Asian Australas. J. Biosci. Biotechnol. 2019, 4 (3), 176-183

Asian-Australasian Journal of Bioscience and Biotechnology
ISSN 2414-1283 (Print) 2414-6293 (Online)
www.ebupress.com/journal/aajbb

Article

Assessment of rice genotypes for salt stress at seedling and reproductive stage by using phenotypic and molecular markers

Mirza Mofazzal Islam[1], Rigyan Gupta[1*] and Md. Syful Islam[2]

[1]Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh-2202, Bangladesh
[2]R& D Station, Lal Teer Seed Limited, Rampal, Bagerhat, Bangladesh

[*]Corresponding author: Rigyan Gupta, Scientific Officer, Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh-2202, Bangladesh. Phone: +8801728651459; E-mail: rigyan2008@gmail.com

Received: 25 November 2019/Accepted: 26 December 2019/ Published: 31 December 2019

Abstract: Screening of salinity tolerance genotypes of rice on the basis of its phenotypic performance alone is not much reliable and will take more time in progress in breeding process. Molecular marker-based screening eases this process. An experiment was carried out with 22 diverse rice genotypes in Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh to study their salt tolerance at both seedling and reproductive stages. On the basis of yield and yield contributing traits, genotypes were categorized as tolerant, moderately tolerant and susceptible. The genotypes RC 227, RC 229, RC 191, Binadhan-8, Binadhan-10, Binadhan-11 and FL-478 were found as tolerant, while Binadhan-7 and BRRI dhan39 were found as susceptible. Grain weight hill^{-1} was found highly significant. Plant height, total tiller hill^{-1}, grain weight panicle^{-1}, 1000 grain weight and all other traits except sterile spikelets panicle^{-1} were showed a positive correlation with grain weight hill^{-1}. Selected four salt linked SSR markers viz. AP3206f, RM1287, RM7075 and RM10793 were used to determine salinity tolerance. The genetic diversity was ranges from 0.6116 to 0.7810 with an average of 0.6663. The highest PIC value was 0.7524 and the lowest was 0.4762 from AP3206f and RM7075, respectively. The UPGMA clustering system generated four genetic clusters. The highest genetically dissimilarity of (Cluster 1) vs (Cluster 3) and the crossing would be helpful for salt tolerant rice development. Thus, selected SSR primers and genotypes would be useful in marker assisted selection (MAS), quantitative trait loci (QTL) mapping, gene pyramiding and ultimately improvement of salt tolerant rice varieties.

Keywords: performance; rice; salt stress; phenotypic and molecular markers

1. Introduction

The staple food for one third of the world's population is Rice (*Oryza sativa* L.) (2n = 24), belonging to the family Graminae and subfamily Oryzoidea. It occupies almost one-fifth of the total land area covered under cereals (Chakravarthi and Naravaneni, 2006). It is crucial to increase rice production in different rice growing ecosystems to feed the increasing world population (Khush, 2005). Approximately 11% of the world's arable land is planted annually to rice, and it ranks next to wheat (Chakravarthi and Naravaneni, 2006). This staple food ranked first position by production (130 Lac Metric Tons) during the year 2013-14 among all cereals in Bangladesh (BBS, 2013).

Salinity is the second most widespread soil problem in rice growing countries after drought and is considered as a serious constraint to increase rice production worldwide (Gregorio *et al*., 1997). It is quite well known that rice show variation for salt tolerance (Sabouri *et al.,* 2009; Sabouri and Biabani, 2009; Habib *et al*., 2013). Salinity is one of the most important abiotic stresses can directly affects plant growth and development (Muhling and Lauchli, 2001; Galvani, 2007; Lauchli and Grattan, 2007; Arshad *et al*., 2012). Over 800 million

hectares of land throughout the world are salt affected, either by salinity (397 million ha.) or the associated condition of sodicity (434 million ha.) (FAO, 2000).

