DAS),  $D_k$  is the DAS at k<sup>th</sup> data collection,  $S_k$  is the survival percentage of k<sup>th</sup> data collection and k=1, 2, 3....n. as reported by Wijerathna et al. [7].

#### 2.7 Statistical Analysis

Relative values of each parameter, SSI, root length, shoot length, root fresh weight, root dry weight and shoot dry weight were analyzed by analysis of variance (ANOVA) using SAS version 9.1.3 [10]. Cluster analysis was done using SSR markers based on Jaccard's similarity coefficient following the unweighted pair group method with arithmetic mean (UPGMA) by SPSS 16.0 version [11]. Alleles obtained from SSR markers were analyzed using PowerMarker 3.25 version [12].

### 3. RESULTS AND DISCUSSION

#### 3.1 Assessment of Salinity Tolerance by Standard Evaluation Score (SES)

The modified standard evaluation score method recommended by International Rice Research Institute, Philippines, was used in rating the visual symptoms of seedlings caused by salt stress. Selected varieties of this study demonstrated all five categories of SES, after 21 days of salinization. Accordingly, the traditional Suwandel variety was highly susceptible to the salinity indicating 9 in SES. Next highest tissue damages were observed in Lanka Samurdhi, Bg357 and Bg352 indicating the SES levels of 8.5 to 8. The minimum visual injuries or the highest tolerance ability was demonstrated in Goda Wee and At354 with SES value of 2 (Table 1).

#### 3.2 Assessment of Salinity Tolerance by Salinity Survival Index (SSI)

It is important to assess survival potential of germplasms under salt stress with a quantitative parameter as such parameter is convenient for the analysis, mapping studies and it is independent of personal skills. A quantitative parameter called salinity survival index was

measured giving maximum weight for the plants that survived throughout whole period while minimum weight was given for the plants that died at earliest possible. In this study, SSI distributed within the range of 0.108 to 0.776 among twenty varieties. The highest SSI was recorded from Goda Wee (0.776), a traditional rice variety, followed by At354 (0.763), a high yielding improved rice variety, and Al Wee (0.738), another traditional variety. The lowest SSI was exhibited by Suwandel (0.108), Lanka Samurdhi (0.152) and Bg352 (0.223) (Table 1), following the same pattern as SES. Two traditional varieties, Maa Wee and Al Wee and an elite variety, At401 showed more tolerability towards salinity stress than the tested accession of Pokkali. This may probably be due to the fact that the prevalence of different accessions of Pokkali, the well known salt tolerant variety, possessing different alleles [6]. Fig. 1 shows the dropping pattern of the survival percentage against the days after salinization that was plotted for the six varieties. Three varieties, Goda Wee, At354 and Al Wee showed sudden drop in survival only at 18<sup>th</sup> day while Suwandel, Lanka Samurdhi and Bg352 showed the same drop at the 8<sup>th</sup> day after salinization.

Standard evaluation score of visual salt injury is the mostly used screening technique for salinity tolerance in rice as reported in previous studies. It was reported that visual symptom rating is adequate to determine the level of tolerance for breeding purposes as it was correlated well with yield performance in saline rice fields [1]. However, in addition to SES, in this study we used a novel parameter, SSI, which is mostly similar to SES, but distributed quantitatively. Although SSI was previously developed for the assessment of salt stress in rice under saline soil [7], the results of this study also showed a vast range of variation among germplasm even with

the saline hydroponic system, proving to be useful in both soil and hydroponics designed for screening for breeding purpose and gene mapping studies.

### 3.3 Assessment of Salinity Tolerance by Relative Growth Parameters

The objective of this assessment was to identify the ability to perform under salt stress condition and to compare the performance among varieties regardless their performance under non-stress condition. Therefore, the parameters measured such as root length, shoot length, root fresh weight, root dry weight and shoot dry weight were analyzed with the relative values. Accordingly, all the relative parameters except relative shoot dry weight were highly significant (Table 3). Also all the parameters were significant under control indicating the prevalence of different genetic variability among germplasm. This genetic variability can be nullified with the relative assessment of each trait. Therefore, the variability of relative values reflects extra genetic potential exhibiting the survival ability under salinity.

