Performance of Rice Landraces Under Salt Stress at the Reproductive Stage Using SSR Markers

Md. Abdullah Al Ibrahim[1], Md. Hasanuzzaman Rani[2], Shamsun Nahar Begum[2], Md. Babul Akter[3]*and Mirza Mofazzal Islam[2]*

[1]Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[2]Plant Breeding Division and [3]Crop Physiology Division, Bangladesh Institute of Nuclear Agriculture (BINA), BAU Campus, Mymensingh-2202, Bangladesh

ABSTRACT

Salinity is the most significant cause of rice yield reduction in many rice-growing areas of the world. The aim of this study was to screen 24 rice genotypes including 20 landraces to find the potential germplasm source for salt tolerance in breeding program. Screening was performed at reproductive stage based on the yield and yield attributes in sustained water bath maintaining the salinity level at 8 dS/m. Three microsatellite markers linked with salt tolerance quantitative trait loci *viz.* RM234, RM134 and RM9 were used for investigation of salt tolerant rice landraces. At the reproductive stage, four landraces *viz.* Kute Patnai, Kashrail, Bazra Muri and Tal Mugur were identified as salt tolerant on the basis of phenotypic evaluation. Besides, eight rice genotypes *viz* Binadhan-8, Patnai, KutePatnai, Bazra Muri, Tal Mugur, Pokkali, Kashrail and FL 378 were found as salt tolerant using SSR marker. Considering both assessment, four rice genotypes *viz.* Kute Patnai, Kashrail, Bazra Muri and Tal Mugur were selected as true salt tolerant lines. Therefore, these identified landraces could be potential germplasm sources for future salt tolerance rice breeding program.

Keywords: Rice germplasm, salinity, yield, microsatellite marker

1. INTRODUCTION

Rice (*Oryza sativa* L.) is an important food crop that feeds more than half of the world's population. Asian farmers contribute about 92% of the total world's rice production [1]. But rice is very sensitive to salinity stress and currently listed as the most salt sensitive cereal crop with a threshold of 3 dS/m for most cultivated varieties [2]. Salinity is a major constraint to rice production that directly regulates the plant growth and development [3-5]. It affects all the growth stages of rice from germination through maturation [6] but early seedling and reproductive stages are the most sensitive to salt stress [7, 34]. Rice yield in salt-affected land is significantly reduced with an estimation of 30–50% yield losses annually [8]. Due to natural salinity and human interferences, the arable land is continuously transforming into saline that is expected to have overwhelming global effects, resulting in up to 50% land loss by 2050 [9,10].

In Bangladesh, 11.37 million hectares of land produces 34.53 million tons of rice [11] and about 1.8 million ha of coastal land is affected by different degrees of salinity. Most of the southern districts of the country are under saline zones which cover an area of 25-30% of the total cultivable land [12]. The population of Bangladesh is still growing by two million every year and may increase by another 30 million over the next 20 years. Thus, Bangladesh will require about 27.26 million tons of more rice for the year 2020 (http://www.knowledgebank-brri.org/riceinban.php). The increase of rice growing areas and the adoption of salt tolerant varieties are important factors that contribute in more rice production. Methods for salinity tolerance screening are also important for the success of a breeding program. To improve the rice yield under saline condition is one of the main targets of plant breeding and screening for salinity tolerance based on agronomical parameters such as growth, yield and yield components is becoming a popular method worldwide [13-16].

The use of molecular markers has been increasing considerably in breeding programs because of their reliability which helps in deciding the distinctiveness of genotypes [17]. Among the molecular marker technologies, microsatellite or simple sequence repeats (SSRs) are widely used in rice genetic studies because of their availability, widespread distribution in the genome, high allelic diversity [18-23], efficient for identification of genes and quantitative trait loci in different rice cultivars [24, 25]. Landraces are currently being used as preferred potential donors of salt tolerance traits because of their local adaptation. Due to genetic similarities among cultivated rice genotypes, the transfer of useful genes from one to another is possible. The presence of markers tightly linked to salt tolerance genes will allow selection and maintenance of the desirable tolerant genotypes in breeding process [26, 27]. Thus, the evaluation of rice landraces could provide valuable sources for genetic improvement of salt tolerant rice variety.

Therefore, the objective of this study was to identify the salt tolerant rice landraces based on phenotype and molecular markers evaluation which can be used further for marker assisted selection in rice breeding program.

2. MATERIALS AND METHODS

2.1 Plant materials

A total of 24 rice lines including 20 landraces, two high yielding varieties developed by BINA (Bangladesh Institute of Nuclear Agriculture) and two advanced lines were used in this study (Table 1). BINA developed salt tolerant variety Binadhan-8 which was used as tolerant while HYV Binadhan-7 was used as susceptible control.

