Phenotypic and Genotypic Screening of Rice Genotypes for Salt Tolerance

[1]Ruby Rawal, [2]Vipul Kumar ,[2]Mohammad Shahid, and [3]S. K. Sharma
[1]Department of Biochemistry, Kurukeshtera University, Kurukeshtra
[2]Biocontrol laboratory, Department of Plant Pathology, C.S.A. University of Agriculture & Technology, Kanpur-208002, India
[3]Central Soil Salinity Research Institute, Karnal – 132001

Abstract:- **In the present study, attempt was made to study molecular and morphological screening of 6 rice genotypes (CARI DHAN 5, NDRK 11-1, NDRK 11-2,NDRK 11-3,NDRK 11-4,NDRK 11-5) for salt tolerance by using SSR marker. Phenotypic and genotypic screenings of these genotypes was done for salinity tolerance. Four traits were selected for phenotypic screening namely grain length, grain width, 100 seed weight and yield per plant. The genotype NDRK 11-1 expressed highest grain width of 3.1cm in control whereas it was 2.9 cm in salinity. The genotype NDRK 11-3 was expressed highest yield per plant of 21.3 g in control whereas NDRK 11-1 had expressed highest yield per plant of 13.6g in saline. Two traits were selected for physiological screening, namely Na and K accumulation. The genotype was expressed highest Na accumulation of NDRK 1-5 (0.27) in control whereas in saline NDRK 11-4 (7.14).The genotype was expressed highest K accumulation of NDRK 11-5 (12.91) in control whereas in saline NDRK 11-4 (10.72). . The results suggest that micro satellite markers could be used for the estimation of genetic diversity and the identification of rice cultivars. The Saltol markers in rice can be proficiently used in tagging salt tolerant genes and identification of salt tolerant rice genotypes.**

Keywords: salinized NDRK,.

I. INTRODUCTION

Salt stress is a major constraint across many rice production areas because of the high sensitivity of modern rice varieties. Salinity is particularly a major problem in coastal regions in the tropics where rice-based farming systems predominate. This is because of the intrusion of brackish water during the dry season and at the start of the wet season. Salt stress is also a worsening problem in inland areas because of the buildup of salinity as a consequence of excessive use of irrigation water with improper drainage coupled with the use of poor quality irrigation water (Ismail et al 2010). Rice is considered sensitive to salinity, particularly during early vegetative and later at reproductive stages decreases grain yield much more than salinity during vegetative stage (Akbar and Ponnamperuma, 1982). Nonetheless, it is one of the few crops that can thrive on salt-affected soils because of its ability to grow well in standing water that can help leach salts from topsoil and is, therefore, recommended as an entry crop for desalinization of salt affected lands (Ismail et al. 2007; Singh et al 2010).

Although, many workers have devised several mechanical and chemical methods to reclaim the salt affected soils, they are either expensive or not readily applicable. Hence, the use of plant species that can tolerate high salt level is important for sustainable crop production on such soil. Therefore, the second approach is ideal and cost effective. This may be achieved by making use of variation in the tolerance both between and within cultivars (Shannon and Francois, 1978). The identification of tolerant genotypes, their ionic uptake and transport properties, produce an initial germplasm base for breeding salt tolerant crops.

Saltol is on rice chromosome 1, and confers salinity tolerance at the seedling stage, which is important for good crop establishment in coastal areas. Researchers mapped Saltol's location by crossing a traditional Indian cultivar with moderate salt tolerance (Pokkali) with a saline-sensitive cultivar (IR29). Once engineered into rice, Saltol and Pup1 can increase rice productivity and improve farmer income. "Both salinity and phosphorus deficiency are widespread and often coexist, especially in the rainfed fields of the poorest farmers," explains Abdelbagi Ismail, principal investigator of the Saltol and Pup1 projects, "Globally, more than 15 million hectares of rice lands are saline, and more than half of all rice lands are phosphorus deficient." Keeping all the points in view the experiment was carried out with following objectives.To evaluate the rice genotypes for morphological traits

- To evaluate the rice genotypes for physiological traits and To screening the rice genotypes using SALTOL markers

II. EXPERIMENTAL SET UP

The research work was conducted during the period from January 2011 to June 2011 in CSSRI, Karnal. The experiment was laid out in a glass house at Central Soil Salinity Research Institute, Karnal (Haryana) during kharif season of 2012.