In Bangladesh, the total saline area is one third of the 9 million hectares of total national cultivated area (Anonymous, 2006). Out of 2.85 million hectares of coastal and offshore land of Bangladesh, about 1.0 million hectares are affected by varying degrees of salinity. The coastal saline soils are distributed unevenly in 64 upazillas of 13 districts, covering portions of eight agroecological zones (AEZ) of the country (Seraj and Salam, 2000). Salinity is one of the major constraints for rice production worldwide. Hence, adoption of salt tolerant rice varieties has been considered as one of the strategies to increase rice production in salinity areas. As soil salinity affects all stages of growth and development of rice plant, so screening for salt tolerance at both vegetative and reproductive stages has been considered to be more useful. The use of physiological characters as selection criteria in salt tolerance breeding requires the identification of the contribution of each individual character to salt tolerance (Sabouri *et al.,* 2009). Panicle weight, effective tiller numbers per plant and harvest index are important agronomic characters for the prediction of rice yield. These yield components are severely affected by salinity (Mojakkir *et al.,* 2015). Breeding for salinity tolerance in rice requires suitable screening techniques and appropriate molecular marker technology (Gregorio et al., 2002). SSR or microsatellite markers are useful for making genetic maps (Islam, 2004; Niones, 2004), assisting selection procedure (Bhuiyan, 2005) and studying genetic diversity of rice germplasms. Microsatellite marker analysis is promising to identify major gene locus for salt tolerance that can be helpful for plant breeders in developing new cultivars. The objective of this study was assessing phenotypic variability of rice genotypes under salt stress at both seedling and reproductive stage and identification of salt tolerant rice genotypes.

2. Materials and Methods

2.1. Plant materials

Twenty-two rice germplasm accessions, with diverse genetic background, were used in this study. Of which eleven were from International Rice Research Institute (IRRI), six were from Bangladesh Institute of Nuclear Agriculture (BINA), and five were from Bangladesh Rice Research Institute (BRRI).

2.2. Phenotypic study of salinity tolerance at seedling stage

The genotypes were screened for salt tolerance at seedling stage in hydroponic system using IRRI standard protocol (Gregorio, 1997). Salinized and non-salinized setups with three replications were maintained. The evaluation was done using Yoshida *et al.* (1976) nutrient solution at the glasshouse. The nutrient solution was salinized by adding crude salt to obtain desired EC (12 dS/m). The modified standard evaluation system was used in rating the visual symptoms of salt toxicity (IRRI, 1997). Visual rating of salinity tolerance was done according to Table 1. This scoring discriminated the susceptible from the tolerant and the moderately tolerant genotypes. Initial and final scoring was done at 13 days and 22 days after salinization. Other observations are seedling height, root length and total dry matter recorded both at salinized and non-salinized conditions.

2.3. Screening of rice genotypes at the reproductive stage:

The genotypes were evaluated for their tolerance to salinity at the reproductive stage under sustained water bath using IRRI standard protocol (Gregorio *et al.*, 1997). The experimental design was completely randomized design with three replications. Two setups were maintained: normal and salinized. Pregerminated seeds of rice genotypes were sown in perforated glass fibre pots. The pots were placed in glass fibre trays with tap water. After 2 weeks, seedlings were thinned and the water level was raised to about 1 cm. The pots were salinized at EC 6 dS /m three weeks after sowing and EC was monitored in every week. Data were recorded for plant height (cm), days to flowering, days to maturity, number of effective tillers/plant, number of field grains, number of unfilled grains, total dry mater (g), percent fertility and grain yield (g).

2.4. Genotyping of salinity tolerant rice genotypes:

Modified CTAB mini prep was used for DNA extraction for 25-dayold seedling (IRRI, 1997). Ten primers were used for this study. Among these primers, three primers were showed polymorphic and clear bands (Table 2). Each PCR reaction carried out with 13.0µl reactions containing 1.5 µl 10x buffer, 0.75 µl dNTPs, 1µl primer forward, 1µl primer reverse, 0.25 µl taq polymerase, 8.25 µl ddH2O and 1.0 µl of each template DNA samples. PCR profile was maintained as initial denaturation at 94^{o}C for 5 min, followed by 34 cycles of denaturation at 94^{o}C for 1 min, annealing at 55^{o}C for 1 min and polymerization at 72^{o}C for 2 min; and final extension by 7 min at 72^{o}C. Then electrophoresis in 2% agarose gel was done after polymorphism in the PCR products and stained in ethidium bromide. Banding patterns were visualized with ultraviolet gel documentation system. The banding

patterns of 22 germplasm were scored compared with tolerant control and susceptible control variety and similar banding pattern with BINA dhan8 were considered as tolerant and BINA dhan7 were considered as salt susceptible.