2.2 Phenotypic evaluation under the saline condition

The genotypes were evaluated for their tolerance to salinity under sustained water bath condition following IRRI standard protocol for salinity screening at the reproductive stage [13]. Completely randomized design (CRD) with three replications was followed for experimental design. Both normal and salinized setups were maintained. At first, pot was prepared by inserting a cloth bag inside the pot and then it was filled up with fertilized soil followed by 50 N, 25 P and 25 K mg kg^{-1} of soil. Initially, the soil level was about 1 cm above the topmost circle of holes and the pots with leveled soil were placed in a plastic tray which serves as water bath. Then the plastic tray was filled up with no saline tap water having EC 0.2 dS/m measured by EC meter. The soil was watered and then left for absorbing water to settle down. To maintain the accurate soil level, additional soil was added after two days. The seeds were kept in the conventional oven for 5 days at 50˚C for breaking the seed dormancy during the soil settlement time. Then the oven treated seeds were soaked with tap water for 24 hours for pre-germination. The pre-germinated seeds were sown (3/4 seeds/pot) on the soil surface of the perforated pot. After 2 weeks, thinning was carried out to maintain two seedlings per pot and then water level was raised up to 1 cm above the soil surface. The experimental pots were observed on daily basis to maintain the level of water, pests and diseases. After 3 weeks of seed sowing, the pots were salinized at EC 8 dS/m by dissolving crude salt and monitored in every week using EC till maturity. In our country, salinity level varies between 6-8 dS/m at reproductive stage of rice [19]. So, we screened our studied genotypes at EC 8 dS/m. Data were recorded on plant height (cm), days to flowering, number of effective tillers/plant, number of field grains and unfilled grains, percent fertility and grain yield (g). The following formula was used for calculating the percent fertility and reduction:

Percent fertility= {(No. of filled grains/ (No. of filled grains+ No. of unfilled grains)} x100

Percent (%) reduction = {(traits in normal - traits in saline)/Traits in normal} x100

2.3 DNA extraction, PCR amplification and molecular marker analysis

Modified CTAB mini prep method was used for genomic DNA extraction from leaf sample of 25 days old seedling [28]. Each PCR reaction carried out with 13.0 µl reactions containing 1.5 µl 10x buffer, 0.75 µl dNTPs, 1µl primer forward, 1µl primer reverse, 0.25 µl taq polymerase, 7.5 µl ddH_2O and 1.0 µl of each template DNA samples. PCR analysis was performed according to previous study by Akter et al. [29] with little modifications. PCR profile was maintained as initial denaturation at 94^oC for 5 min., followed by 34 cycles of denaturation at 94^oC for 30 second, annealing at 55^oC for 30 second and extension at 72^oC for 1min., and a final extension of 7 min. at 72^oC. Ten primers were surveyed and among them three primers showed polymorphism between the parents (Table 2). Finally, three polymorphic SSR markers (Table 2) were used for genotyping the 24 rice landraces. The amplified

PCR products were separated in a 2.5 % agarose gel and then stained in 0.1 g/ml ethidium bromide containing water. Banding patterns were visualized with ultraviolet gel documentation system. The banding patterns of 24 genotypes were scored by comparing with tolerant and susceptible controls.

3. RESULTS AND DISCUSSION

3.1 Phenotypic performances of rice landraces at reproductive stage

A wide range of significant phenotypic variation was observed at reproductive stage among the rice genotypes under 8 dS/m salinity stress. Normal growth and development was observed in control but some confrontational symptoms were found in salinized condition like cracked and dried leaves, stunted plant growth and early flowering & maturity. Rice genotypes showed significant differences in reduction of plant height, panicle length and number of filled grains.

The percentage of plant height reduction ranged from 6.55 to 29.24 and the highest reduction rate was observed in Volanath (29.24%) followed by Rupessor (28.59%), Binadhan-7 (27.42%) and Koicha binni (26.88%). On the other hand, Pokkali (6.55%) followed by Binadhan-8 (6.61%), Kashrail (7.54%), FL-378 (8.17%), Tal Mugur (8.84%), Bazra Muri (8.96%), FL-478 (9.43%), Kute Patnai (10.63%), Nona Bokra (10.74%), Jamai naru (12.44%) and Patnai (12.77%) showed comparatively lower reduction rate (Table 3). Therefore, the reduction might be occurred due to salt stress during growth and development. The similar results were also reported by Rubel *et al.* [30], Bhowmik *et al.* [31] and Choi *et al.* [32].

Percent reduction in panicle length was ranged from 6.88 to 22.61. Considering the panicle length, Volanath (22.61%), Binadhan- 7 (21.91%), Rupessor (21.35%) and Koicha Binni (21.56%) showed heigher reduction. Besides, Kashrail (6.88%), Pokkali (7.11%), Binadhan-8 (7.20%), FL-478 (7.43%) Patnai (7.69%), FL-378 (8.19%), Bazra Muri (8.72%), Nona Bokra (8.99%), Kute Patnai (9.13%), Tal Mugur (9.40%) and Jamai Naru (9.60%) displayed lower reduction (Table 3).