A. Sowing of rice seeds:

The 6 different rice genotypes(table 1) namely, CARI DHAN 5, NDRK 11-1, NDRK 11-2, NDRK 11-3, NDRK 11-4, and NDRK 11-5 were collected from Central Soil Salinity Research Institute, Karnal (Haryana). After collecting the genotypes, they were sown on floating grid in hydroponics (sowing of seed) and culture solution (table 1)to collect the sample for extraction of DNA from all the genotypes.

Table1: Rice Culture Solution (Modified Yoshida Solution)

Stock	Reagent	Grams/litre	Grams/10litre
1	NH4NO3	91.4	914
2	K2SO4	71.4	714
3 (a)	KH2PO4	23.1	231
(b)	K2HPO4	4.3	43
4	CaCl2.6H2O	175	1750
5	MgSO4.7H2O	324	3240
Minor Nutrients			
6 (a)	MnCl2.4H2O	1.5	15
(b)	(NH4)6Mo7O24 4H2O	0.074	0.74
(c)	H3BO3(Boric Acid)	0.93	9.30
(d)	ZnSO4.7H2O	0.035	0.35
(e)	CuSO4.5H2O	0.03	0.30
7 or	FeNaEDTA*	10.5	105
8	FeSO4	2.5	25

* Freshly prepared

Steps involved in hydroponic sowing:

1. A proposed 20 seeds of each genotype were counted and uniformly spread on well labelled floating grids (24x10 c.m.) which are divided in to four parts.
2. The floating grids were then carefully floated in a tank containing 160 litres of water and then shrink the water on seed from upper part because it is mostly dry and it affect the plant germination.
3. The seeds were allowed to germinate for about 7 days in a Glass House at 250C with more than 80% humidity.
4. After three days, water in the tank was replaced with a freshly prepared Yoshida solution (pH=4.5) and the germinated seedlings were allowed to grow for 20-30 days.
5. Also, at an interval of every three days solution of the tank was replaced for better result by another yoshida solution tank.

B. Collection of plant samples:

After one month of sowing, leaf samples of various rice genotypes were cut 8-10 cm above the grid using a clean scissor, from the nursery. The leaf samples of different rice genotypes were wrapped in separate aluminum foils and were labeled properly using a permanent marker. While collection of samples, samples were stored in an ice box containing packs of solid Co_2 to protect the samples from getting destroyed. After sample collection all the samples collected were stored at -20 $^{\circ}$c.

C. DNA Isolation

Total genomic DNA was isolated from leaves following a cetyl trimethyl ammonium bromide (CTAB) method. 5 grams of leaves were ground in liquid nitrogen, then added 2 ml extraction buffer and incubated at 65°C for 40 minutes. Added 1 ml solution of chloroform:isoamyl alcohol (24:1) v/v and mixed for 5 sec. This solution was then separated using of centrifuge with the speed of 12000 rpm for 10 minutes at room temperature. The supernatant was separated from the pellet by putting into on new eppendorf tube. The DNA in the supernatant was purified using 1ml of choloroform: isoamyl alcohol (24:1) and centrifuge it. The DNA was precipitated with chilled 1 ml isopropanol and incubated at -20 c for 30 minutes. The DNA precipitate was washed with 70% ethanol. The pelleted DNA was air dried and resuspended in 2 ml 1X TE buffer (10mM Tris-HCl pH 7.5, 10mM EDTA) or milli Q water.

D. . PCR (POLYMERASE CHAIN REACTION):

The PCR amplification was carried out according to the following programme (table 3).

Table 2: PCR Programme

Stage	Step	Temperature (^{o}C)	Duration (min)	No of cycles
I	Initial denaturation	94	5	1
II	1. Denaturation	94	1	35
	2. Annealing	36	1	-
	3. Extension	72	2	-
III	Final extension	-	5	1

After the completion of required cycles of amplification the samples were stored at 4°C in a refrigerator and the contents were loaded on to metaphor gels for electrophoresis. After 1:30 to 2:00 hours of electrophoresis, the electric current was turned off and the leads were removed. The lid of tank was opened and photographed using gel documentation system (BIO-RAD).

III. RESULTS AND DISCUSSION:

A. Grain Length

The performance of grain length (cm) is presented in the table 7. Grain length did not differ significantly among different genotypes of rice. The genotype was expressed highest grain length of NDRK 11-4 (10.4cm) under control and (10.0) saline stress. The next best was NDRK 11-3 (9.4) in both expressed lowest grain length of CARI DHAN 5(7.2) in control and saline stress.

