Article

Screening Aromatic Rice Genotypes by RAPD Markers for Breeding Salinity Stress Tolerant Rice Variety

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Abstract: Salinity is an abiotic stress, which causes adverse environmental conditions for rice cultivation. In particular, the local aromatic rice cultivation is heavily influenced by soil salinity stress, which has impact on global food security. This study aimed to screen local aromatic rice genotypes in a hydroponic experiment using Yoshida solutions to evaluate the effect of increasing NaCl concentrations on the early growth stages of rice seedlings. Genetic diversity along with phylogenetic relationship was assessed using the random amplified polymorphic DNA (RAPD) markers. Out of 20 RAPD markers, 17 markers produced reproducible polymorphic bands. Individuals of all genotypes shared 88 (89.80%) of the 98 total RAPD elements amplified. The genetic distance-focused similarity index ranged from 0.05 to 0.94. The highest genetic distance (0.94) was discovered between genotypes *Nayanmoni* and *Kalijira Barisal*, and the lowest between *Badshabhog* and *Kataribhog* (0.05). In addition, the OPS 3(510bp) and OPA 14(1100bp) markers could be used to identify salt-tolerant genotypes. According to genetic distance, the salt stress tolerant check genotype, *Pokkali* was genetically related to *Chinigura* as well as *Kalijira Barisal*. This study established a simple and consistent method for evaluating variability across various aromatic rice genotypes, which will benefit in genotype selection for breeding salinity stress tolerant aromatic rice variety in Bangladesh.

Keywords: aromatic rice; salt screening; RAPD marker; genetic diversity

1. Introduction

Rice (*Oryza sativa* L.) (2n = 24) belonging to the family Poaceae, serves as an important food for more than 3.5 billion people, which is about 63% of the world's population [1,2] and the largest source of calories among the food grains with sufficient nutrients in the developing countries [3]. Bangladesh has a collection of over 8,000 rice germplasms of which nearly 100 are aromatic [4]. These aromatic rice are cultivated across the country that are closely related to the social and cultural heritage in Bangladesh [5]. Rice accounts for about 48% of rural employment in Bangladesh, two-thirds of total calorie supply, and one-half of total protein consumption for the people of Bangladesh. It generates one-half of agricultural GDP and one-sixth of national revenue [6,7].

Aromatic rice is a type of rice that emits scent from the entire grain. Furthermore, it retains its aroma after cooking and is high in amino acids, proteins, and other nutrients, making it popular among consumers worldwide [8]. In Bangladesh, farmers are encouraged to plant fragrant rice because of the appealing aroma and mouthwatering taste, which leads to high prices both locally and internationally [9]. Aromatic rice has a lesser production potential than high-yielding modern rice varieties. Therefore, farmers prefer to produce modern rice varieties. Aromatic rice varieties are less resistant to biotic and

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abiotic stressors in general. Because of their scent, and low amylose content, they are also susceptible to disease and insects [10,11]

Milled rice production is expected to be around 37.61 million tons in fiscal year 2020-21. Aman rice contributed for 14.438 million tons of overall rice output in 2020-2021 [12] with aromatic rice accounting for 12.50 percent of all transplanted Aman rice [13]. Bangladesh has an excellent potential of earning foreign cash by selling high-quality rice. The vast majority of aromatic rice landraces in Bangladesh are indigenous, adaptable, photoperiod-sensitive, and grown in a rainfed lowland ecology during the Transplanted Aman season (July to December). In Bangladesh, the average yield of high-yielding rainfed lowland rice is 3.4 t ha^{-1}, while aromatic rice yields 2.0-2.3 t ha^{-1} [14]

In global context, salinity is a broad phrase that refers to the presence of high quantities of salts in soil and water, such as sodium chloride, magnesium and calcium sulphates, and bicarbonates with over 830 million hectares (ha) of salt-affected lands [15,16,17]. Salt stress is one of the abiotic limitations that causes significant yield losses [11,18,19]. Because of global climate change, soil salinity has arisen as a severe threat to food security [20]. Salinity stress is predicted to harm around 62 million hectares, or 20% of the world's irrigated land. [21,22]. During the rainy season, over 30% of Bangladesh's arable land is located along the shore, where low lands are directly affected by tidal flooding and storm surges. During the dry season, salinity transfer from ground and surface water is detected along the shoreline [23,24]. The central coast of Bangladesh is the most active in comparison to other coasts since it is so irregular and fractured [25]. The World Bank (2000) anticipated 0.10, 0.25, and 1 m rise in sea levels in Bangladesh by 2020, 2050, and 2100, respectively [26]. Soil salinity and its consequences are largely influenced by three factors: direct contamination with salt water, tidal flooding during the wet season (June-October), and upward or lateral migration of saline spring water owing to evaporation [27]. Soils of Satkhira district is severely affected by salinity, with soil salinity levels ranging from 4 to 16 dS/m in 70% of the area [28]. Patuakhali, Barguna, Barisal, and Pirojpur are moderately saline (2-4 dS/m), but there are some non-saline areas. Noakhali has a moderate salinity (4–8 dS/m) and covers a bigger area than Lakshmipur and Feni. The coastal line continues south into Chittagong and Cox's Bazaar, where there are pockets of high salinity and mild levels [28].