Considering the number of filled grains per panicle, Volanath (76.35%), Rupessor (69.91%), Binadhan-7 (72.12%) and Koicha Binni (68.94%) showed higher reduction and Patnai (27.56%), Bazra Muri (34.44%), Pokkali (37.69%), Kashrail (39.32%), Binadhan-8 (43.14%), Kute Patnai (43.23%), FL-378 (44.46%), Tal Mugur (45.05%) and FL-478 (47.92%) exhibited lower reduction (Table 3).

Under salt stress condition, about 80 unfilled grains panicle^{-1} was found in Volanath, Rupessor, Koicha Binni, and Holde Gotal whereas Kashrail, Pokkali, Binadhan-8, FL-478, Patnai, FL-378, Bazra Muri, Kute Patnai, Tal Mugur and Nonabokra produced less than 50 unfilled grains per panicle (Table 4). But under normal growth condition, the range of unfilled grain was found about 15 to 35 per panicle except Binadhan-7 and Bashful Balam.

Considering the effective tiller plant^{-1} Bashful Balam, Chinikani, Volanath, Rupessor and Fulkainja showed higher (>30) reduction. But Kashrail, Pokkali, Nona Bokra, Kute Patnai, Patnai, Bazra Muri, Kalo Mota, Binadhan-8 and Kashrail showed lower reduction (< 20) (Table 4).

Under salinized condition, the rice genotypes Binadhan-8, Kashrail, Pokkali, FL-478, Nona Bokra, Kute Patnai, Tal Mugur, Patnai, FL-378 and Bazra Muri showed higher fertility (> 60%) and Rupessor, Koicha Binni, Volanath, Jamainaru, Ghunshi and Holde Gotal exposed lower fertility (< 45%) (Table 5). All the genotypes exhibited more than 70% fertility at control.

Under normal growth condition all the genotypes produced about 10 g or more yield plant^{-1} but less than 10 g yield plant^{-1} in salinized condition revealed that grain yield production was reduced due to salt stress. Jamai Naru, Kute Patnai, Holde Gotal, Bazra Muri, Kalo Mota, Tal Mugur, Binadhan-8, FL-378, Kashrail and Pokkali produced more than 8 g yield plant^{-1} and Ghunshi, Volanath, Binadhan-7, Rupessor and Jolkumri produced less than 5 g yield plant^{-1} (Table 4). The same result was reported by Asch *et al.* [33] where 80 rice cultivars were used. This result suggests that the salt tolerant cultivars are different from susceptible in up taking salt and yield production.

3.2 SSR marker survey for salt tolerance rice genotypes

In this experiment, initially ten primers namely, RM314, RM140, RM1594, RM9, RM407, RM510, RM51, RM121, RM134 & RM234 were screened for polymorphism survey using twenty four rice landraces. Of them, three SSR markers *viz.*, RM19, RM134 and RM234 showed highly polymorphism and were selected to evaluate 24 rice germplasms for salt tolerance. According to the phenotypic performance, Binadhan-8 was considered as tolerant and Binadhan-7 was considered as susceptible. The genotypes having banding pattern similar to Binadhan-8 were considered as tolerant and those similar to Binadhan-7 were considered as salt susceptible (Table 6).

As compared to Binadhan-8, the genotypes Patnai, Kute Patnai, Chinikani, Tal Mugur, Ghigoj, Bazra Muri, Ghunshi, Kashrail, Pokkali and FL-378 were found tolerant when the DNA samples were amplified with RM9 as produced the band in the same level of Binadhan-8. Besides, Holde Gotal, Bashful Balam, Volanath, Rupessor and FL 478 were found susceptible comparing with Binadhan-7 (Fig. 1). Previously, RM9 marker was also used for identification of salinity tolerance rice genotypes [35].

In case of RM134 primers, BazraMuri, Patnai, Kute Patnai, Holde Gotal, Nona Bokra, Kashrail, Pokkali and FL 378 were found as tolerant and Volanath, Rupessor, and Jolkumri were identified as susceptible (Fig. 2). Regarding to RM234 primers, KutePatnai, BazraMuri, Tal Mugur, Kashrail, Pokkali and FL-478 were identified as tolerant. Patnai, Ghunshi, Chinikani, Volanath Nona Bokra and Rupessor were found susceptible (Fig. 3). Recently, the screening of rice genotypes was done using Binadhan-8 rice variety for salt tolerance using RM234 markers [36].

The results revealed that all the primer pairs detected polymorphism among the rice genotypes. The microsatellite loci were also multiallelic (nine to twelve allele per locus with a mean of 11.33/locus) and the alleles were co-dominant suggesting their relative superiority in detecting DNA polymorphism over some other markers with different allele size. These markers were also reported as highly polymorphic for tagging salt tolerant genes [19,21]. So, the studied three markers might be useful for identifying salt tolerance rice but it should be confirmed for further use.