B. Grain Width

The performance of grain width (cm) is presented in the table 7. Grain width did not differ significantly between different genotypes of rice under both control and saline conditions.

The genotype was expressed highest grain width of NDRK 11-1 (3.1) in control whereas in saline (2.9). The next best was NDRK 11-2 in control (2.8) whereas in saline (2.6). NDRK 11-5 genotype was expressed lowest grain width (1.8) under control and (1.7) saline stress.

C. 100 seed weight

The performance of 100 seed weight (gm) is presented in the table 7. 100 seed weight did not differ significantly between different genotypes of rice under both control and saline conditions.

The genotype was expressed highest 100 seed weight of NDRK 11-5 genotype (2.6) under control and NDRK 11-3 (2.4) saline conditions. The next best was NDRK11-3(2.5) in control (1.9) whereas in saline NDRK 11-5 (2.0). The genotype was expressed lowest 100 seed weight of NDRK 11-2 in control (1.5) whereas in saline (0.8).

D. Yield reduction from normal to saline

The performance of yield per plant (gm) is presented in the table 7. Yield per plant differ significantly between different genotypes of rice under control and saline stress. The genotype was expressed highest yield per plant of NDRK 11-3 in control (21.9) whereas in saline NDRK 11-1 (13.6). The next best was NDRK 11-5 in control (20.6) whereas in saline NDRK 11-3 (9.0). The genotype was expressed lowest yield per plant of CARI DHAN 5 (10.6) under control and (3.4) in saline conditions. These results imply that salt tolerant genotypes (having lower salt tolerance score) exhibited higher plant height and root length under salt stress(table 4). Peng et al. (1999) reported that increasing plant height would allow greater biomass production. Zhang et al. (2004) found similar results from their studies on doubled haploid (DH) population consisting of 81 DH lines. They reported that increase of plant height was mainly responsible for the increase in biomass which might increase the yield potential (fig 1).

Table 3: Performance of rice genotypes for different traits control and salinity (EC_{iw} 10)

Sl. No	Designation	Genotypes	Grain Length		Grain Width		100 seed weight (g)		Yield / plant (g)	
			Control	*Saline*	*Control*	*Saline*	*Control*	*Saline*	*Control*	*Saline*
1	STBN 9	CARI Dhan 5	7.2	7.2	2.7	2.7	2.2	2.0	10.6	3.4
2	STBN 10	NDRK 11-1	7.9	7.9	3.1	2.9	1.7	1.4	18.2	13.6
3	STBN 11	NDRK 11-2	8.6	8.6	2.8	2.6	1.5	0.8	15.7	5.6
4	STBN 12	NDRK 11-3	9.4	9.4	2.7	2.6	2.5	2.4	21.9	9.0
5	STBN 13	NDRK 11-4	10.4	10.0	2.8	2.6	2.2	1.5	19.6	6.6
6	STBN 14	NDRK 11-5	9.3	9.2	1.8	1.7	2.6	2.0	20.6	7.1
	Mean		8.8	8.7	2.7	2.5	2.1	1.7	17.8	7.5
	Max		10.4	10.0	3.1	2.9	2.6	2.4	21.9	13.6
	Min		7.2	7.2	1.8	1.7	1.5	0.8	10.6	3.4