The DNA markers provide a wide range of uses for strengthening a plant's genetic structure, including genetic identification of parents, genetic variation assessment, and high-resolution identification, genetic confirmation, and establishment of genetic linkage groups. A wide variety of molecular markers are available for crop genetic investigation [29,30]. Random amplified polymorphic DNA (RAPD) and ISSR are two robust DNA fingerprinting technologies among all the DNA markers [31,32]. In RAPD analysis, sample preliminary sequence data are not required. With limited resources, numerous loci from many individuals can be studied for screening purposes. Because of their simple experimental methods and effective genetic screening of intra- and interspecific hybrids, RAPDs are widely used [29]

Traditional techniques such as phenotype-based screening of plant genotype selection for salt tolerance are difficult due to the extensive influences of the environment and the low narrow-sense heredity of salt tolerance [33]. It also impedes the development of an accurate, quick, and reliable screening strategy for screening of salt tolerant genotypes. The objectives of this research were to i) screen local aromatic rice genotypes under saline and non-saline circumstances at the seedling stage; ii) assess genetic diversity and relatedness among different local aromatic rice genotypes using RAPD markers, with an emphasis on salt-tolerant features; and iii) determine the phylogenetic relationships among the local aromatic rice genotypes.

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2. Results and discussion

2.1. Tolerance and vegetative growth of rice seedlings under different salinity stress conditions

For salinity tolerance testing, all rice seedlings were exposed to four treatments viz. control, 6 dS/m NaCl, 9 dS/m NaCl, and 12 dS/m NaCl, each with three replications. Rice seedlings were normal in every phenotypic behavior in the control condition (Figure 1a), however, various indications of salt injury were identified in the salinity stress conditions, including yellowing of leaves, leaf rolling, tip whitening, drying of leaves, and reductions in root growth, shoot growth, and stem thickness, and in some cases, death of the seedlings (Figures 1 b-d).

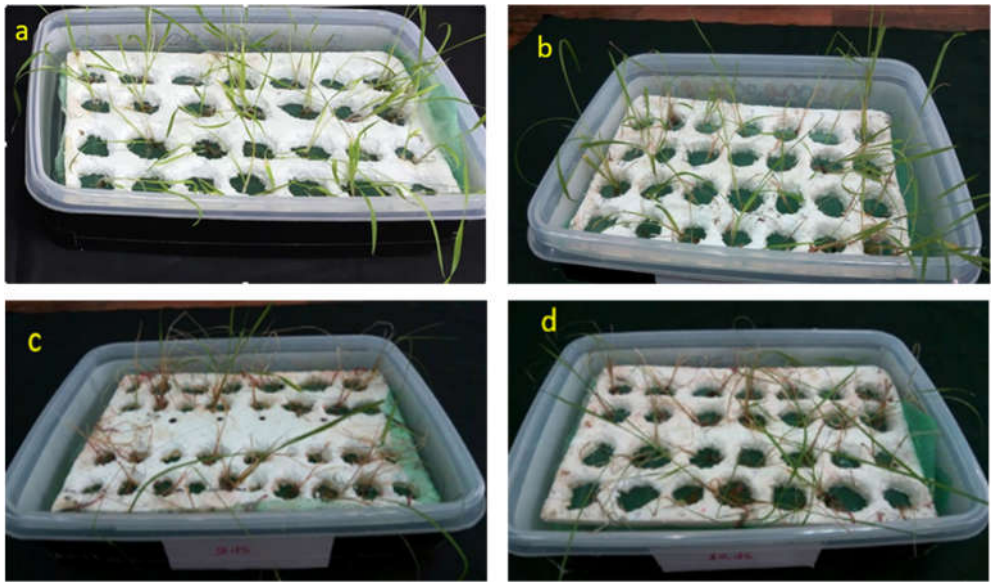

Figure 1. The growth performances of local aromatic rice genotypes seedlings under varying levels of salinity stress. (**a)** control condition; (**b)** 6 dS/m NaCl; (**c**) 9 dS/m NaCl; (**d**) 12 dS/m NaCl using hydroponic system.

