



Genetic variation in microsatellite DNA, physiology and morphology of coastal saline rice (*Oryza sativa* L.) landraces of Bangladesh

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Abstract

Genetic variation of *Oryza sativa* L. landraces (LRs) collected from the saline coastal belt of Bangladesh, modern varieties (MVs), as well as Pokkali, Nona Bokra and salt tolerant modern varieties (SMVs) derived from the last two were analyzed with 60 evenly distributed rice microsatellite DNA markers. A total of 196 reproducible polymorphic alleles were identified from the band loci. Heterozygosity among the 31 LRs was found to be 0.57, 0.46 in the 5 MVs and 0.40 in the 8 SMVs. Computation of genetic similarity with this data, using Jaccard's coefficient followed by UPGMA clustering, divided the landraces into 6 distinct groups. Three groups were composed of LRs only from the highly saline southwest. Two groups consisted of LRs from the mild to moderately saline mid-east and northeast coasts. The sixth group was heterogeneous, with LRs from the northeast, LRs from the southwest and Nona Bokra. Pokkali and Gunshi, a LR of the southwest, branched out individually. When all the 46 *O. sativa* L. cultivars were clustered together, most of the MVs and SMVs were found to be linked within the heterogeneous group. The measure of seedling Na and K concentration, Na/K ratios, affected leaf area as well as survival under salinity stress in hydroponics identified 6 LRs from the highly saline southwest as the most tolerant. These group with Pokkali when UPGMA clustering using the Pearson product-moment correlation coefficient suitable for the quantitative physiological data on seedling saline stress was computed. Morphological observations of plant type and height, days to maturity and yield components in non-saline soil indicated low variability among the different LRs. When yield performance as well as tolerance scores were considered, 7 LRs from the southwest and 1 LR from the mid-northeast show potential as donors for breeding salt tolerant rice. The microsatellite fingerprinting analysis thus revealed that some of the salt tolerant landraces of the coastal region have unique polymorphic loci, quite distinct from the popular salt tolerance donor Pokkali. The similarity matrices between the *O. sativa* L. cultivars chosen for the study can be used as a valuable tool for the proper choice of parents for mapping or breeding purposes.

Abbreviations: BARC – Bangladesh Agricultural Research Council; BRRI – Bangladesh Rice Research Institute; CTAB – Hexadecyltrimethyl ammonium bromide; DMRT – Duncan's multiple range test; DU – University of Dhaka; Het – heterogeneous; IRIS – International Rice Information System; IRRI – International Rice Research Institute; LRs – land races; MNE – mid & north east; MVs – modern varieties; SMVs – salt tolerant modern varieties; SRDI – soil resources Development Institute; SSR – simple sequence repeat; SW – south west.

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Introduction

The Sundarban forest area spans one-third of the southern portion of 3 districts in Bangladesh and is known to be highly saline. From the west to east, these are respectively, Satkhira, Khulna and Bagerhat. Salinity levels gradually decline from west to east; Satkhira being highly saline with 70% of the area having soil salinity levels of 4–16 dS/m (Karim and Iqbal, 2001). Further eastwards, there are the coastal regions of Patuakhali, Barguna, Barisal and Pirojpur. These areas are slightly saline (2–4 dS/m), with some pockets being non-saline. The coastline extends north-eastwards, where there are the districts of Lakshmipur, Noakhali and Feni. The salinity in Noakhali is moderate (4–8 dS/m), and extends to larger areas compared to Lakshmipur and Feni. The coastal line moves southwards into Chittagong and Cox's Bazaar, where there are pockets of high salinity as well as mild levels (Karim and Iqbal, 2001). In general, coastal salinity levels start gradually increasing from November at the beginning of the dry season and peaks in March until start of the annual rains, from April till October. Variations in the salinity levels from the west to the east coast are due to rivers flowing into the basin from the north and through into the Bay of Bengal (Karim and Iqbal, 2001). In the moderate to highly saline southwest coastal areas, farmers can only grow a single rice (*O. sativa* L.) crop during the monsoon season when the salinity levels are relatively low. In the rain-fed lowlands of Satkhira and Khulna in the southwest, landraces still account for 80% of the top 20 rice varieties, despite introduction of modern varieties (Bose et al., 2001). Soil salinity in this region can be moderate even during the monsoon season, if there is lower than average or early rainfall. The popular landraces of this region are therefore well adapted to the prevailing soil stresses, including soil salinity. In the slight to moderate saline northeastern coast, farmers grow Aus rice, which is planted in the dry season in late March, when soil and canal water salinity levels are known to be moderate (Panaullah, 1993). Landraces in the eastern coast of Noakhali and Feni form 60% of the top 20 varieties grown there in the dry season (Bose et al., 2001). These varieties should therefore possess some resistance to salt stress, particularly at the seedling stage.