4. CONCLUSION

Based on phenotypic observation, Binadhan-8, Kute Patnai, Kashrail, FL-378, Tal Mugur, Bazra Muri were found as tolerant while Binadhan-7, Rupessor, Koicha Binni, Volanath were found as susceptible. This phenotypic observations support the genotypic findings for identification of salt tolerant rice genotypes. The selected salt tolerant landraces can be used further in rice breeding program to develop salt tolerant high yielding varieties.

ACKNOWLEDGEMENTS

This work was supported by strengthening research activities and sub-stations development project of BINA, Mymensingh-2202, Bangladesh.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Authors MAI and MMI designed the study, wrote the protocol and did the statistical analysis. Authors MHR, SNB and MBA managed the literature searches, wrote the first draft of the manuscript and final proof submission. All authors read and approved the final manuscript.

REFERENCES

1. Mitin A. 2009. Documentation of selected adapted strategies to climate change in rice cultivation. East Asia Rice Working Group, pp. 25-28.
2. USDA. 2013. *Bibliography on Salt Tolerance. Fibres, Grains and Special Crops*. Riverside, CA: George E. Brown, Jr. Salinity Lab. US Department Agriculture, Agriculture Research Service.
3. Galvani A. 2007. The challenge of the food sufficiency through salt tolerant crops. Rev. Env. Sci. Biotechnol., 6: 3-16.
4. Lauchli A, Grattan SR. 2007. Plant growth and development under salinity stress. In: *Advances in molecular breeding toward drought and salt tolerant crops*, (Eds.): M.A. Jenks, P.M. Hasegawa and S.M. Jain. Springer, Dordrecht, Netherlands.
5. Arshad, M, Saqib M, Akhtar J, Asghar M. 2012. Effect of calcium on the salt tolerance of different wheat (*Triticum aestivum* L.) genotypes. Pak. J. Agri. Sci., 49: 497-504.
6. Manneh B. 2004. Genetic, physiological and modeling approaches towards tolerance to salinity and low nitrogen supply in rice (*Oryza sativa L*.). PhD Thesis, Wageningen University, Wageningen, The Netherlands, pp. 260.
7. Yokoi S, Bressan RA, Hassegawa PM (2002) Salt Stress tolerance of Plant. JIRCUS Working Report pp. 25-33.
8. Eynard A, Lal R, Wiebe K. 2005. Crop response in salt-affected soils. J. Sustain.Agric. 27: 5-50.
9. Saha P, Chatterjee P, Biswas AK. 2010. NaCl pretreatment alleviates salt stress by enhancement of antioxidant defense and osmolyte accumulation in mungbean (*Vigna radiata* L. Wilczek). Indian J. Exp. Biol., 48:593-600.
10. Hasanuzzaman M, Nathan K, Fujita M. 2013. Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages. In: Ahmad, P., Azooz, M.M., Prasad, M.N.V. (Eds.), Ecophysiology and responses of plants under salt stress. Springer, New York, pp. 25-87.
11. BBS. 2014. Agriculture Wing. Bangladesh Bureau of statistics, Ministry of planning, Government of the People's Republic of Bangladesh, Dhaka.
12. SRDI. 2012. Saline soils of Bangladesh. Soil Resources and Development Institute. Ministry of Agriculture, Farmgate, Dhaka-1215.
13. Gregorio GB, Senadhira D, Mendoza RD. 1997. Screening rice for salinity tolerance. IRRI discussion paper series no. 22. Manila (Philippines): International Rice Research Institute. pp. 1-30.
14. Zeng L, Shannon MC, Grieve CM. 2002. Evaluation of salt tolerance in rice genotypes by multiple agronomic parameters. Euphytica, 127: 235-245.