At 9 dS/m salt conditions, salinity stress tolerant check genotype, Pokkali had very little chlorophyll damage (nearly 10%-20%). However, chlorophyll damage in Chinigura, Khasa mukpura, and Nayanmoni were 30%-50%, 50-75% and 90-100%, respectively. In salty soils, NaCl accounts for 50-80% of total soluble salts [34] resulting in high and potentially hazardous Na+ and/or Cl concentrations in the plant. Many enzymes and biological activities, such as photosynthetic signaling systems, are affected by these ions [35,36]. Saline soils inhibit plant growth due to osmotic stress, ionic toxicity, and a diminished ability to absorb critical minerals [37]. Due to the hyperosmotic pressure of the soil solution, root cells may lose water rather than absorb it in extreme circumstances. Water shortages affect a cascade of physical, signaling, gene expression, metabolic, and physiological pathways and processes, resulting in diminished cell elongation, wilting, and, eventually, plant mortality; these salinity-related negative consequences can be categorized as "water-deficit effects."[38,36] Salinity harmed the stems of rice plants also. The decrease in stem size could be attributed to a decrease in cell division and expansion [14] (Table 1, Figure 2**).**

Salt impeded the growth of barley and wheat plants, causing wilting, necrosis, and chlorosis, as well as decreased plant biomasses [17,36]. Plants exposed to metalloids in salt stresses are likely to have a smaller root system and a smaller leaf area. Furthermore, root browning, foliar chlorosis, necrotic patches, and other toxicity symptoms are commonly associated with slower growth. Chlorosis occurs more frequently in younger leaves, but necrotic areas occur more frequently in older leaves with higher metalloid concentrations [17].

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Table 1. Salinity condition (NaCl) scoring on local aromatic rice seedlings.

Name of the Variety	Score			Tolerance
	6ds/m	9ds/m	12ds/m	
Nayon moni	3	7	7	HS
Khuti cikon	3	5	9	HS
Radhuni pagol	3	3	9	HS
Badshabhog	3	5	7	S
Rajbhog	1	3	5	MT
Kolomala	3	5	9	HS
Bashmoti 370	1	3	5	MT
Khasa mukpura	1	5	7	S
Bhatir cikon	3	5	9	HS
Kataribhog	3	5	7	S
Pokkali	1	1	3	T
Chinigura	1	3	5	MT
Kalijira Barisal	1	3	5	MT
Kalijira normal	1	3	5	MT

Note: T = Tolerant, MT = Moderately Tolerant, S = Susceptible, HS = Highly Susceptible, 1-9 scale, where 1 = Highly Tolerant and 9 = Highly Susceptible.

2.2. *Phenotypic variation and anatomical observations of aromatic rice genotypes under salinity stress conditions*

Following the IRRI standard evaluation approach **[**33**]** , rice genotypes were classified into five groups based on biochemical and morphological alterations caused by salinity: highly resistant (score 1), tolerant (score 3), moderately tolerant (score 5), susceptible (scoring 7) and very susceptible (score 9). (SES). The SES score for genotype Pokkali was found to be 3 at EC 12 dS/m, which is commonly acknowledged as a salt tolerant cultivar. Five genotypes (Rajbhog, Basmati 370, Chinigura, Kalijira Barisal, and Kalijira normal) received a score of 5 and were regarded to be somewhat salt tolerant. SES score 7 was discovered for four genotypes (Nayanmoni, Badshahbhog, Khasa mukpura, and Kataribhog) that were thought to be susceptible to saline stress. The remaining four genotypes (Khuti cikon, Radhuni pagol, Kolomala, and Bhatir cikon) with a SES score of 9 were classified as very sensitive to saline stress during the seedling stage (Table 1, Figure 2) [39]. Leaf structural change is thought to be an important trait of halophytic plants in order to avoid higher salty conditions. Anatomical features of rice, especially leaf tissues of the epidermis, contain bulliform cells, which pose a large vacuole [40], that may serve as a dump for high amounts of Na/Cl-ions that are transported to the leaves under stress conditions, to protect physiologically active mesophyll cells [21,41].

Salt-induced rice plants produced thick cell walls in the leaves and stems in the hypodermis and cortex. Salt stress reduced chlorophyll fluorescence by limiting thylakoid electron transport while increasing membrane viscosity and restricting plastoquinone diffusion [39,42,43] which could be related to the complex of photosystem-II and electron transport chains that leads to a decrease in total chlorophyll content in leaves [39,44]. As a result, chlorophyll content could be employed as an essential biomarker of salinity stress responses in different plants to identify tolerant genotypes [39,45] Leaf rolling was observed in all the saline treated (6 dS/m, 9 dS/m, and 12 ds/m) seedlings. This feature is affected by the turgidity of the bulliform cells [46]. Therefore, saline-treated leaves possessed more compact mesophyll tissue than control (Figure 2). Bulliform cells play an important role in leaf rolling to avoid water loss during drought stress [41,40]. Extensive leaf rolling was observed in the salt range ecotype; therefore, it can safely be referred to as an important adaptive defensive strategy against salt stress [47, 48].