With regards to the relative root length, maximum relative root length under stress was obtained by Maa Wee and it has exceeded the root length even than the control. The higher reduction of relative root length was displayed in Suwandel, Basmati, Bg357, Bg352 and Muhudu Rella when compared to the non-stress condition and these performances are compatible with respective SSI and SES. Kuruluthuda, Pokkali, Maa Wee and At401 showed increased relative root length under salinity than under the control. This fact indicates that these varieties have the ability to increase the root biomass under stress condition, probably may be due to a strategy existed to overcome salt stress.

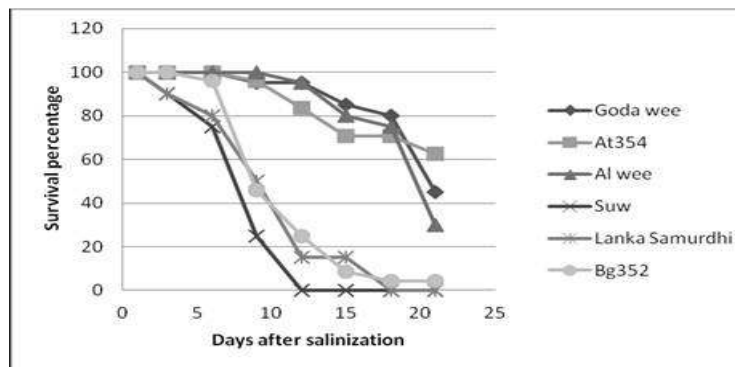


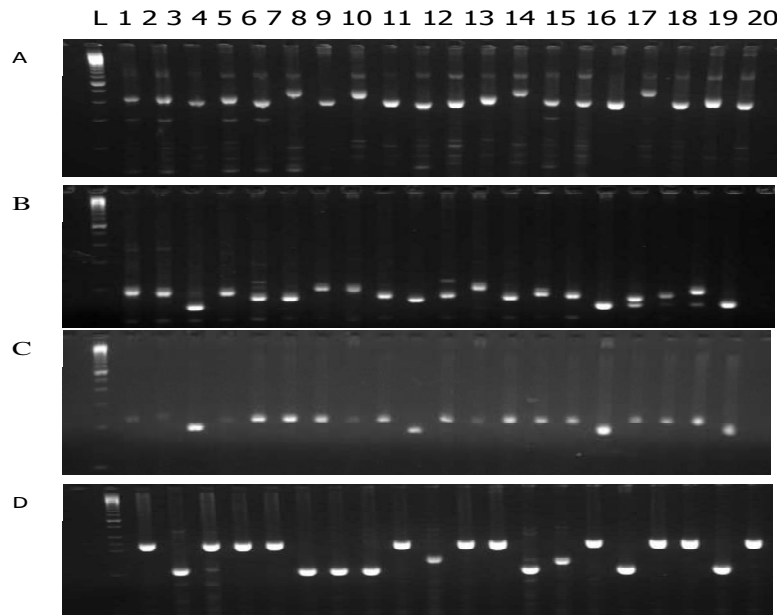
Fig. 1. Survival percentages of six varieties with the days after salinization

With respect to the relative shoot length, Suwandel displayed the maximum reduction and Bw400 displayed the minimum reduction which exceeded even under the control. Also Bw400 exhibited minimum reduction in other traits such as relative root fresh weight, relative shoot dry weight and relative root dry weight under salinity stress. Obviously, under salt stress, reduction of biomass is experienced in most of the crop plants [13]. In this study, we observed different reduction capacities in terms of root biomass and shoot biomass, in twenty rice germplasm under salt stress condition and found that there are some germplasm showing minimum biomass reduction capacity, namely Bw400, At354 and At401, performing better than the tested accession of Pokkali. Bw400, At354 and At401 also were able to categorize as 'Tolerant' or above based on SES. Moreover, Ma Wee, Al Wee and Goda Wee also showed considerably high amount of relative shoot and root biomasses under salt stress compatible to the results of SSI.