15. Lee, IS, Kim DS, Lee SJ, Song HS, Lim YP, Lee YI. 2003. Selection and characterizations of radiation-induced salinity tolerant lines in rice. Breed. Sci. 53:313-318.
16. El-Hendawy SE, Ruan Y, Hu Y, Schmidhalter U. 2009. A Comparison of screening criteria for salt tolerance in wheat under field and controlled environmental conditions. J. Agronomy Crop Sci. 195, 356-367.
17. Mani P, Bastin J, Arun kumar R, Ahmed ABA. 2010. RAPD analysis of genetic variation of four important rice varieties using two OPR primers. ARPN J. Agric. Biol. Sc., 5: 25-31.
18. Garland SH, Lewin L, Abedinia M, Henry R, Blakeney A. 1999. The use of microsatellite polymorphism for the identification of Australian breeding lines of rice. Euphytica, 108: 53-63.
19. Islam MM. 2004. Mapping salinity tolerance genes in rice at reproductive stage. Ph. D. Dissertation. University of the Philippines Los Banos, College, Laguna, Philippines. p. 150.
20. Bhuiyan MAR. 2005. Efficiency in evaluating salt tolerance in rice using phenotypic and marker assisted selection. M.S. Dissertation, Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh, Bangladesh. pp. 96.
21. Niones JM. 2004. Fine mapping of the salinity tolerance gene on chromosome 1 of rice (*Oryza sativa L.*) using near-isogenic lines. M.S. Dissertation. University of the Philippines Los Banos, Laguna, Phil. P 78.
22. Cho YG, Ishii T, Temnykh S, Chen X, et al. (2000). Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.). Theor. Appl. Genet., 100: 713-722.
23. Harrington S. 2000. A survey of genetic diversity of eight AA genome species of *Oryza* using microsatellite markers. MS thesis Cornell University, Ithaca.
24. Bres-Patry C, Loreux M, Clément G, Bangratz M, et al. (2001). Heredity and genetic mapping of domestication-related traits in temperate japonica weedy rice. Theor. Appl. Genet., 102: 118-126.
25. Moncada P, Martinez CP, Borrero J, Châtel M, et al. 2001. Quantitative trait loci for yield and yield components in an *Oryza sativa* x *Oryza rufipogon* BC_2F_2 population evaluated in an upland environment. Theor. Appl. Genet. 102: 41-52.
26. Hittalmani S, Foolad MR, Mew T, Rodriguez RL, et al. 1995. Development of PCR based markers to identify rice blast resistance gene, Pi-2(t), in a segregation population. Theor. Appl. Genet. 91: 9-14.
27. Naqvi NI, Chattoo BB. 1996. Development of a sequence characterized amplified region (SCAR) based indirect selection method for a dominant blast-resistance gene in rice. Genome, 39: 26-30.
28. IRRI. 1997. International Rice Research Institute. Annual Report for 1997. Los Banos, Laguna, Philippines. P. 308.
29. Akter MB, Kim B, Lee Y, Koh E, Koh H-J. 2014. Fine mapping and candidate gene analysis of a new mutant gene for panicle apical abortion in rice. Euphytica, 197:387-398.
30. Rubel MH, Hassan L, Islam MM, Robin AHK, Alam MJ. 2014. Evaluation of rice genotypes under salt stress at the seedling and reproductive stages using phenotypic and molecular markers. Pak. J. Bot., 46(2): 423-432.
31. Bhowmik SK, Islam MM, Emon RM, Begum SN, Sultana S. 2007. Identification of salt tolerant rice cultivars via phenotypic and marker-assisted procedures. Pak. J. Biol. Sci., 10 (24): 4449-4454.
32. Choi WY, Lee KS, Ko JC, Choi SY, Choi DH. 2003. Critical saline concentration of soil and water for rice cultivation on a reclaimed saline soil. Korean J. Crop Sci., 48: 238-242.
33. Asch F, Dingkuhn M, Wittstock C, Doerffling K. 1998. Sodium and Potassium uptake of rice panicles as affected by salinity and season in relation to yield and yield components. J. Plant Soil, 207: 133-145.
34. Singh RK, Gregorio GB, Jain RK. 2007. QTL mapping for salinity tolerance in rice. Physiol. Mol. Biol. Plants., 13: 87-99.

35. Sudharani M, Reddy PR, Reddy GH. 2013. Identification of genetic diversity in rice (*oryza sativa* L) Genotypes using microsatellite markers for salinity tolerance. Inter. J. Sci. Innov. Discov., 3 (1): 22-30.
36. Emon RM, Gregorio GB, Nevame AYM, Islam MM, Islam M, Ye-Yang F. 2015. Morpho-Genetic Screening of the Promising Rice Genotypes under Salinity Stress. J. Agril. Sci., 7(5): 94-111.

Table 1. List of rice genotypes used in the experiment

Sl. No.	Genotypes	Type	Source of collection
1.	Jamai Naru	Bangladeshi Landrace	BINA
2.	Patnai	Bangladeshi Landrace	BINA
3.	Kute Patnai	Bangladeshi Landrace	BINA
4.	Holde Gotal	Bangladeshi Landrace	BINA
5.	Bashful Balam	Bangladeshi Landrace	BINA
6.	Bazra Muri	Bangladeshi Landrace	BINA
7.	Ghunshi	Bangladeshi Landrace	BINA
8.	Chinikani	Bangladeshi Landrace	BINA
9.	Binadhan 7	HYV	BINA
10.	Volanath	Bangladeshi Landrace	BINA
11.	Rupessor	Bangladeshi Landrace	BINA
12.	Kalo Mota	Bangladeshi Landrace	BINA
13.	Nona Kochi	Bangladeshi Landrace	BINA
14.	Tal Mugur	Bangladeshi Landrace	BINA
15.	Ghigoj	Bangladeshi Landrace	BINA
16.	Fulkainja	Bangladeshi Landrace	BINA
17.	Koicha binni	Bangladeshi Landrace	BINA
18.	Nona Bokhra	Indian local cultivar	IRRI
19.	Binadhan 8	Salt tolerant HYV	BINA
20.	FL 378	Salt tolerant exotic line	IRRI
21.	Kashrail	Bangladeshi Landrace	BINA
22.	Jolkumri	Bangladeshi Landrace	BINA
23.	Pokkali	Indian local cultivar	IRRI
24.	FL 478	Salt tolerant exotic line	IRRI