2.5. SSR data analysis

The size of most intensely amplified fragments was determined by comparing the migration distance of amplified fragments relative to the molecular weight of known size markers, 100 base pairs (bp) DNA ladder using Alpha-Ease FC 5.0 software. The number of alleles per locus, major allele frequency, gene diversity and PIC values were calculated using Power Marker version 3.25 (Liu and Muse, 2005). NTSYS-pc was used to construct a UPGMA (un-weighted pair group method with arithmetic averages) dendrogram showing the distance-based interrelationship among the genotypes.

3. Results and Discussion

3.1. Screening of genotypes for salt tolerance at seedling stage

All genotypes grew robust and were uniform in color and height in the non-salinized condition. In salinized condition, the genotypes showed wide ranging variation in phenotypes from score 1 (highly tolerant) to 9 (highly susceptible). The most salt tolerant genotypes were RC 227, RC 229, RC 191, Binadhan-8, Binadhan-10, Binadhan-11 and FL-478. Susceptible genotypes were Binadhan-7 and BRRI dhan39.

3.2. Screening of rice genotypes at the reproductive stage

At reproductive stage, Binadhan-8 showed the highest plant height and the lowest value was found in Binadhan-7 and ranges from 93.00 to 147.7 cm (Table 2). Highest number of tiller was recorded in Binadhan-8 while the lowest was recorded in Binadhan-7. Effective tillers hill^{-1} was observed ranges from 2.58 to 8.41. The longest panicle length was observed in Binadhan-8 cm while the lowest panicle length was shown in Binadhan-7. The highest filled grains were observed in Binadhan-8 (127.1) while the lowest number was observed in Binadhan-7 (39.02). Maximum unfilled grain was observed in Binadhan-7 while minimum unfilled grain was observed in Binadhan-8. The highest % fertility was observed in Binadhan-8 while lowest % fertility was recorded in Binadhan-7. The highest grain weight per panicle was found in Binadhan-8 while the lowest value was found in Binadhan-7. Maximum grain wt. was found in Binadhan-8 while the lowest grain wt. was found in Binadhan-7. The highest 1000 (33.48 g) grain wt. was recorded in Binadhan-8 while the lowest value was recorded in Binadhan-7 (10.82 g) (Table 2).

At the reproductive stage, highly significant and positive correlation found between plant height and total tiller hill^{-1}, grain weight panicle^{-1}, grain weight hill^{-1}, 1000 grain weight and all other traits except sterile spikelets panicle^{-1}. Total tiller hill^{-1} also positively correlated with panicle length, fertility percentage, 1000 grain weight and all other traits except sterile spikelets panicle^{-1}. A highly negative correlation was found between sterile spikelets panicle^{-1} and all other remaining traits (Table 3).

3.3. Genotypic screening

Using 4 SSR markers, a total of 17 alleles were detected among 22 rice lines. Average number of allele per locus was 4.25, with a range of 3 (RM7075) to 5 (AP3206f & RM1287). Rare alleles were observed at all of SSR loci with an average of 1.25 rare alleles per locus. RM1287 detected the highest number of alleles (5) and rare alleles (2) (Table 4). The size of the different major alleles at different loci ranges from 113bp (RM10793) to 163 bp (RM1287) (Table 4). On average, 46.59 % of the 22 rice lines shared a common major allele ranging from 36.36% (AP3206f) to 55 % (RM7075) common allele at each locus. Highest gene diversity (0.7810) was observed in loci AP3206f while lowest gene diversity (0.5620) was observed in loci RM7075 (Table 5). PIC values ranged from 0.4762 (RM7075) to 0.7524 (AP3206f). These results revealed that markers AP3206f would be best in screening 22 rice lines followed by RM1287, RM10793 and RM7075. The banding patterns of marker AP3206f (Highest PIC) and RM7075 (Lowest PIC) were showed in Figure 1 and Figure 2.