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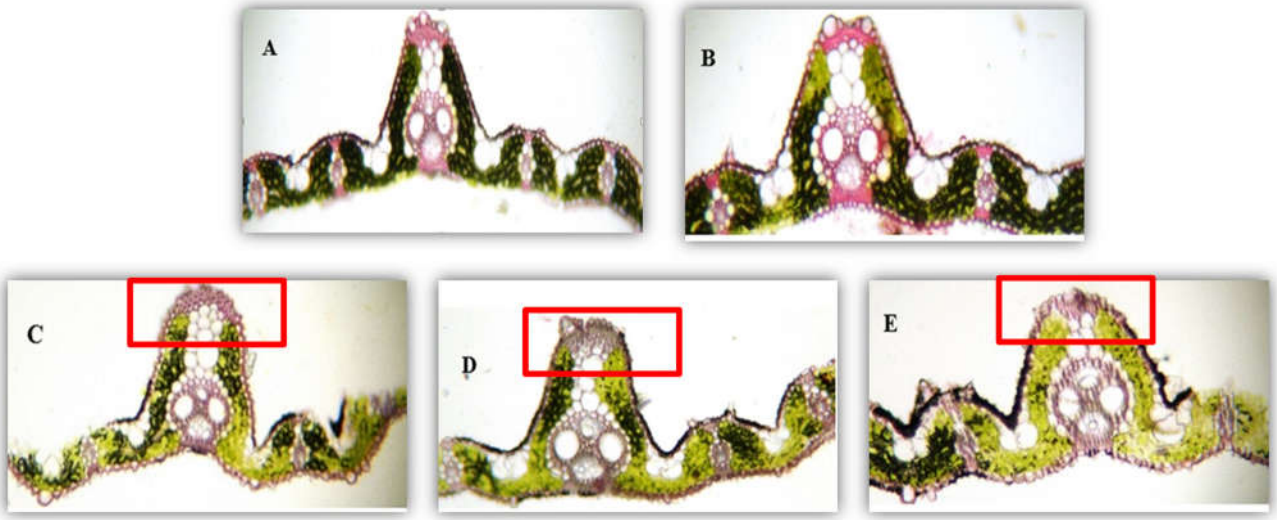

Figure 2. Transverse sections of rice leaf showing anatomical visualization of local aromatic rice genotypes the effects of salt accumulations. A. Treatment under control condition; B. Pokkali (salt tolerant variety) under 9 dS/m NaCl; (C) Chinigura local variety under 9 dS/m NaCl; (D) Chinigura variety under 9 dS/m NaCl; (E) Nayanmoni variety under 9 dS/m NaCl. Red boxes indicating the salt accumulation regions.

2.3. Amplification of DNA and genotype identifications by three distinct primers

The banding patterns of different rice genotypes using all seventeen primers are shown in **Figure 3.**

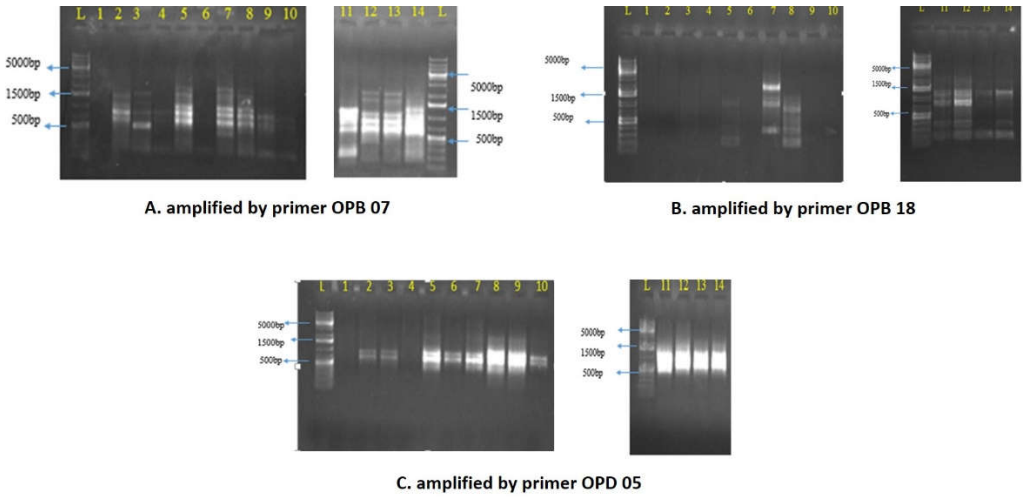

Figure 3. DNA profiles of aromatic rice genotypes amplified by three distinct primers. A. amplified by primer OPB 07, B. amplified by primer OPB 18, and C. amplified by primer OPD 05.

In this research, 20 RAPD primers were initially screened, which generated 98 alleles with sizes ranging from 100–3500 bp. Out of the 98 alleles, 88 alleles (89.79%) were polymorphic and 10 alleles (10.21%) were monomorphic [49] (Table 2).

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Table 2. Distribution of genetic polymorphism of aromatic rice genotypes.