Landraces have been shown to be excellent sources of genes for novel alleles (Evenson and Gollin, 1997; Guevarra et al., 2001; Hoisington et al., 1999; Jackson, 1999; Tanksley and McCouch, 1997). More

than 4000 traditional Bangladesh rice accessions or landraces have been collected and registered at a rice gene bank in the Bangladesh Rice Research Institute (BRRI) for medium-term storage and an identical set is held in trust at IRRI for longer storage (Bashar and Sarker, 1997; Jackson, 1999). Core selections of rice germplasm from many countries, including Bangladesh are being analyzed at IRRI for identification of new alleles and molecular markers for future use in enhancing the productivity of rice cultivars (IRRI; Medium Term Plan 2002–4). There has however been no systematic study of the traditional cultivars from Bangladesh. This paper is an effort at identifying traditional landraces from the coastal saline zones of Bangladesh, which could be donors for salt tolerance traits.

Breeding for salt stress tolerance in rice has been moderately successful (Mishra et al., 2001; Senadhira et al., 2002). However, the genetic base for salinity tolerance in internationally released cultivars has originated mainly from two common salt tolerance donors, Pokkali and Nona Bokra, particularly in case of improved genotypes released for coastal salinity (Gregorio et al., 2002). Initial breeding programs using these donors resulted in moderately salt tolerant, improved cultivars. More complex crosses using a somaclonal variant of Pokkali, TCCP 266-2-49-B-B-3, which had better agronomical properties, resulted in improved cultivars with salt tolerance levels comparable to their donor parents (Gregorio et al., 2002) (www.cgiar.org/irri/iris). BRRI has released two moderately salt tolerant modern varieties, photosensitive BRRI dhan 40 and BRRI dhan 41, originating from advanced IRRI lines and suitable for growth in the coastal areas of Bangladesh only in the monsoon season (BRRI, 2003). Therefore, there is a need to widen the genetic base for donors of salt tolerance traits for adaptability to different agroecological conditions common in most coastal areas. Bangladesh coastal areas are affected by both tidal saline intrusion as well as water stagnation because of lowlands in the monsoon season (July to October), upward or lateral movement of saline ground water during the dry season (November to May) and willful inundation with brackish water for shrimp cultivation (Karim and Iqbal, 2001). Soil profiles in the coastal areas of Bangladesh have an excess of magnesium, calcium, and sulphate and are generally deficient in zinc (Panaullah, 1993; Karim and Iqbal, 2001). Characterization of the landraces adapted to the variable nature of the saline coastal region of Bangladesh will help

identify useful donors for new salt tolerance traits into modern varieties or indicate approaches for improving the yields of the landraces. Any improved rice variety will make major impacts in the livelihoods of the resource-poor farmers of the region, since it has been shown that introduction of modern rice varieties resulted in the production of staple food for 46% of the Bangladesh population between the years 1985–1997 (Hossain, 1998).

Use of simple sequence repeats or SSRs as genetic markers for rice germplasm assessment has gained popularity because of the technical efficiency of its use, its codominance and multi-allelic, highly polymorphic nature as well as its uniform distribution throughout the genome (McCouch et al., 1997; Temnykh et al., 2000). SSRs have been found to be of particular value in analysis of closely related rice genotypes (Nagaraju et al., 2002; Thanh et al., 1999). This manuscript explores variation in 31 landraces at the molecular, physiological and morphological levels along with 15 released rice varieties, seeking to identify locations of prevalence of these landraces as well as sources of tolerance distinct from Pokkali and Nona Bokra, the standards presently used by breeders.