3.4. UPGMA Dendrogram

UPGMA method was used for cluster analysis to differentiate the studied lines into groups based on similarity coefficient. Twelve clusters were made at genetic similarity level of 0.11to 0.58. All of the 22 rice lines were grouped in four main clusters. Tabkhkar *et al.* (2012) also grouped 48 rice lines with SSR markers into four main clusters**.** Cluster 1 was the biggest group which contained eleven genotypes viz. G01, G02, G03, G07, G 11, G12, G14, G16, G18, G19 and G21. This cluster had two separate additional sub-clusters within it. Cluster 2 contains two rice genotypes viz. G22 and G04, cluster 3 contains eight rice genotypes viz. G10, G17, G06, G09,

G13, G05, G15 and G20 and cluster 4 was the smallest group which contain only G08 (Figure 3). Siwach *et al.* (2004) observed the allelic diversity among Basmati and non-basmati long grain *indica* rice varieties using microsatellite markers, which supports this clustering analysis. Indian aromatic high-quality rice germplasms also showed similar trend in their DNA fingerprinting and phylogenic analysis (Jain *et al.*, 2004).

Table 1. Modified standard evaluation score of visual salt injury at seedling stage [Method adapted from Gregorio *et al.*, (1997)].

Score	Observation	Response category
1	Normal growth with no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
5	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Moderately tolerant
7	Complete cessation of growth; most leaves dry; some plants dying	Susceptible
9	Almost all plants dead or dying	Highly susceptible

Table 2. Performance of rice genotypes under salinity treatments.

Genotypes	Plant height (cm)	Total tillers hill^{-1} (no.)	Effective tillers hill^{-1} (no.)	Panicle length (cm)	Filled grains panicle^{-1} (no.)	Sterile spikelet panicle^{-1} (no.)	Fertility (%)	Grain weight panicle^{-1} (g)	Grain weight hill^{-1} (g)	1000 grain weight (g)
RC 227	114.8	8.75	6.61	24.96	53.25	37.42	60.07	1.17	7.63	21.89
RC229	110.4	6.50	4.75	26.00	66.83	25.92	71.36	1.65	6.61	25.69
RC 225	100.8	7.33	5.91	23.92	81.08	17.33	81.25	2.03	9.51	26.65
RC 217	126.3	8.33	5.83	23.17	70.58	23.00	74.00	1.74	8.15	25.40
RC 222	104.2	6.08	5.25	26.08	65.67	23.33	71.73	1.52	6.37	21.75
RC 191	97.50	5.05	4.08	21.75	65.42	51.92	53.91	1.30	3.46	20.90
RC 249	102.9	6.75	5.83	25.83	57.42	21.42	69.27	1.44	7.47	23.46
RC 251	105.8	6.75	5.66	26.17	72.70	36.00	63.52	1.53	5.49	21.35
BRRI dhan29	109.4	5.50	4.83	26.83	71.58	29.08	71.20	1.58	5.94	22.15
Pajam	124.7	8.19	6.96	25.83	87.56	22.50	79.98	1.96	10.00	23.41
BRRI dhan39	119.3	7.91	6.41	26.91	79.42	18.50	81.32	1.85	10.00	23.30
RC 221	102.6	5.19	4.50	24.75	63.00	21.42	74.78	1.37	5.01	20.82
RC 192	114.2	5.58	5.00	25.98	75.83	70.75	54.42	1.57	4.97	19.35
RC 193	104.3	6.66	6.16	23.75	83.50	21.55	79.76	1.69	7.42	20.32
Ciherang	95.79	5.23	3.66	19.13	61.25	37.91	71.34	0.70	1.94	11.35
Binadhan-12	107.0	8.23	2.91	19.67	58.33	24.96	68.57	1.34	3.34	20.02
Binadhan-11	101.8	7.58	5.41	22.67	58.50	28.17	63.51	1.02	4.14l	16.39
BR11	115.3	7.00	6.08	26.46	60.25	38.58	61.17	1.39	6.38	23.33
Binadhan-7	93.00	4.50	2.58	18.50	39.02	71.08	44.56	0.64	1.76	10.82
Binadhan-8	147.7	11.4	8.41	28.92	127.1	9.417	90.02	2.89	16.9	33.48
Binadhan-10	134.3	10.4	7.75	28.17	98.58	13.58	85.34	2.46	11.7	28.83
FL- 478	130.5	9.25	7.08	27.75	92.00	17.17	82.46	2.12	10.7	27.35
$LSD_{(0.05)}$	5.09	0.373	0.250	0.797	2.91	1.77	3.23	0.111	0.344	0.660

Table 3. Phenotypic Correlation among the different traits of rice genotypes.