Primer Code	Sequence 5'-3'	Allele size (bp)	Total Alleles	Polymorphic Alleles	Percentage (%) polymorphism
OPA 01	CAGGCCCTTC	350-3000	7	6	85.71
OPA 08	GTGACGTAGG	350-3500	6	4	66.67
OPA12	TCGGCGATAG	700-1800	4	3	75.00
OPA 14	CAGGCCCTTC	200-2000	4	4	100.00
OPA 16	AGCCAGCGAA	600-2000	6	4	66.67
OPA 17	TCGGCGATAG	200-2000	7	7	100.00
OPA 19	CAATCGCCGT	200-3000	9	9	100.00
OPB 07	GGTGACGCAG	300-2000	7	7	100.00
OPB 18	CCAGCAGCTT	100-3000	9	8	88.88
OPC 01	GTGACGTAGG	700-2000	5	5	100.00
OPD 05	TGAGCGGACA	300-1500	5	4	80.00
OPJ 05	CAGGCCCTTC	500-1500	3	3	100.00
OPJ 07	CCAGCAGCTT	200-2500	6	5	83.33
OPF 14	TGCTGCAGGT	500-900	3	3	100.00
OPL 15	AAGAGAGGGG	400-1500	5	5	100.00
OPAK 10	CAAGCGTCAC	200-500	5	4	80.00
OPS 3	CAGAGGTCCC	200-3000	7	7	100.00
Total			98	88	
Average			5.76	4.47	89.80

The number of polymorphic bands was found to vary depending on the primers and cultivars used [50]. Three primer did not produce any bands. The primers OPA 14, OPA 17, OPA 19, OPB 07, OPC 01, OPJ 05, OPF 14, OPL 15, OPS 3 produced a higher level of polymorphism (100%) [50] On the other hand, the primers OPA 08 and OPA 16 generated lower polymorphic bands (66.67%) (Table 3). In the present study, 88 of the RAPD bands were found to be polymorphic.

Some previous study [50] discovered 100% polymorphism in their experiment, which used eight primers to generate 255 amplification products. Another team [49] also did RAPD analysis on two cultivars using nine primers, yielding 77 amplification products containing 83.3% of which were polymorphic [5] amplified a total of 69 alleles, 66 (95.65%) of which were polymorphic. [5] likewise employed five primers to generate 84 reproducible bands ranging from 240 to 1090 bp, with 73% of the bands being polymorphic[51] performed RAPD analysis on eight different types.

The genetic diversity values of seventeen primers for local aromatic rice genotypes are given (Supplemental data 1). Furthermore, the aggregate Nei's gene diversity and Shannon's information index for all loci were estimated to be 0.3405 and 0.5110, respectively. The loci OPA 17-04 and OPC 01-04 had high gene diversity and a high Shan-Information index (0.6929), whereas the loci OPB18-4 and OPB18-08 had the lowest (0.0000). This study suggests that there is genetic variation among local rice genotypes.[52]

2.4. Genetic diversity of studied rice genotypes

The average estimated gene flow (Nm) across all loci was 0.0000, while the coefficient of gene differentiation (Gst) was 1.0000. (Supplemental data 2) The average heterozygosity (Ht) across all primers was 0.3405 and the family history (Hs) was 0.0000. The RAPD marker, on the other hand, showed the same amount of differentiation (Gst) in all genotypes studied.

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A similarity matrix was used to estimate the level of relatedness of the aromatic rice genotypes, and pairwise genetic similarity indices were generated (Table 3). The estimations of pair-wise similarity ranged from 0.05 to 0.94.

Table 3. Nei's (1972) genetic distance values among studied 14 rice genotypes.

Pop ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	****													
2	0.34	****												
3	0.15	0.30	****											
4	0.07	0.24	0.14	****										
5	0.56	0.31	0.44	0.44	****									
6	0.14	0.34	0.15	0.13	0.39	****								
7	0.62	0.33	0.46	0.53	0.34	0.47	****							
8	0.39	0.47	0.51	0.37	0.42	0.36	0.47	****						
9	0.37	0.53	0.39	0.30	0.37	0.31	0.56	0.37	****					
10	0.14	0.31	0.20	0.05	0.42	0.22	0.47	0.33	0.23	****				
11	0.82	0.41	0.62	0.66	0.36	0.56	0.41	0.60	0.51	0.60	****			
12	0.79	0.60	0.68	0.77	0.58	0.79	0.45	0.66	0.73	0.84	0.41	****		
13	0.95	0.49	0.77	0.82	0.62	0.90	0.49	0.84	0.73	0.90	0.41	0.33	****	
14	0.73	0.44	0.58	0.62	0.56	0.64	0.47	0.64	0.66	0.64	0.42	0.41	0.20	****

Note PopID (1-14): Nayanmoni (1), Khuti cikon (2), Raduni pagol (3), Badshabhog (4), Rajbhog (5), Kolomala (6). Basmati-370 (7), Khasa mukpura (8), Bhatir cikon (9), Kataribhog (10), Pokkali (11), Chinigura (12), Kalijira Barisal (13), Kalijira normal (14).

The pairwise genetic distance was observed from 0.05 to 0.94. [49,50] The highest genetic diversity revealed in RAPD analysis was determined between Nayanmoni and Kalijira Barisal (0.94) and the lowest genetic diversity revealed between Badshabhog and Kataribhog (0.05) (Table 3). Among the genotypes, the lowest genetic diversity with salt tolerant non-aromatic Pokkali was closely related to Chinigura and Kalijira Barisal.