Materials and methods

Collection of Oryza sativa L., landraces from coastal Bangladesh

Seeds were collected from farmer's fields in the saline-prone districts of Satkhira and Khulna in the southwest as well as from districts of Feni and Noakhali in the northeastern coast of Bangladesh. Farmers grow the rice (*O. sativa* L.) crop in the southwest during the monsoon season, from June till the end of December. Seeds and soil samples were collected from the southwest in the first week of December 2001 at crop maturity. Samples from various fields within a designated location as well as several locations within the two districts were collected and labeled separately. Seeds collected from each location were planted separately and plants visually scored for morphological uniformity. Leaves from plants of one location were used for isolation of DNA. For the northeastern coast, soil samples were collected as above from the farmer's field in April of 2002. Since farmers plant seeds at this time, seeds were collected from their stock. Rice (*O. sativa* L.) landraces grown in the coastal areas were also obtained from the gene bank at

the Bangladesh Rice Research Institute (BRRI). These lines were collected in 1973 and were assigned accession numbers when entered into the gene bank. This information was not provided until after the fingerprinting to ensure unbiased molecular evaluation. Out of the 31 landraces tested, 20 varieties were directly from the farmer's field or seed supplies; the rest from the BRRI gene bank. The total list of 31 LRs along with their location is in Table 4. Seeds of Pokkali, Nona Bokra, 8 salt tolerant MVs derived from these and 5 salt-sensitive BRRI and IRRI modern varieties were obtained from the BRRI breeding division. The SMVs were IRRI varieties, IR50184-3B-18-2B-1, IR51491-AC5-4, IR52724-2B-6-2B-1-1, IR58440-3B-9-2-2, IR60494-2B-18-3-2-3, IR65195-3B-13-2-3, and BRRI varieties, BRRIIdhan 40, BRRIIdhan 41. The MVs were IRRI varieties, IR29, IR36 and BRRI varieties, BRRIIdhan 28, BRRIIdhan 29 and BRRIIdhan 36. Origin of the BRRI varieties is given in Table 1A and salt tolerance donors of the SMVs summarized in Table 1B.

DNA isolation

Leaves (0.5–1 gm) of the 46 *O. sativa* L. cultivars were obtained from a pooled sample containing at least 10 seedlings (Parsons et al., 1997). These were cut finely, crushed to powder in liquid nitrogen and subjected to DNA isolation using the CTAB method (Doyle and Doyle, 1990). Slight modifications to the protocol included use of sodium acetate instead of ammonium acetate to precipitate DNA after RNase treatment and additional phenol:chloroform and chloroform purification steps after preliminary DNA isolation.

Amplification of DNA using SSR primers

A total of 60 microsatellite primers, 5 primers from each chromosome were used to amplify DNA from the leaves of the *O. sativa* L. cultivars. List of the primers used, chromosome number and the maximum number of alleles obtained is given in Table 2. The PCR reactions conducted in a volume of 25 μ l contained 10 mM Tris-HCl (pH 8.2), 50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin, 200 μ M dNTPs, 60 ng/ μ l primer, 1 unit Taq DNA Polymerase (Life Technologies, USA) and 75–100 ng Template DNA. Amplification reaction consists of preheating for 5 min at 94 °C and of 35 cycles of 1 min at 94 °C (denaturation) 1 min at 55 °C–61 °C (annealing) and 3 min at 72 °C (elongation) followed by 7 min

Table 1A. Origin of BRRIdhan-derived varieties

Sl. no.	Variety	Origin
1	BRRIdhan 28	IR28 × Purbachi
2	BRRIdhan 29	BG90-2* × BR10; BR10 → IR5 × IR20
3	BRRIdhan 36	IR54791
4	BRRIdhan 40	IR4595-4-1-13 × BR10;
5	BRRIdhan 41	BR5828 → BR23* × BR1185*;

*Origin at IRIS site: www.iris.irri.org.

Table 1B. Salt tolerance trait donors of the SMVs

Sl. no.	Variety	Salt tolerance donors
1	IR50184-3B-18-2B-1	Pokkali & SR26B
2	IR51491-AC5-4	Pokkali & Pokkali
3	IR52724-2B-6-2B-1-1	Pokkali
4	IR58440-3B-9-2-2	Pokkali & Pokkali
5	IR60494-2B-18-3-2-3	Nona Bokra & Pokkali
6	IR65195-3B-B-2-3	Nona Bokra & TCCP266-B-B-B-10-3-1

Source: www.iris.irri.org.

at 72 °C in a Mastercycler Gradient PCR system (EPPENDORF) (Panaud et al., 1996). Amplified products were separated in 4% Typing Grade agarose gel (Life Technologies, USA.), containing 0.5 ng/ml EtBr (Ethidium Bromide). Separated PCR products were visualized under UV light and photographed using Kodak Electrophoresis Documentation & Analysis System, when 2–4 polymorphic bands were visualized. When no polymorphism was observed in agarose gels, the products were separated on long denaturing 6% polyacrylamide gels with a thickness of 0.4 mm (<http://www.irri.org/GRC/GRChome/IRGmanual/section8.pdf>). Gels were pre-run, samples were denatured in loading buffer and electrophoresed for about 2 h or until the bromophenol blue remained visible. The molecular weight markers used, were 25–bp ladder (Life Technologies). Gels were stained with silver nitrate, according to the protocol by Promega. After staining, gels were dried in air and photographed.