Traits	Total tiller hill^{-1} (no.)	Effective tiller hill^{-1} (no.)	Panicle length (cm)	Filled grain panicle^{-1} (no.)	Sterile spikelets panicle^{-1} (no.)	Fertility percentage	Grain weight panicle^{-1} (g)	Grain weight hill^{-1} (g)	1000 grain weight (g)
Plant height (cm)	0.859**	0.802**	0.694**	0.799**	-0.480*	0.615**	0.835**	0.865**	0.787**
Total tiller hill^{-1} (No.)		0.790**	0.515*	0.698**	-0.641**	0.650**	0.755**	0.833**	0.742**
Effective tiller hill^{-1} (No.)			0.813**	0.773**	-0.599**	0.671**	0.807**	0.911**	0.785**
Panicle length (cm)				0.657**	-0.477*	0.534*	0.762**	0.762**	0.790**
Filled grain panicle^{-1} (no.)					-0.558**	0.780**	0.930**	0.869**	0.764**
Sterile spikelets panicle^{-1} (No.)						-0.907**	-0.640**	-0.670**	-0.670**
Fertility percentage							0.784**	0.780**	0.690**
Grain weight panicle^{-1} (g)								0.930**	0.923**
Grain weight hill^{-1} (g)									0.888**

Note: **= Significant at 1% level of probability; *= Significant at 5% level of probability

Table 4. Data on Major alleles (size and Frequencies) and Polymorphism Information Content (PIC) found among 22 rice lines for 4 microsatellites (SSR) marker.

Locus name	*Major allele		PIC
	Size (bp)	Frequency (%)	
AP3206f	154	36.36	0.7524
RM1287	163	45.45	0.6729
RM10793	113	50.00	0.5407
RM7075	151	54.55	0.4762
Mean	145.25	46.59	0.61055

Table 5. Summary information on 4 SSR markers used in the present study.

Marker	Chromo. location	No. of Allele found	Frequency	*Rare alleles	Size range (bp)	Gene diversity
AP3206f	1	5	0.1727	0	154-164	0.7810
RM1287	1	5	0.1818	2	163-175	0.7107
RM10793	1	4	0.1591	3	113-126	0.6116
RM7075	1	3	0.3333	0	149-159	0.5620
Mean		4.25	0.211725	1.25		0.666325

Note: Chromosome, motif of the SSR markers, position, number of repeats and size range as previously published (http://archive.gramene.org/db/markers/marker_view)

*Rare alleles are defined as alleles with a frequency less than 5%.

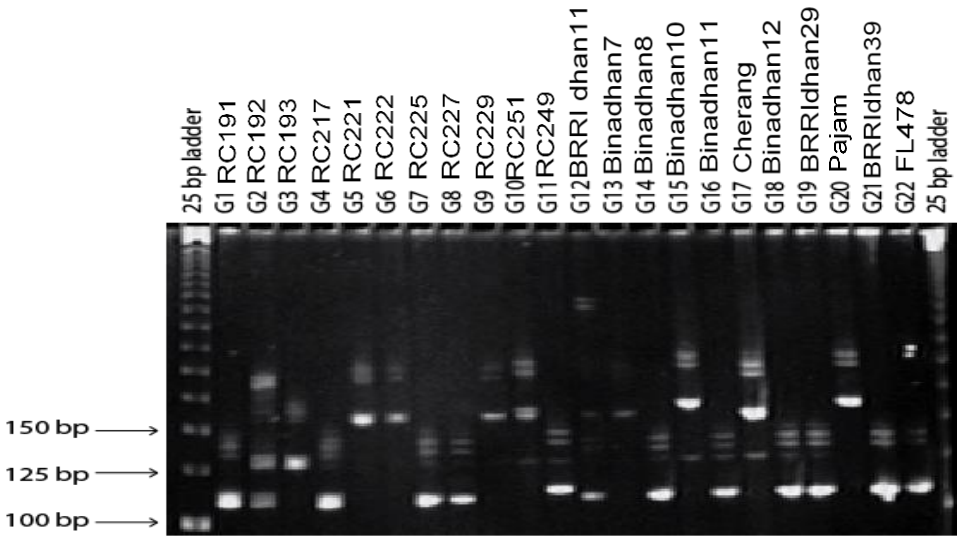

Figure 1. DNA profile of 22 advanced rice lines using primer AP3206f (Highest PIC).