Table 2. The sequence and size of the microsatellite markers used for screening salt tolerant rice lines

Primer name	Expected PCR product size (bp)	Primer sequence		Annealing Temperature (^oC)
RM234	156	For.	ACAGTATCCAAGGCCCTGG	55
		Rev.	CACGTGAGACAAAGACGGAG	
RM134	93	For.	ACAAGGCCGCGAGAGGATTCCG	55
		Rev.	GCTCTCCGGTGGCTCCGATTGG	
RM9	136	For.	GGTGCCATTGTCGTCCTC	55
		Rev.	ACGGCCCTCATCACCTTC	

Table 3. Effects of salinization (EC 8dS/m) on plant height, panicle length and number of filled grains at reproductive stage of the rice germplasm grown in sustained water bath at BINA

SL No.	Genotypes	Plant height (cm)			Panicle Length (cm)			No. of filled grains/ panicle		
		Non-salinized (mean)	Salinized (mean)	% Reduction	Non-salinized (mean)	Salinized (mean)	% Reduction	Non-salinized (mean)	Salinized (mean)	% Reduction
1	Jamai Naru	144.40	122.40	15.24	19.80	17.90	9.60	89.30	39.20	56.10
2	Patnai	134.70	117.50	12.77	20.80	19.20	7.69	112.10	81.20	27.56
3	Kute Patnai	136.40	121.90	10.63	20.80	18.90	9.13	102.70	58.30	43.23
4	Holde Gotal	125.50	105.50	15.94	22.63	20.03	11.49	99.20	47.30	52.32
5	Bashful Balam	138.60	111.70	19.41	22.90	20.10	12.23	122.20	64.10	59.56
6	Bazra Muri	129.40	117.80	8.96	19.50	17.80	8.72	78.10	51.20	34.44
7	Ghunshi	141.10	116.40	17.51	21.10	18.50	12.32	88.20	44.80	60.54
8	Chinikani	123.20	100.30	18.59	18.60	15.40	17.20	101.30	41.20	59.33
9	Binadhan 7	100.30	72.80	27.42	17.80	13.90	21.91	99.70	27.80	72.12
10	Volanath	139.20	98.50	29.24	23.00	17.80	22.61	122.20	28.90	76.35
11	Rupessor	147.60	105.40	28.59	21.87	17.20	21.35	146.90	44.20	69.91
12	Kalo Mota	138.50	118.90	14.15	23.17	20.40	11.96	116.30	48.40	58.38
13	Nona Kochi	141.50	118.00	16.61	23.50	21.00	10.64	106.20	46.60	56.12
14	Tal Mugur	123.30	112.40	8.84	23.40	21.20	9.40	104.10	57.20	45.05
15	Ghigoj	146.33	115.50	21.07	23.40	19.20	17.95	114.20	57.40	49.78
16	Fulkainja	138.00	105.40	23.62	17.50	13.89	20.63	99.70	37.60	62.29
17	Koicha binni	138.40	101.20	26.88	21.80	17.10	21.56	114.60	35.60	68.94
18	Nona Bokra	131.30	117.20	10.74	22.03	20.05	8.99	98.80	53.70	45.65
19	Binadhan 8	87.70	81.90	6.61	21.12	19.60	7.20	131.20	74.60	43.14
20	FL 378	83.20	76.40	8.17	21.13	19.40	8.19	135.40	75.20	44.46
21	Kashrail	131.30	121.40	7.54	21.23	19.77	6.88	112.30	67.70	39.72
22	Jolkumri	134.00	116.20	13.28	22.30	19.80	11.21	133.20	69.60	47.00
23	Pokkali	131.20	122.60	6.55	23.48	21.81	7.11	120.20	74.90	37.69
24	FL 478	85.90	77.80	9.43	20.20	18.70	7.43	103.50	53.90	47.92
	$LSD_{(.05)}$	3.51	3.1		0.96	1.06		3.01	1.94	

Table 4. Mean values of number unfilled grain/plant, effective tiller/plant, days to flowering of studied rice germplasm under salinized (EC 8dS/m) and non-salinized condition at reproductive stage