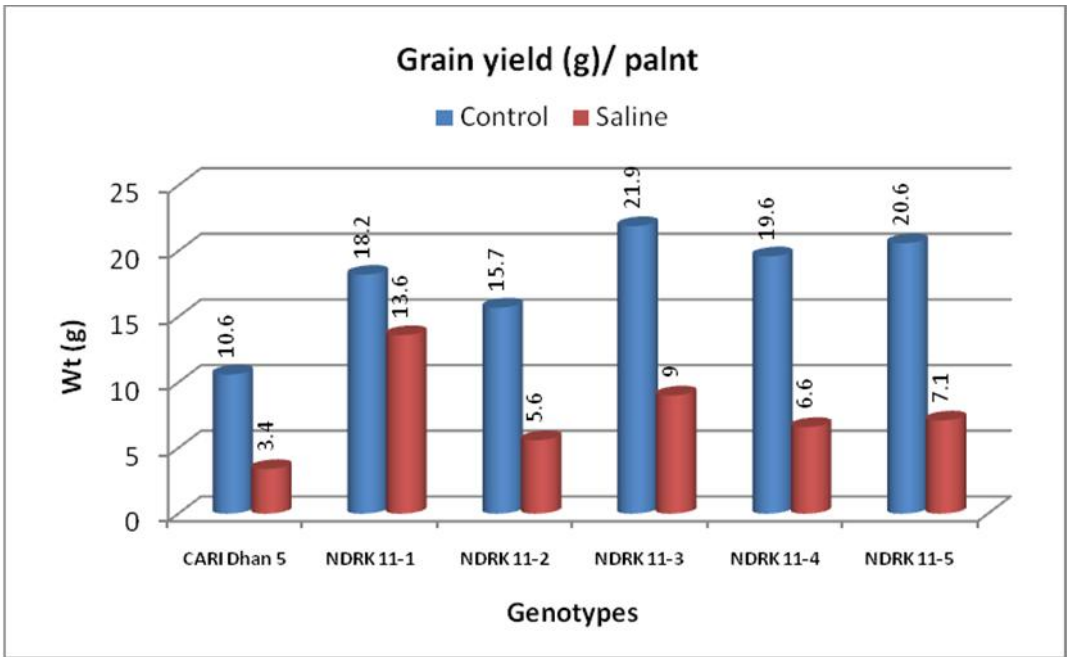

Fig. 1: Yield performance of rice genotypes in control and saline stress.

E. Physiological parameters in rice (Oryza sativa) genotypes

3.5.1 Sodium Content (mmol g/dry wt): The performance of Na accumulation (mmol g/dry wt) is presented in the table 8. Sodium accumulation was differing significantly between different genotypes of rice under control and saline stress.

The genotype was expressed highest Na accumulation of NDRK 11-5 (0.27) in control whereas in saline NDRK 11-4 (7.14). The genotype was expressed lowest sodium content in NDRK 11-2-1n control (0.13) whereas in saline CARI DHAN 5 (2.55).

***3.5.2 Potassium Conten*t (mmol g/dry wt):** The performance of K accumulation (mmol g/dry wt) is presented in the table7. Potassium accumulation was differing significantly between different genotypes of rice under control and saline conditions.

The genotype was expressed highest K accumulation of NDRK 11-5 (12.91) in control whereas in saline NDRK 11-4 (10.72). The genotype was expressed lowest K accumulation CARI Dhan 5 in control (5.27) whereas in saline NDRK 11-1 (4.95) (table 5&6) and fig 2&3.

Table 4: Sodium and potassium content in rice genotypes under control and saline stress .

Sl. No	Designation	Genotypes	Na Content (Leaf) mmol g /dry wt		K Content (Leaf) mmol g /dry wt	
			Control	Saline	Control	Saline
1	STBN 9	CARI Dhan 5	0.20	2.55	5.27	6.39
2	STBN 10	NDRK 11-1	0.20	3.09	5.90	4.95
3	STBN 11	NDRK 11-2	0.13	6.03	9.13	5.41
4	STBN 12	NDRK 11-3	0.20	6.46	12.51	8.36
5	STBN 13	NDRK 11-4	0.22	7.14	12.35	10.72
6	STBN 14	NDRK 11-5	0.27	7.04	12.91	7.86
	Mean		0.20	5.38	9.68	7.28
	Max		0.27	7.14	12.91	10.72
	Min		0.13	2.55	5.27	4.95