2.5. Cluster analysis based on UPGMA dendrogram

Cluster analysis is used to determine the genetic link between genotypes using alleles discovered by RAPD markers. The dendrogram based on Nei's (1972) genetic distance utilizing the Unweighted Pair Group Method of Arithmetic Means (UPGMA) clustered fourteen genotypes into two main clusters with a similarity coefficient of 55% in the current study (Figure 4).

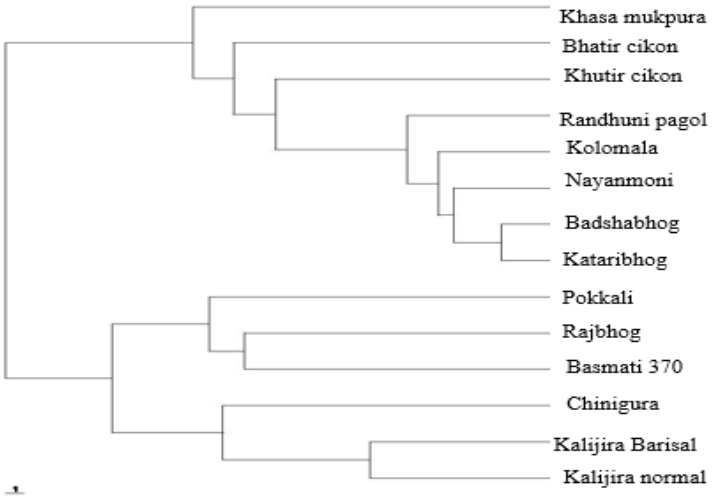

Figure 4. A dendrogram based on Nei's (1972) genetic distance generated by 17 RAPD markers of 14 local genotypes.

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Cluster 1 belongs to Khasa mukpura, Bhatir cikon, Khutir cikon, Randhuni pagol, Kolomala, Nayanmoni, Badshabhog, kataribhog. Cluster II is comprised of Pokkali, Rajbhog, Basmati 370, Chinigura, Klalijira Barisal, and Kalijira normal. Moreover, Cluster I and Cluster II are segregated into sub-clusters and sub-units (Figure 6). In Cluster II, Pokkali in sub cluster 1 and Rajbhog and Basmati 370 under the sub unit of sub cluster 1. Kalijira Barisal, Chinigura and Kalijira normal showed the highest similarity and formed a different sub unit of sub cluster 2. Genotypic variations based on molecular characterization indicated that genotypes belonging to different clusters were characterized by their different genetic sequences. Some scientists [5] observed UPGMA cluster analysis for the combined data of RAPD markers revealed three broad clusters: Cluster I with two landraces i.e. Panbira and Dular; Cluster II with only one landrace i.e Boalia, Cluster 3 with three landraces i.e Maloti, BRRI Dhan 53 and Hashikalm.

2.6. Evaluation of the RAPD markers OPS 3 and OPA 14

Among the polymorphic fragments observed, OPS $3_{(510bp)}$ and OPA $14_{(1100bp)}$ were considered to be associated with the salt tolerant ability of rice. However, it could not be generalized that both fragments were undoubtedly associated with salt tolerance since these fragments were absent in a few tolerant accessions (**Figure 5).**

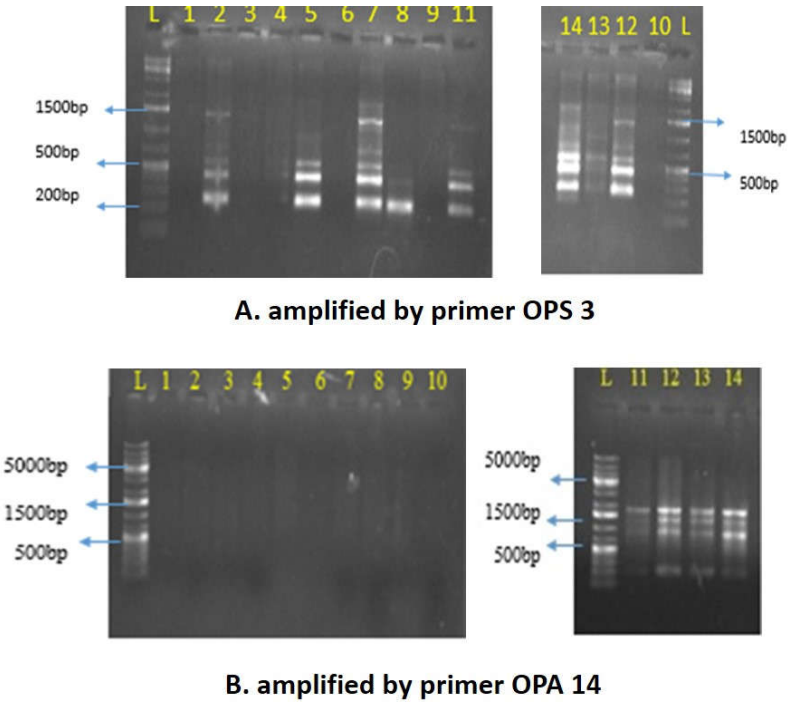

Figure 5. DNA profiles of fourteen aromatic rice genotypes two primers. A. amplified by primer OPS 3, and B. amplified by primer OPA 14.