### 3.4 Assessment of Allelic Variation at *Saltol* Region among Germplasms

One objective of this study was to identify SSR markers that are linked with *Saltol* QTL located

on chromosome 1 in a diverse germplasm collection. Knowing of the polymorphic markers closer to *Saltol* region would also be useful to select parental lines in developing mapping population and breeding programs. Also polymorphic markers are useful to select background and foreground of known genes to be used in MAS [14,15]. Therefore, twenty rice germplasms were evaluated for the genetic diversity of the region closer to the promising QTL *Saltol* in chromosome 1 using SSR markers. Fig. 2 indicates the gel images showing different banding patterns of amplified DNA. Five markers used were highly polymorphic and 23 alleles were amplified with the band size of 100-300 bp. The highest number of alleles was found in RM 1287 (7), followed by RM493 (5), RM10772, RM10694 (4) and RM 10864 (3) which gave the lowest number of alleles (Table 4). Locus RM 1287 showed the highest polymorphism information content (PIC) value (0.8078) followed by RM493 (0.7470), RM10772 (0.6454), RM10694 (0.6142) and RM10864 (0.4824). The average PIC value was 0.6594 and it ranged from 0.4824 to 0.8078 (Table 4). Therefore, out of 5 markers selected in the 10-15 Mb of *Saltol* region on chromosome 1, RM1287 would be more useful for screening of rice germplasm.



**Fig. 2. SSR marker profile of rice germplasms generated by (A) RM10772, (B) RM 1287, (C) RM10694 and (D) RM10864**

Lane L: 100 bp ladder, Lane 1: KH1, Lane 2:KH2, Lane 3: LS, Lane 4: KT, Lane 5: Suw, Lane 6: Pok, Lane 7: Maa, Lane 8: Al, Lane 9: Goda, Lane 10: Mor, Lane 11: MR, Lane 12: Kivul, Lane 13: RH, Lane 14: Bas, Lane 15: Bw400, Lane 16: At401, Lane 17:Bg357, Lane 18: Ld356, Lane 19: At354, Lane 20: Bg352

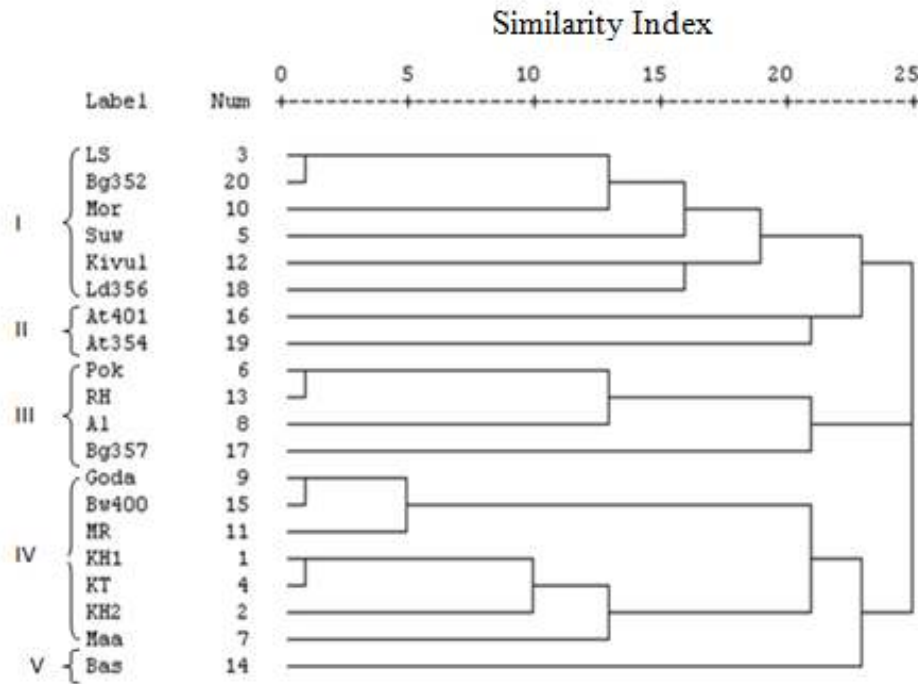
**Table 3. Means of the growth performances and relative values measured under salinity stress and control conditions**