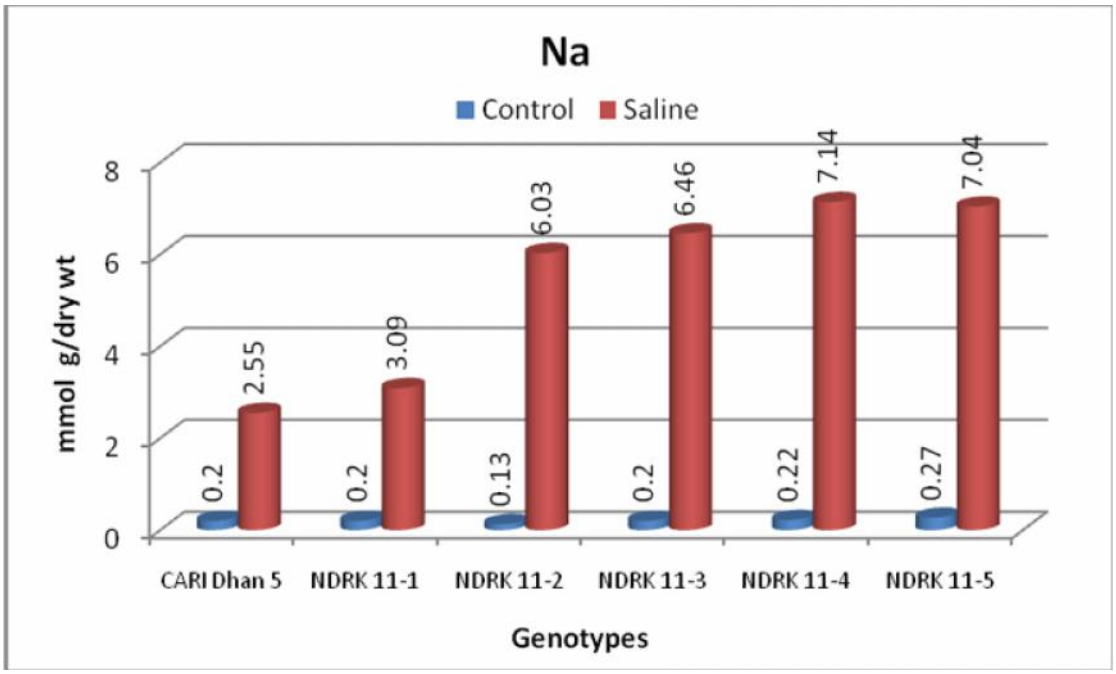

Fig. 2: Accumulation of Na content in rice genotypes under control and saline stress.

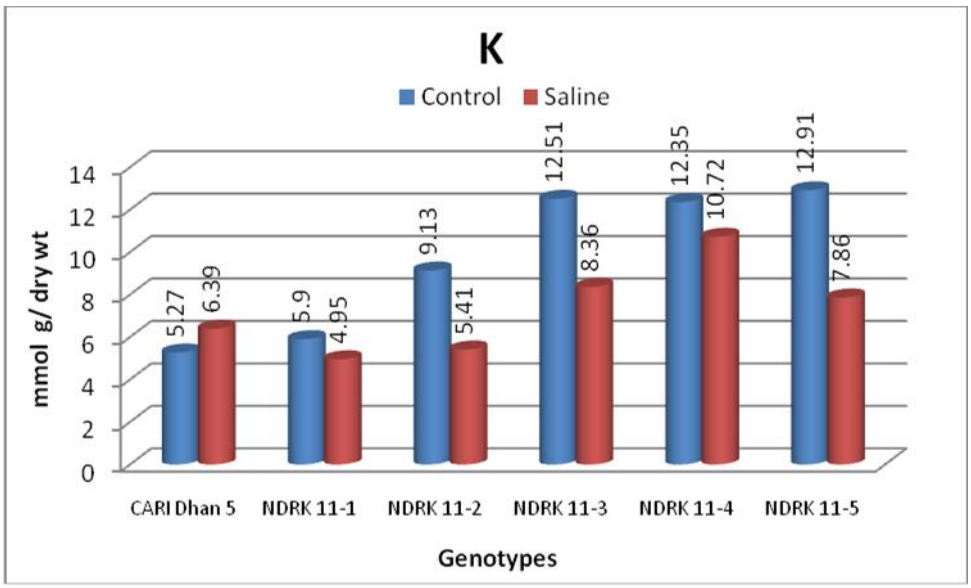

Fig. 3: Accumulation of K content in rice genotypes under control and saline stress.

Table 5: Performance of rice genotypes for different qualitative traits.

Sl. No	Designation	Genotypes	Husk Colour		Seed coat colour	
			Control	Saline	Control	Saline
1	STBN 9	CARI Dhan 5	Golden	Golden	Light brown	Light brown
2	STBN 10	NDRK 11-1	Golden	Golden	Light brown	Light brown
3	STBN 11	NDRK 11-2	Golden	Golden	White	White
4	STBN 12	NDRK 11-3	Golden	Golden	Light brown	Light brown
5	STBN 13	NDRK 11-4	Golden Brown	Golden	Light brown	Light brown
6	STBN 14	NDRK 11-5	Golden Brown	Golden Brown	Light brown	Light brown

All genotypes were grown robustly and showed uniform husk colour and seed coat colour in the salinized and non salinized conditions.