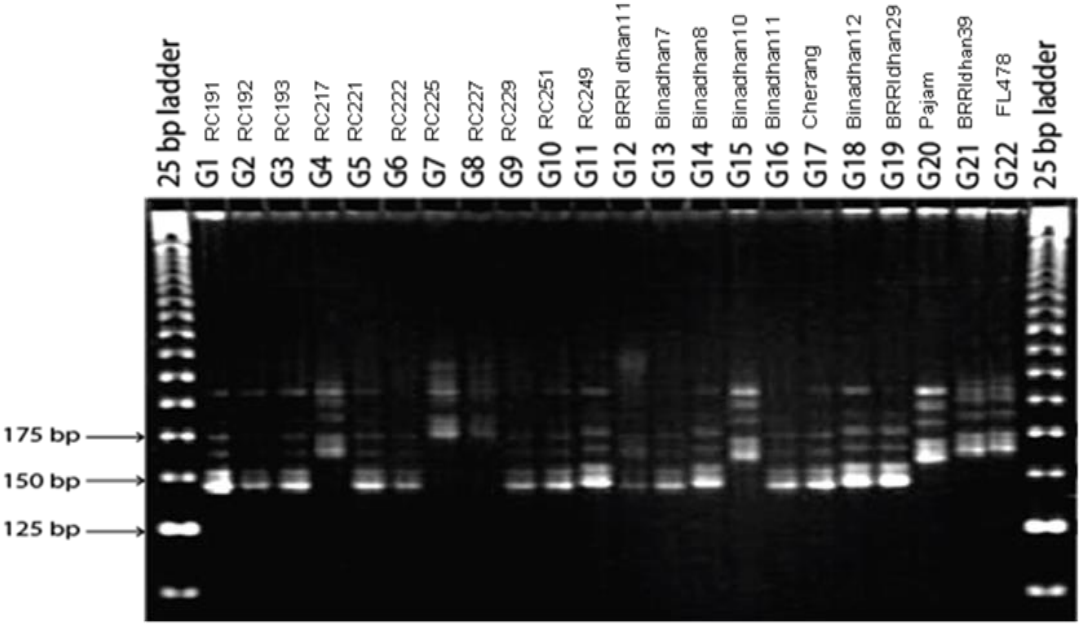

Figure 2. DNA profile of 22 advanced rice lines using primer RM7075 (Lowest PIC).