Table 4. Primer OPS 3 and OPA 14 used and number of different amplified fragments observed among 14 rice genotypes.

Primer Code	Sequence 5'-3'	Allele size (bp)	Total Alleles	Polymorphic Alleles	Size ranged of DNA fragments (kb)
OPA 14	CAGGCCCTTC	200-2000	4	4	2.2-0.2
OPS 3	CAGAGGTCCC	200-3000	7	7	2.2-0.2
Total			11	11	

The primer OPS 3 was used as a specific marker for detecting salt-tolerant genotypes and produced specific fragments at 510 bp that were distinctly and prominently with high

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intensity in 17 out of 22 salt-tolerant varieties [53] This band was either totally absent in many or faintly visible in a few moderately tolerant and sensitive varieties. In our study, out of 20 primers, OPS 3 produced specific fragments at 510 bp in both the Pokkali variety and the Chinigura variety. Besides that, OPA 14 was selected for the comparison of the salt-tolerant Pokkali variety with other local aromatic rice genotypes that produced distinct polymorphic bands at 1100bp. Interestingly, these higher intensity bands were either totally absent in many or very faintly seen in a few moderately tolerant and sensitive varieties. Earlier researchers in soybeans observed similar outcomes [54]. In the case of OPA 14 primer, a polymorphic band with high intensity in the salt tolerant Pokkali variety and three moderately tolerant (Chinigura-lane 12, Kalijira Barisal-lane 13, and Kalijira normal-lane 14) genotypes, it was absent in all others (Plate 1-10). The absence of bands can be caused by a variety of factors, including deletion, duplication, inversion, transversion, point mutation, and so on [55] Both fragments identified in response to salt stress were present in genotypes proven to be salt tolerant like Pokkali, and moderate tolerant like Rajbhog, Basmati 370, Chinigura, Kalijira Barisal, and Kalijira normal and absent in salt sensitive varieties like Nyanmoni, Khuti cikon, Randhuni pagol, Badshabhog, Kolomala, Khasa mukpura, Bhatir cikon, and Kataribhog (Figure 7 and Figure 8). Therefore, this needs to be verified through segregate analyses using salt tolerant and susceptible accessions selected for a specific trait for salt tolerance as parents [56]. This will be a useful approach towards marker-assisted selection (MAS) for salt tolerance improvement. These two primers were further used in screening of rice varieties to detect the RAPD fragments co-segregating with salt tolerance (Table 4).

2.7. Dendrogram produced by OPS 3 and OPA 14 Primers

In the present study, the dendrogram based on Nei's (1972) genetic distance grouped fourteen genotypes into two main clusters at a similarity coefficient of 55% (Figure 6).

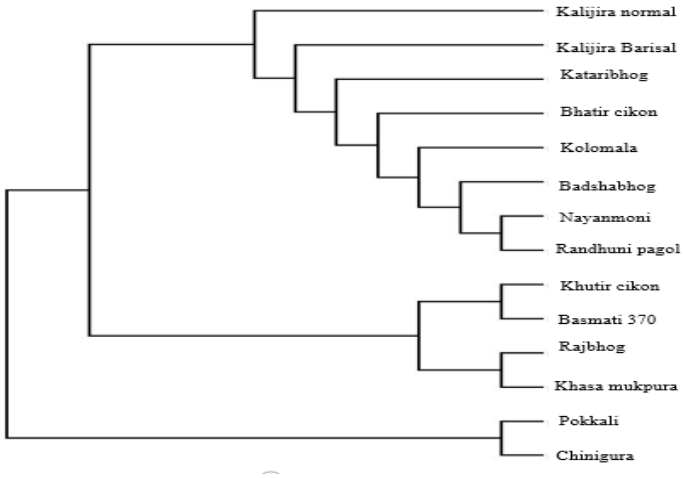

Figure 6. A dendrogram generated by OPS 3 and OPA 14 primers for 14 rice genotypes for the identification of salt tolerant rice.

In the case of cluster analysis, Cluster I belong to two sub-clusters. Sub-cluster 1 includes Nayanmoni, Randhuni pagol, Badshabhog, Kolomala, Bhatir cikon, kataribhog, Klalijira Barisal, and Kalijira normal. Sub-cluster 2 is sub-divided into 2 sub-units. Khutir cikon, Basmati 370 belong to sub unit 1 and Khasa mukpura and Rajbhog belong to sub unit 2 of sub cluster 2. Cluster II is comprised of Pokkali and Chinigura (Figure 6). It is obvious that there is a clear difference between the results of the physiological screening and molecular screening of rice since the number of rice genotypes falling into different groups in physiological screening was not similar to that of clusters in dendrogram. Hence, the RAPD markers OPS 3 and OPA 14 that co-segregate with the salt tolerant genotypes could be used to identify the salt tolerant genotypes in the segregating population,

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especially in breeding programs in Bangladesh where facilities for investigating other molecular markers are limited.