F. Assessment of genetic diversity using SSR markers

In this experiment, SALTOL markers were used to assess the genetic diversity among the rice genotypes. The SSR profiles of rice genotypes generated by four *saltol* markers: RM 10793, RM 8094, RM 10829, and RM 493. The SSR marker RM 10793 was found to be polymorphic in nature. The markers RM 10829, RM 8094 AND RM 493 are monomorphic in nature(Fig. 4-7).

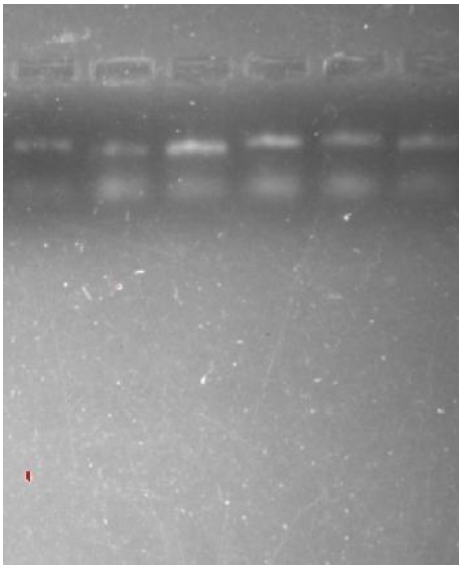

Fig. 4: SSR profile generated by saltol primer RM 493. Lane 1: STBN 9, Lane 2: STBN 10, Lane 3: STBN 11, Lane 4: STBN 12, Lane 5: STBN13, Lane 6: STBN14.

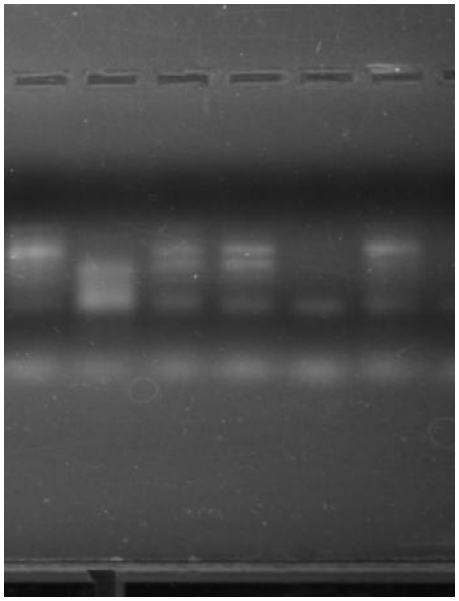

Fig. 5: SSR profile generated by saltol primer RM 8094. Lane 1: STBN 9, Lane 2: STBN 10, Lane 3: STBN 11, Lane 4: STBN 12, Lane 5: STBN13, Lane 6: STBN14.

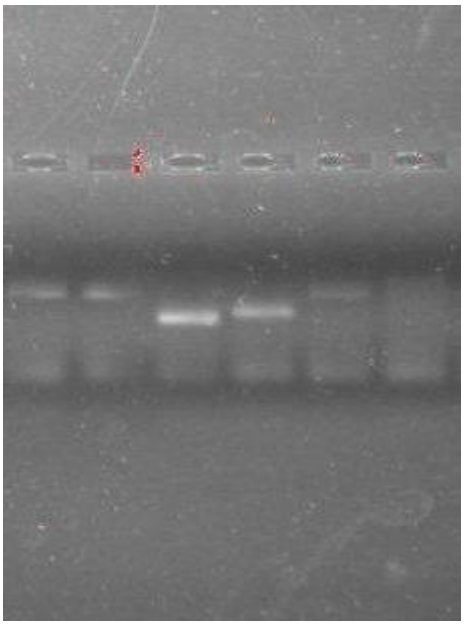

Fig. 6: SSR profile generated by saltol primer RM 10793. Lane 1: STBN 9, Lane 2: STBN 10, Lane 3: STBN 11, Lane 4: STBN 12, Lane 5: STBN13, Lane 6: STBN14.