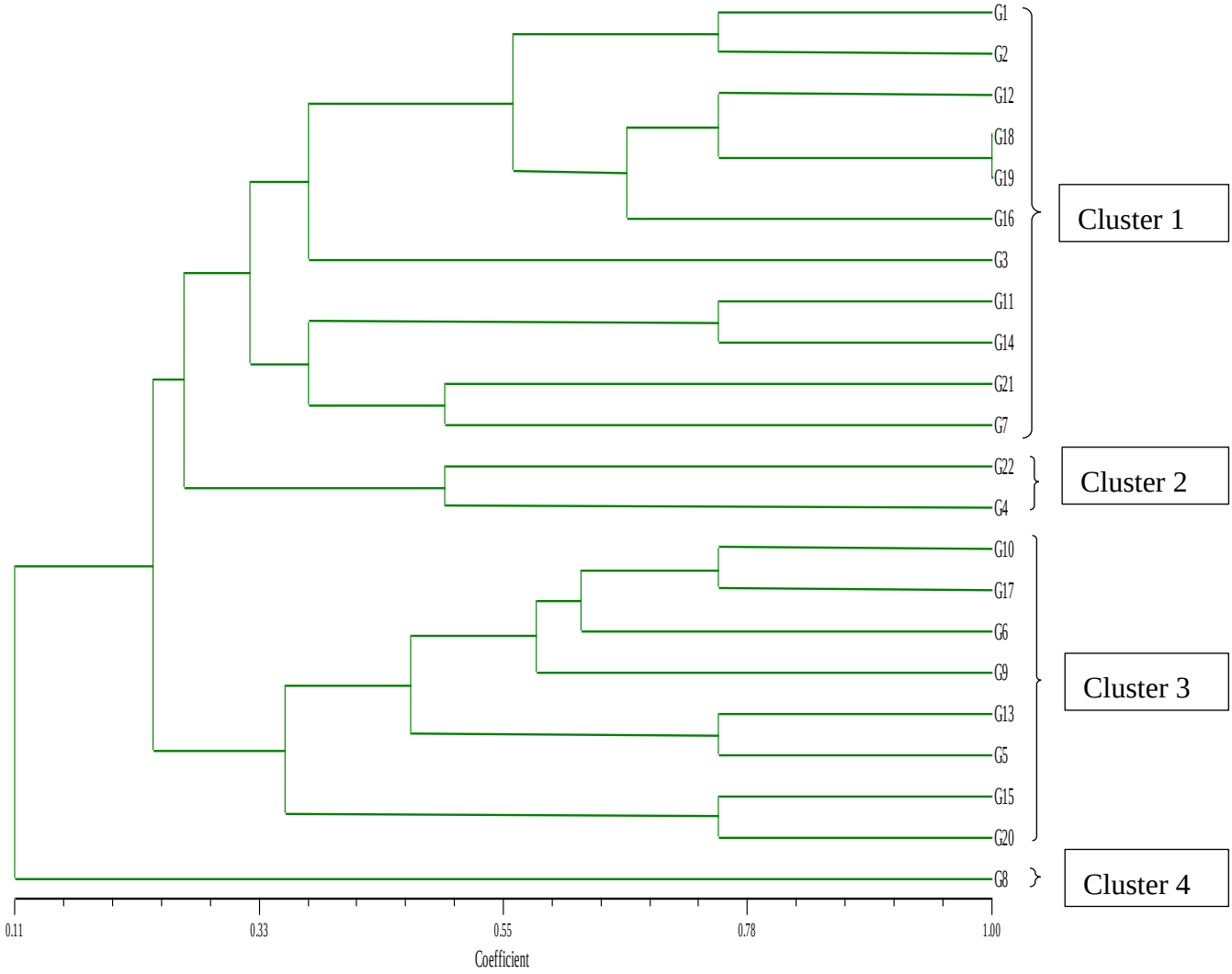

Figure 3. UPGMA dendrogram showing the genetic relationship among rice genotypes.

Here, G1-RC 191, G2–RC 192, G3-RC 193, G4-RC 217, G5-RC 221, G6-RC 222, G7- RC 225, G8-RC 227, G9-RC 229, G10-RC 251, G11-RC 249, G12-BR11, G13-Binadhan-7, G14-Binadhan-8, G15-Binadhan-10, G16-Binadhan-11, G17-Ciherang, G18-Binadhan-12, G19-BRRI dhan29, G20-Pajam, G21-BRRI dhan39, G22-FL478

4. Conclusions

At seedling stage, using the IRRI standard protocol for screening the salt tolerance the genotypes RC 227, RC 229, RC 191, Binadhan-8, Binadhan-10, Binadhan-11 and FL-478 were found highly tolerant and Binadhan-7 and BRRI dhan39 were found as susceptible genotypes. Screening these genotype at reproductive stage by using the IRRI standard protocol for salt stress the genotype Binadhan-8 found highly tolerant and the genotype Binadhan-7 was the most susceptible. The salt linked SSR markers viz. AP3206f, RM1287, RM7075 and RM10793 could be used to determine salinity tolerance of the genotypes. The genetic diversity of the genotypes with an average of 0.6663 and was ranges from 0.6116 to 0.7810. The highest PIC value was found 0.7524 from AP3206f and the lowest was 0.4762 from RM7075. In UPGMA clustering system, the highest genetically dissimilarity was found in (Cluster 1) vs (Cluster 3) among the four clusters. Thus, these for the improvement of salt tolerant rice varieties these selected SSR primers and genotypes would be useful in marker assisted selection (MAS), quantitative trait loci (QTL) mapping and gene pyramiding.

Conflict of interest

None to declare.

References

Anonymous, 2006. ABSPII. Drought tolerant rice and salinity tolerance rice. Agril. Biotechnol. Sup. P. II-South Asia, 26.

Arshad M, M Saqib, J Akhtar and M Asghar, 2012. Effect of calcium on the salt tolerance of different wheat (*Triticum aestivum* L.) genotypes. Pak. J. Agril. Sci., 49: 497-504.

BBS (Bangladesh Bureau of Statistics), 2013. Statistical Yearbook of Bangladesh. Statistics Division, Min. Plan. Govt. P. Rep. Bang., 189-258.