Variety	RL			SL			RFW			SDW			RDW		
	Stressed (cm)	Control (cm)	Relative ...	Stressed (cm)	Control (cm)	Relative ...	Stressed (mg)	Control (mg)	Relative ...	Stressed (mg) ns	Control (mg)	Relative ns	Stressed (mg)	Control (mg)	Relative ...
KH1	26.11	26.34	0.99	28.82	66.38	0.43	124.31	848.40	0.15	46.78	310.20	0.15	10.73	64.40	0.17
KH2	16.56	19.72	0.84	33.70	65.20	0.52	90.80	459.00	0.20	74.76	241.75	0.31	14.58	44.25	0.33
LS	13.8	16.64	0.83	15.59	43.48	0.36	164.50	450.00	0.37	62.40	107.00	0.58	15.07	35.40	0.43
KT	23.21	22.78	1.02	27.84	60.32	0.46	306.73	529.40	0.58	67.40	188.00	0.36	14.17	33.80	0.42
Suw	9.97	15.4	0.65	16.00	52.75	0.30	171.50	288.00	0.60	65.40	124.00	0.53	10.16	21.00	0.48
Pok	17.43	16.52	1.05	36.94	65.70	0.56	167.39	495.40	0.34	79.91	187.80	0.43	13.54	31.80	0.43
MW	20.85	19.7	1.06	28.20	57.33	0.49	110.34	414.33	0.27	44.40	143.67	0.31	9.79	25.67	0.38
Al	20.19	29.16	0.69	43.63	65.17	0.67	163.91	458.75	0.36	80.62	171.33	0.47	11.74	30.50	0.39
Goda	18.14	23.88	0.76	31.55	56.76	0.56	168.29	609.00	0.28	64.00	187.20	0.34	14.44	37.40	0.39
Mor	18.7	24.96	0.75	22.52	47.86	0.47	81.26	546.40	0.15	41.60	171.00	0.24	8.90	36.40	0.24
MR	10.79	18.94	0.57	26.20	59.34	0.44	100.29	507.20	0.20	53.20	215.80	0.25	9.00	49.00	0.18
KH	18.34	22.58	0.81	40.37	72.30	0.56	223.85	582.60	0.38	91.16	283.50	0.32	13.39	45.20	0.30
RH	16.05	19.18	0.84	36.28	67.85	0.53	194.91	609.73	0.32	88.50	265.00	0.33	46.44	52.50	0.88
Bas	9.63	14.08	0.68	22.00	57.00	0.39	127.90	512.60	0.25	47.12	234.60	0.20	9.12	45.60	0.20
Bw400	14.35	15.75	0.91	28.42	21.25	1.34	234.80	195.50	1.20	104.60	144.00	0.73	18.41	22.50	0.82
At 401	19.66	18.6	1.05	29.46	48.88	0.60	246.78	556.00	0.44	193.72	303.25	0.64	18.56	46.00	0.40
Bg 357	11.62	20.33	0.57	19.63	45.10	0.44	145.85	435.75	0.33	117.10	191.75	0.61	21.53	37.50	0.57
Ld 356	15.06	19.9	0.76	23.10	46.85	0.49	188.74	361.50	0.52	65.00	155.00	0.42	12.22	27.50	0.44
At354	21.96	24.86	0.88	29.78	46.48	0.64	164.50	188.50	0.87	67.60	119.67	0.56	16.30	20.33	0.80
Bg352	16.25	25.8	0.63	22.54	51.55	0.44	112.00	157.20	0.71	61.95	109.40	0.57	15.87	22.20	0.71