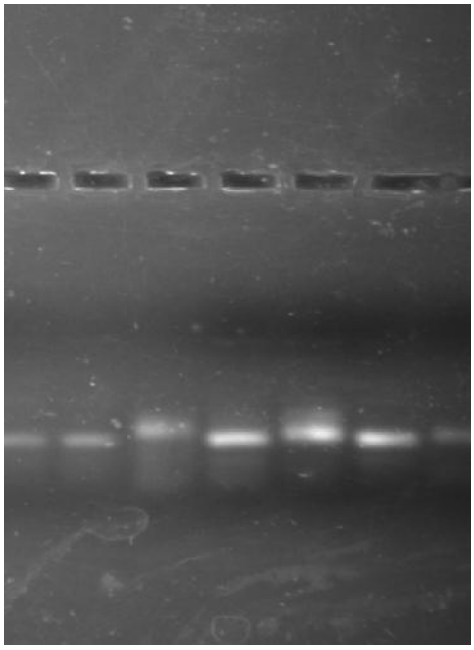

Fig. 7: SSR profile generated by saltol primer RM 8094. Lane 1: STBN 9, Lane 2: STBN 10, Lane 3: STBN 11, Lane 4: STBN 12, Lane 5: STBN13, Lane 6: STBN14.

Four markers (RM 8094, RM 493, RM3412, RM10793, and RM10829) were used to estimate the genetic diversity among six genotypes (RAU -1428-13-7, RAU-1-16-48, CR 2218-207-3-1-1, CR 2461-1-122-2-1, CR 2219-44-2, CARI Dhan 2). A cluster analysis using UPGMA based on similarity coefficients was done to resolve the phylogenetic relationships among the different rice genotypes considered for the present study. Sequential agglomerative hierarchical non-overlapping (SAHN) clustering was performed on squared Euclidean distance matrix and similarity matrix using Jacquard's coefficient utilizing the unweighted pair group method with arithmetic averages (UPGMA) method. Data analysis was done using the software NTSYSpc version 2.02. At 50 % similarity co-efficient, rice genotypes were grouped into major two clusters. In cluster I, four rice genotypes were grouped together whereas, another rice genotype was grouped in cluster II. The first branch of cluster consists of four genotypes (CARI DHAN 5, NDRK 11-5, NDRK 11-1, NDRK 11-4) and the NDRK 11-3, NDRK 11-2 were grouped separately in different Clusters i. e Cluster II and Cluster III, respectively at 37% of similarity. The second branch the binary data matrix was subjected to cluster analysis. The first cluster was sub divided into two main clusters (clusters I and clusters II) and the cluster I consists of two genotypes namely, RAU -1428-13-7 and CR 2461-1-122-2-1. The cluster II consists of CR 2219-44-2 and it is clustered with CARI Dhan2 with similarity co efficient of 0.54. The second cluster was subdivided into one cluster. Cluster III is subdivided into two clusters were RAU-1-16-48 and CR 2218-207-3-1(Fig. 8)