Bhuiyan MAR, 2005. Efficiency in evaluating salt tolerance in rice using phenotypic and marker assisted selection. MS Dissertation, Dept. Gen. and Pl. Breed., Bang. Agril. Univ., Mym. Bang.

Chakravarthi BK and R Naravaneni, 2006. SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa* L.). Afr. J. Biotechnol., 5: 684-688.

FAO, 2000. Global network on integrated soil management for sustainable use of salt effected soils. FAO L. Pl. Nutri. Manage. Ser., Rome, Italy.

Galvani A, 2007 .The challenge of the food sufficiency through salt tolerant crops. Reviews in Env. Sci. and Biotechnol., 6: 3-16.

Gregorio GB, 1997. Tagging salinity tolerant genes in rice using amplified fragment length polymorphism (AFLP). Ph. D. Dissertation. Univ. Philip. L. Bañ., Col., Lag., Philip., 118.

Gregorio GB, D Senadhira and RD Mendoza, 1997. Screening rice for salinity tolerance. IRRI Discussion Paper Series no. 22. Manila (Philippines): Int. R. Res. Ins., 1-30.

Habib N, M Ashraf and M Shahbaz, 2013. Effect of exogenously applied nitric oxide on some key physiological attributes of rice (*Oryza sativa* L.) plants under salt stress. Pak. J. Bot., 45: 1563-1569.

International Rice Research Institute (IRRI), 1997. Rice Almanae. IRRI-WARDA-CIAT, Los Baños, Lag., Philip.

Islam MM, 2004. Mapping salinity tolerance genes in rice (*Oryza satiya* L.) at reproductive stage. Ph. D Dissertation. Univ. Philip., Los Banos, Col., Lag., Philip..

Jain S, RK Jain and SR McCouch, 2004. Genetic analysis of Indian aromatic and quality rice (*Oryza sativa* L.) Germplasm using panels of fluorescently-labeled microsatellite markers. Theo. App. Gen., 109: 965-977.

Khush GS, 2005. What it will take to feed five billion rice consumers by 2030. P. Mol. Bio., 59: 1-6.

Lauchli A and SR Grattan, 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. Sprin., Dord., Nethe.

Liu K and SV Muse, 2005. Power Marker: an integrated analysis environment for genetic marker analysis. Bioinf., 21: 2128-2129.

Mojakkir AM, MZ Tareq, MA Mottalib, ABMZ Hoque and MA Hossain, 2015. Effect of salinity at reproductive stage in rice. Int. J. B. S. Sci. Res., 3: 7-12.

Muhling KH and E Lauchli, 2001. Physiological traits of sodium toxicity and salt tolerance. Pl. Nut., 92: 378-379.

Niones JM, 2004. Fine mapping of the salinity tolerance gene on chromosome 1 of rice (*Oryza sativa* L.) using near-isogenic lines. MS Dissertation. Univ. Philip. L. Ban., Col., Lag., Philip.

Sabouri H and A Biabani, 2009. Toward the mapping of agronomic characters on a rice genetic map: Quantitative Trait Loci analysis under saline condition. Biotechnol., 8: 144-149.

Sabouri H, AM Rezai, A Moumeni, A Kavousi, M Katouzi and A Sabouri, 2009. QTLs mapping of physiological traits related to salt tolerance in young rice seedlings. Biol. Plant., 53: 657-662.

Seraj, ZI, and MA Salam, 2000. Growing rice in saline soils. The Biotechnol. Direct.. Mac. Ref. Ltd., Porters South, Crinan street, London.

Siwach P, S Jain, N Saini, VK Chowdhury and RK Jain, 2004. Allelic diversity among basmati and non-basmati long-grain Indica rice varieties using microsatellite marker. J. Pl. Biochem. and Biotechnol., 13: 25-32.

Tabkhkar N, B Rabiei, A Sabouri, 2012. Genetic diversity of rice cultivars by microsatellite markers tightly linked to cooking and eating quality. Aust. J. Cr. Sci., 6: 980-985.

Yoshida S, DA Forno, JH Cook and AK Gomez, 1976. Laboratory manual for physiological studies of rice. Int. R. Res. Ins. (IRRI), L. Bañ., Lag., Philip., 61-66.