\*\*\* Significant at P = 0.001, \*\* Significant at P = 0.01, <sup>ns</sup> not significant (Significant level of each parameter was obtained from ANOVA) ; RL (root length), SL (shoot length), RFW (root fresh weight), SDW (shoot dry weight) and RDW (root dry weight)

**Table 4. Allelic variability of five markers located near *Saltol* region on chromosome 1**

Marker	Allele no	Major allele frequency	Gene diversity	PIC
RM10772	4.0000	0.4000	0.7000	0.6454
RM1287	7.0000	0.2500	0.8300	0.8078
RM10694	4.0000	0.4211	0.6759	0.6142
RM493	5.0000	0.3158	0.7812	0.7470
RM10864	3.0000	0.5500	0.5650	0.4824
Mean	4.6000	0.3874	0.7104	0.6594



**Fig. 3. UPGMA dendrogram using Jaccard's similarity coefficient among twenty rice germplasms based on five SSR markers in the *Saltol* QTL region of chromosome 1**

The dendrogram (above Fig. 3) obtained from 23 alleles of 5 SSR primers showed 5 major clusters at similarity levels of 23. More susceptible germplasms like Suwandel, Bg352 and Lanka Samurdhi were grouped in cluster I. Two improved, highly salinity tolerant varieties, At354 and At401 were separately clustered in cluster II. Cluster III contained two sub clusters of which one sub cluster contained susceptible variety Bg357 while other sub cluster represent tolerant and moderately tolerant varieties. The highest tolerant cultivar, Goda Wee, was clustered together with another tolerant cultivar, Bw400, in a sub cluster of major cluster IV suggesting a similar genetic background at the tested region, *Saltol* of chromosome 1. These two varieties exhibited favorable phenotypic traits for salt tolerance although both were originated from two

completely different backgrounds. When the main cluster IV is considered, regardless the sub clusters, it can be noted that all the varieties included are either salinity tolerant or moderately tolerant ones. They are four tolerant varieties, Goda Wee, Bw400, Kaluheenati1 and Maa Wee and, three moderately tolerant varieties, namely Muhudu Rella, Kaluheenati2 and Kuruluthuda. In this cluster except Bw400, all other six varieties are traditional varieties suggesting that these varieties might have been originated from the same ancestor grown in Sri Lanka. It was interesting to note that a salinity susceptible imported variety, Basmathi, was separately clustered in cluster V.

Diversity analysis based on SSR marker alleles linked with *Saltol* clustered the salinity tolerant



and salinity susceptible germplasms as compatible with the distribution pattern of salinity survival index. Comparison of the SSR allele results revealed interesting relationships related to the overall level of salinity tolerance in germplasms and their origin. Based on these alleles, salinity susceptible varieties and tolerant varieties were able to be separately sub-clustered in some main clusters of the dendrogram, which appears to be useful in selecting parental lines for breeding on salt tolerance.

#### 4. CONCLUSION

In this study, of twenty cultivars, two traditional varieties, Goda Wee and Al Wee and, an improved variety, At354 were highly tolerant to the salt level of 12 dS/m, showing more than 0.7 of salinity survival index. Also traditional varieties, Pokkali, Ma Wee, Kivul Handiran and Kaluheenati1 and improved varieties, Bw400 and At401, were categorized as tolerant varieties. All these nine germplasms showed minimum reduction capacities of root and shoot biomass under salinity stress of 12 dS/m confirming their comparative salt tolerant nature. Five markers selected in the 10-15 Mb of *Saltol* region on chromosome 1 were highly polymorphic and of them, RM1287 would be more useful for screening of rice germplasm for salinity tolerance.