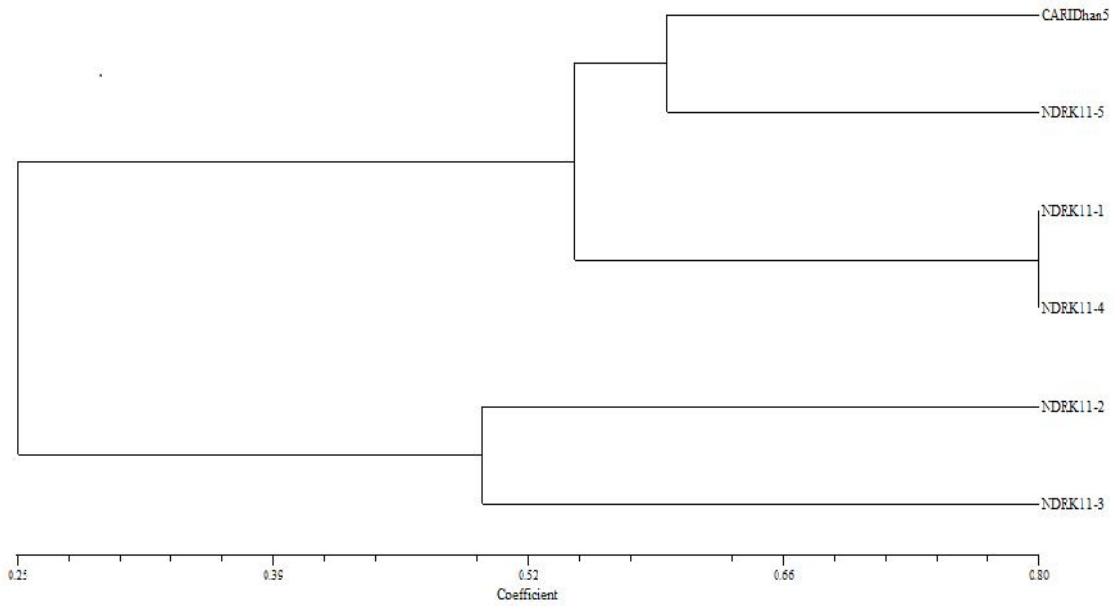

Fig. 8: UPGMA based dendogram of rice genotypes

Molecular markers help to identify alleles that are associated with key phenotypic traits (Xu et al. 2006). Nguyen et al. (2001) found that the marker had association with NaCl tolerant alleles in the seedling population (IR64/ChengHui 448, IR64/OM1706 and IR64/FR13A) under EC 18 dS/m and salt stress genes were located at loci in chromosomes 1 and 8.

3.7 Physiological parameters

Table 6: Sodium and potassium content in rice genotypes under control and saline stress.

Sl. No	Designation	Genotypes	Na Content (Leaf)mmol g /dry wt		K Content (Leaf) mmol g /dry wt	
			Control	Saline	Control	Saline
1	STBN 9	CARI Dhan 5	0.20	2.55	5.27	6.39
2	STBN 10	NDRK 11-1	0.20	3.09	5.90	4.95
3	STBN 11	NDRK 11-2	0.13	6.03	9.13	5.41
4	STBN 12	NDRK 11-3	0.20	6.46	12.51	8.36
5	STBN 13	NDRK 11-4	0.22	7.14	12.35	10.72
6	STBN 14	NDRK 11-5	0.27	7.04	12.91	7.86
	Mean		0.20	5.38	9.68	7.28
	Max		0.27	7.14	12.91	10.72
	Min		0.13	2.55	5.27	4.95

IV. CONCLUSION

The SSR profiles of rice genotypes was generated by four *saltol* markers: RM 10793, RM 8094, RM 10829, and RM 493 all were found polymorphic. The rice genotypes CARI DHAN 5 and NDRK 11-5 were found to be distantly related with **NDRK**-11-3, NDRK 11-2, with other genotypes, this might be because of adaptation of genes in different locations. The present study revealed a moderate to high level of diversity at molecular level among the set of rice genotypes studied. The results suggest that micro satellite markers could be used for the estimation of genetic diversity and the identification of rice cultivars. The *Saltol* markers in rice can be proficiently used in tagging salt tolerant genes and identification of salt tolerant rice genotypes.

REFERENCES

[1] Ismail A.M., Thomson M.J., Vergara G.V., Rahman M.A., Singh R.K., Gregorio G.B., 2010. Designing resilient rice varieties for coastal deltas using modern breeding tools. Wallingford: CAB; 154–65.

[2] Hoanh CT, Szuster BW, Pheng KS, Ismail AM, Nobel AD, editors. Tropical Deltas and coastal zones: food production, communities and environment at the land-water interface.

[3] Akbar M., Ponnamperuma F.N., 1982. Saline soil of South and Southeast Asia as potential rice lands. In: IRRI, ed. Rice Research Strategies for the Future."IRRI, Manila, the Philippines: 256–281.

[4] Ismail A.M., Heuer S., Thomson M.J., Wissuwa M., 2007. Genetic and genomic approaches to develop rice germplasm for problem soils. Plant Mol Biol. 65, 547–70.

[5] Singh R.K., Flowers T.J., 2010. The physiology and molecular biology of the effects of salinity on rice. In: Pessarakli M, editor. Handbook of plant and crop stress. 3rd ed. Florida: Taylor and Francis, 901–942.

[6] Shannon C., Francois, M., 1978. Testing salt tolerance variability among tall wheat cross line. *Agronomy Journal.* 70, 719-722.

[7] Nguyen Trong Luong., Vuong Đinh Tuan ., Pham Van Ro. 2001. Results of the regional rice mutant multilocation trials in Mekong Delta of Viet Nam. The Second meeting on “Reviewing Results and Planning of regional rice mutant multilocation trials. Malaysia Sept. 174-180.

[8] Xu K., Xu X., Fukao T., Canlas P., Maghirang-Rodriguez R., Heuer S.,Ismail A.M., Bailey-Serres J., Rald P.C., Mackill D.J.2006. Sub1A is an ethylene-response-factor-like gene that confers submergence tolerance to rice.Nature. 442, 705–708.

[9] Peng, J., et al. 1999. b. Green revolution’ genes encode mutant giberellin response modulators. Nature .400, 256–261