3. Materials and Methods

3.1. Plant materials and growth condition

Thirteen local aromatic rice seeds were collected from the department of Genetics and Plant Breeding, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) and a universal salt tolerant genotype of Pokkali seeds was collected from the Bangladesh Rice Research Institute (BRRI) gene bank (Table 5). A hydroponic system[33] was used at the glasshouse to evaluate the salinity performance of the thirteen aromatic rice genotypes with Pokkali using nutrient solutions [57]

Table 5. Cultivation information with accessions and local name of some Bangladeshi Local Aromatic rice genotypes.

Sl. No.	Local name of Aromatic rice	Accessions no.	Cultivation area	Kernel size and shape
1	Nayonmoni	461	Rajshahi	Short, medium
2	Khuti cikon	4107	Cumilla	Short, bold
3	Radhuni Pagol	6711	Rajshahi	Short, medium
4	Badshahbhog	5349	Bagerhat	Short, bold
5	Rajbhog	4360	Khulna	Short, medium
6	Kolomala	1886	Kishoreganj	Short, medium
7	Basmoti 370	4904	Pakistan	Medium, slender
8	Khasa mukpura	7586	Khagrachari	Short, medium
9	Bhatir cikon	774	Chittagong	Short, medium
10	Kataribhog	7082	Dinajpur	Short, medium
11	Pokkali	17905	India	Long, medium
12	Chinigura	4867	Mymensingh	Short, bold
13	Kalijira Barsal	4357	Barisal	Short, bold
14	Kalijira	4357	Khulna	Short, medium

For the anatomy studies, rice seedlings were selected to study the anatomical structure of leaves and roots by cross section

3.2. Genomic DNA Isolation and Polymerase Chain Reaction (PCR)

Genomic DNA was extracted from 25-day-old rice by using a modified sodium decyl sulfate (SDS) method. For the quality assessment, the yield of DNA per gram of leaf tissue was electrophoresed on a 0.4% agarose gel. RAPD markers were performed following the protocol: 5µl (10X) Reaction Buffer, 2.0µl (10µM) dNTPs, 2.0µl (10µM) $MgCl_2$, 36µl Nuclease free ddH_2O and 2µl genomic DNA was added with 45µl PCR cocktail, then 2.5µl primer was added and finally 0.5µl *Taq* Polymerase (5 U/µL) was used. The total volume was 50µl using a thermal cycler. After initial denaturation for 5 minutes at 94°C, each cycle comprises 1 minute of denaturation at 94°C, 1 minute of annealing at 36°C, and 1:30 minute of extension at 72°C with a final extension of 5 minutes at 72°C at the end of 40 cycles. The PCR product was preserved at 4°C in the thermal cycler. The amplified products were separated electrochemically on a 0.4% agarose gel and conducted in 0.5X TBE buffer at 80V for 45 minutes. A 1 KB plus DNA ladder was used.

3.3. Statistical analysis

Amplification products in the gel images were scored for presence (1) or absence (0). Missing and doubtful cases were scored. The size of each band was calculated using Alpha Ease FCT™ software (version 4.0). The data was then pooled to create a single data matrix. This was used to estimate polymorphic loci, Nei's gene diversity, population differention, genetic distance (D) and to construct a UPGMA (Unweighted Pair Group Method with

Arithmetic Means) dendrogram among populations using the computer programs POP-GENE (Version 3.5) and TREEVIEW [58].

4. Conclusions

Considering hydroponic screening, five local aromatic rice varieties such as Rajbhog, Basmati 370, Kalijira Barisal, Chinigura, and Kalijira normal were scrutinized as moderate salt tolerant on the basis of the performance of Pokkali, the model salt tolerant variety, and the rest were classified as the susceptible and highly susceptible genotypes. However, according to the molecular RAPD marker technique, three other genotypes including Chinigura, Kalijira Barisal, and Kalijira normal were chosen as moderate salt tolerant genotypes and Chinigura was closely related to Pokkali. Nayonmoni, Badshahbhog, Khasa mukpura, Kataribhog Khuti cikon, Radhuni pagol, Kolomala, Bhatir cikon were categorized as susceptible to highly susceptible to salinity stress at seedling stage of rice. The RAPD markers OPS $3_{(510bp)}$ and OPA $14_{(1100bp)}$ may serve as strong putative, which could be helpful in identifying the salt-tolerant rice varieties. This study laid a foundation for future diversity assessment and genetic analysis of aromatic rice for designing breeding program for development of salinity stress tolerant aromatic rice variety.

Supplementary Materials: Figure S1: title; Table S1: title; Video S1: title.

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