#### ACKNOWLEDGEMENTS

Grant, RG/2011/BT/02 of National Science Foundation, Sri Lanka is greatly acknowledged.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

#### REFERENCES

- Gregorio GB, Senadhira D, Mendoza RD. Screening rice for salinity tolerance, IRRI Discussion Paper Series International Rice Research Institute Los Banos Laguna Philippines. 1997;22.
- hiruchelvam S, Pathmarajah S. An economic analysis of salinity problems in the Mahaweli river system H irrigation scheme in Sri Lanka. Economy and Environment program for Southeast Asia Research Report Series; 1999.
- Sirisena DN, Rathnayake WMUK, Herath HMA. Productivity enhancement of saline paddy fields in Angiththamkulam yaya, Sri Lanka a case study. *Pedologist*. 2010; 96-100.
- Heenan DP, Lewin LG, McCaffery DW. Salinity tolerance in rice varieties at different growth stages. *Australian Journal of Experimental Agriculture*. 1988;28: 343-49.
- De Costa WAJM, Wijeratne MAD, De Costa DM. Identification of Sri Lankan rice varieties having osmotic and ionic stress tolerance during the first phase of salinity stress. *Journal of National science Foundation Sri Lanka*. 2012;3:251-80.
- Thomson MJ, De Ocampo M, Egdane J, Rahman MA, Sajise AG, Adorada DL, Tumimbang-Raiz E, Blumward E, Seraj ZI, Singh RK, Gregorio GB, Ismail AM. Characterizing the *Saltol* quantitative trait locus for salinity tolerance in rice. *Rice*. 2010;3:148-60.
- Wijerathna YMAM, Kottearachchi NS, Gimhani DR, Sirisena DN. Exploration of relationship between fragrant gene and growth performances of fragrant rice (*Oryza sativa* L.) seedling under salinity stress. *Journal of Experimental Biology and Agricultural Sciences*. 2014;1:7-15.
- Chattopadhyay K, Nath D, Mohanta RL, Bhattacharyya S, Marndi BC, Nayak AK, Singh BP, Sarkar RK, Singh ON. Diversity and validation of microsatellite markers in *Saltol* QTL region in contrasting rice genotypes for salt tolerance at the early vegetative stage. *Australian Journal of Crop Science*. 2014;8(3):356-62.
- Islam MR, Gregorio GB, Salam MA, Collard BCY, Singh RK, Hassan L. Validation of *Saltol* linked markers and haplotype diversity on chromosome 1 of rice. *Molecular Plant Breeding*. 2012;3(10): 103-14.
- SAS Institute Inc. SAS 9.1.3 Output delivery system: user's guide. Cary, NC: SAS Institute Inc. 2006;1.
- SPSS Inc. Released. SPSS for Windows. 16.0 Chicago. SPSS Inc; 2007.
- Liu K, Muse SV. Power Marker: Intergrated analysis environment for genetic marker data. *Bioinformatics*. 2005;21:2128-212.
- Munns R, Tester M. Mechanisms of salinity tolerance. *Annual Review of Plant Biology*. 2008;59:651-81.
- Vu HTT, Le DD, Ismail AM, Le HH. Marker assisted backcrossing (MABC) for

- improved salinity tolerance in rice (*Oryza sativa* L.) to cope with climate change in Vietnam. Australian Journal of Crop Science. 2012;6(12):1649-54.
15. Jena KK, Mackill DJ. Molecular markers and their use in marker assisted selection in rice. Crop Science. 2008;48(4):1266-76.

---

© 2015 Dahanayaka et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Peer-review history:*  
*The peer review history for this paper can be accessed here:*  
<http://sciencedomain.org/review-history/11232>