SL No.	Genotypes	No. of unfilled grain		No. of effective tiller/plant			Days to flowering	
		Non-salinized	Salinized	Non-silanized	Salinized	% Reduction	Non-salinized	Salinized
	Jamai Naru	25	74.23	12	9	25.00	133	123
1	Patnai	30	42.78	10	8	20.00	118	115
2	Kute Patnai	33	36.45	12	11	8.33	108	105
3	Holde Gotal	26	91.45	11	8	27.27	114	108
4	Bashful Balam	70	78.4	11	6	45.45	113	107
5	Bazra Muri	18	28.34	12	10	16.67	126	123
6	Ghunshi	22	54.68	7	5	28.57	128	123
7	Chinikani	20	51.09	10	7	30.00	116	111
8	Binadhan 7	45	69.2	9	6	33.33	106	101
9	Volanath	25	101.6	11	7	36.36	126	121
10	Rupessor	30	99.1	12	8	33.33	103	97
11	Kalo Mota	17	68.3	11	9	18.18	131	127
12	Nona Kochi	30	54.3	11	9	27.27	128	124
13	Tal Mugur	29	44.34	10	8	20.00	92	89
14	Ghigoj	38	56.34	7	5	28.57	108	105
15	Fulkainja	25	67.45	12	8	33.33	98	92
16	Koicha binni	42	88.45	11	8	27.27	96	90
17	Nona Bokhra	28	41.23	10	9	10.00	103	99
18	Binadhan 8	30	48.98	12	10	16.67	91	88
19	FL 378	28	43.8	13	9	25.00	93	89
20	Kashrail	31	46.7	9	8	11.11	94	91
21	Jolkumri	32	54.3	10	8	20.00	93	90
22	Pokkaly	26	35.78	13	11	15.38	96	93
23	FL 478	25	41.45	14	11	27.27	95	92
24	LSD$_{(.05)}$	1.35	2.2		0.34	0.95		

Table 5. Fertility (%), yield/plant of rice landraces under salnized (EC 8dS/m) and non-salinized condition at reproductive stage

SL No.	Genotypes	Fertility (%)		Yield/plant (g)	
		Non-salinized	Salinized	Non-salinized	Salinized
1	Jamai Naru	78.13	45.99	10.34	8.45
2	Patnai	78.89	60.16	16.95	7.36
3	Kute Patnai	79.18	69.88	18.97	8.34
4	Holde Gotal	79.23	43.81	17.34	8.87
5	Bashful Balam	72.89	56.08	16.19	6.19
6	Bazra Muri	81.27	64.28	13.99	9.95
7	Ghunshi	80.04	47.16	11.75	4.77
8	Chinikani	83.51	56.07	9.80	5.83
9	Binadhan -7	68.90	57.61	6.32	2.34
10	Volanath	81.78	44.68	15.34	4.23
11	Rupessor	83.04	50.35	13.67	4.89
12	Kalo Mota	87.25	51.46	18.72	8.38
13	Nona Kochi	77.97	56.53	19.17	5.12
14	Tal Mugur	78.21	51.54	17.34	8.05
15	Ghigoj	77.93	61.87	16.42	5.06
16	Fulkainja	79.95	47.73	11.41	5.59
17	Koicha binni	58.89	43.98	17.35	5.27
18	Nona Bokhra	77.92	64.25	13.35	7.96
19	Binadhan -8	81.39	64.62	19.38	8.11
20	FL 378	69.29	58.70	15.61	8.13
21	Kashrail	70.06	61.79	15.86	8.97
22	Jolkumri	82.44	65.61	10.92	4.67
23	Pokkali	82.22	73.43	14.43	9.33
24	FL 478	69.70	55.90	14.08	6.96
	$LSD_{(.05)}$	1.82	1.22	0.69	0.53

Table 6. Genotypic performance of twenty four rice germplasm using SSR markers

Genotypes	Salt tolerance with SSR markers		
	RM9	RM134	RM234
Binadhan-8, Patnai, KutePatnai, BazraMuri, Tal Mugur, Pokkali, Kashrail and FL 378	T	T	T
Binadhan-7, Bashful, Balam, Volanath, Rupessor, Nona Kochi and Koichabinni	S	S	S
Holde Gotal, Kalo Mota, Nona Bokra and FL- 478	S	T	S
Ghunshi	T	S	T
Chinikani	T	S	S
Ghigoj	T	T	S
Fulkainja and Jolkumri	S	S	T
Jamai naru	S	T	T

Where, S=Susceptible and T=Tolerant

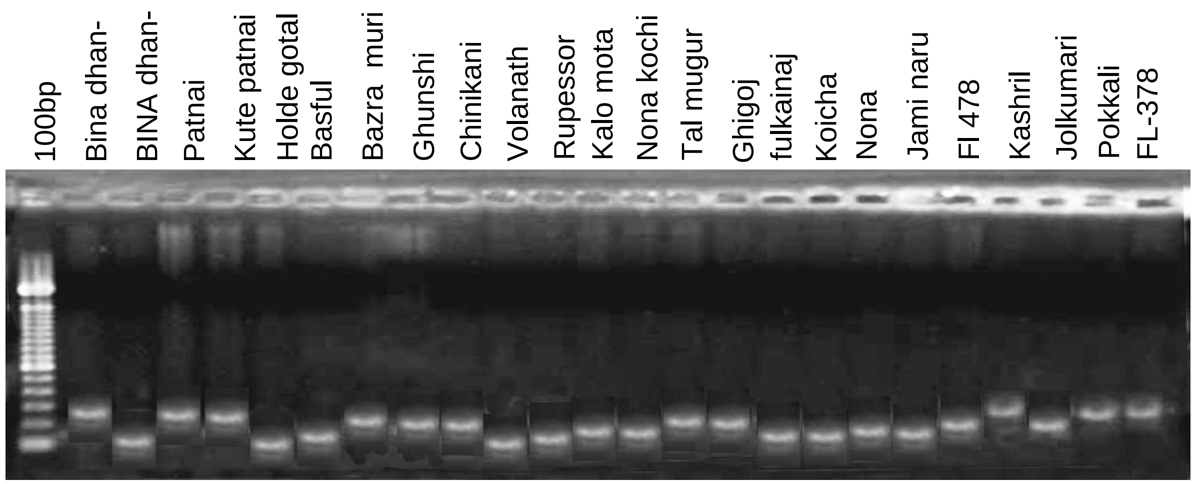

Fig.1. Banding profiles of 24 rice germplasm using RM9 primer

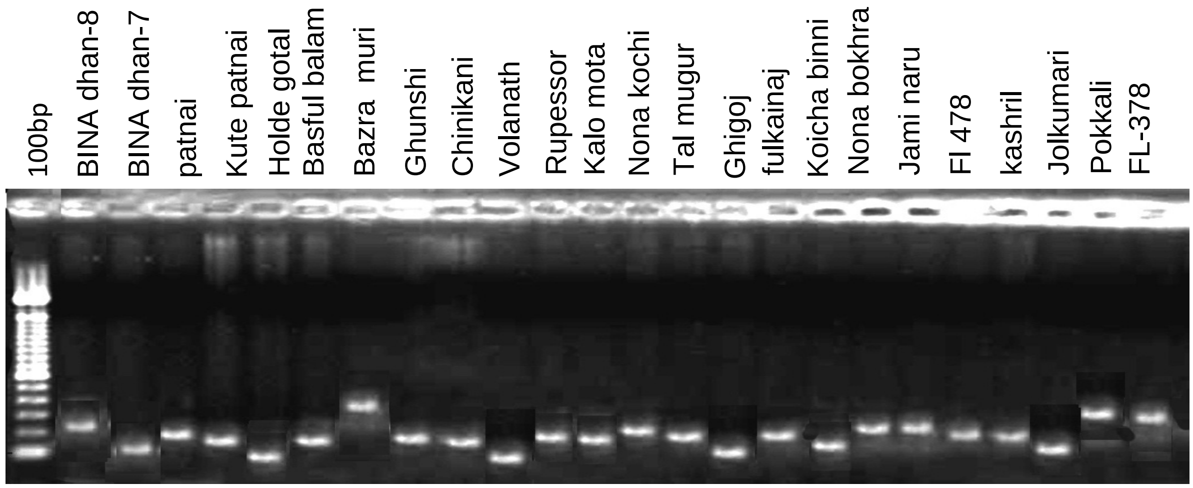

Fig. 2. Banding profiles of 24 rice germplasm using primer RM134

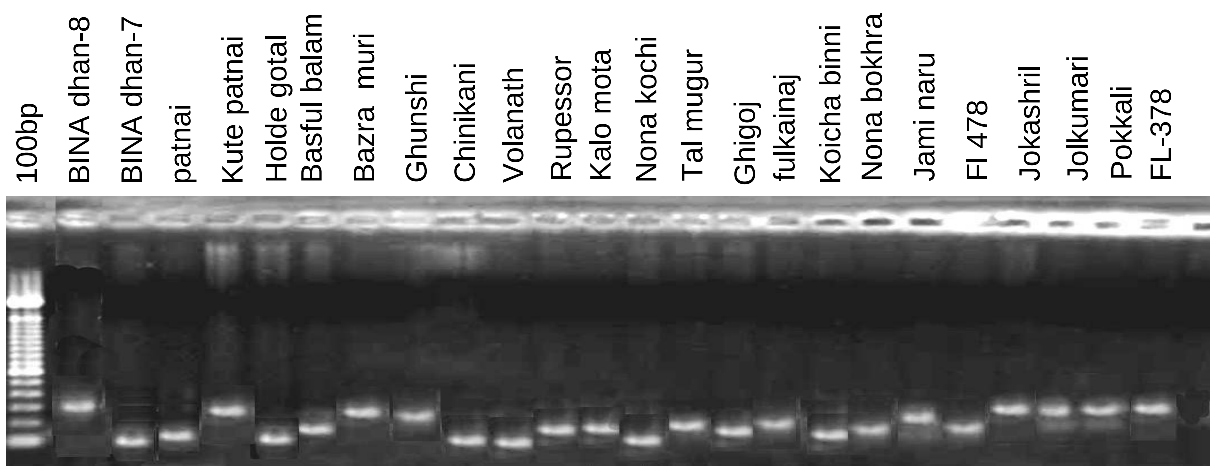

Fig. 3. Banding profiles of 24 rice germplasm using primer RM234