Data analysis

For the microsatellite DNA fingerprinting of the of the *O. sativa* L. cultivars, polymorphisms were scored for the presence or absence of bands on agarose or polyacrylamide gels on a 1/0 basis and the data analyzed using the NTSYS-PC version 2.11f (Rohlf, 2000). The average proportion of alleles (bands on gels)

that were shared between any two accessions was used to measure similarity using the Jaccard's similarity coefficient (Jaccard, 1908), which is suitable for qualitative data (Rohlf, 2000). Data on screening of seedlings for tolerance to salinity, such as, survival, area of leaf affected, reduction in leaf height, Na and K concentration and Na/K ratio under saline stress in hydroponics, were subjected to measure of similarity using the Pearson product-moment correlation coefficient (Pearson, 1896), suitable for quantitative data (Ludbrook, 2002). Cluster analysis on the marker and physiological data was done separately based on the similarity matrix obtained in each case, using the unweighted pair group method with arithmetic mean (UPGMA) (Sneath and Sokal, 1973). The efficiency of the UPGMA for estimation of genetic relatedness in each case was determined by computing the cophenetic correlation coefficients (Mantel, 1967). Expected Heterozygosity with respect to SSR marker bands obtained for the different rice varieties was calculated from the sum of squares of allele frequencies (Nei, 1973) as follows:

$$H = \sum (1 - \sum P_{ij}^2) / n,$$

where, P_{ij} is the frequency of the j th allele for marker i and the summation extends over n alleles.

Table 2. List of the total microsatellite primers used, type of gel and the polymorphism in band levels obtained for the 46 rice cultivars

Serial no.	Primer	Chr. no.	No. of polymorph. bands	Type of gel used	Serial no.	Primer	Chr. no.	No. of polymorph. bands	Type of gel used
1	RM5	1	4	A	31	RM184	10	2	A
2	RM9	1	4	A	32	RM185	4	2	A
3	RM14	1	3	A	33	RM189	9	4	P
4	RM17	12	3	A	34	RM204	6	4	A
5	RM18	7	3	A	35	RM208	2	3	A
6	RM19	12	3	A	36	RM209	11	3	A
7	RM21	11	4	A	37	RM210	8	2	A
8	RM24	1	4	A	38	RM211	2	3	A
9	RM26	5	4	P	39	RM217	6	3	A
10	RM29	2	2	A	40	RM218	3	3	A
11	RM31	5	3	A	41	RM219	9	3	A
12	RM32	8	3	A	42	RM221	2	3	P
13	RM72	8	7	P	43	RM222	10	6	P
14	RM80	8	3	A	44	RM224	11	3	A
15	RM101	12	2	A	45	RM227	3	2	A
16	RM103	6	4	P	46	RM228	10	3	A
17	RM107	9	4	P	47	RM229	11	3	P
18	RM117	12	3	P	48	RM232	3	3	A
19	RM122	5	3	A	49	RM233	5	3	A
20	RM124	4	3	P	50	RM238	6	2	A
21	RM125	7	3	A	51	RM244	10	3	P
22	RM131	4	4	P	52	RM256	8	2	A
23	RM134	7	2	A	53	RM257	9	3	P
24	RM140	1	4	P	54	RM273	4	3	P
25	RM143	3	2	A	55	RM279	2	3	A
26	RM150	6	3	A	56	R M270	12	3	P
27	RM169	5	3	A	57	RM293	3	5	P
28	RM171	10	3	A	58	RM303	4	3	A
29	RM172	7	4	P	59	RM328	9	3	A
30	RM180	7	7	A	60	RM332	11	2	A

A = 4% typing grade agarose gel electrophoresis; P = 6% denaturing polyacrylamide gel electrophoresis.

Na/K evaluation for assessment of salinity tolerance at the seedling stage

Sodium and potassium uptake by the shoots, Na/K ratio and seedling survival rates are well correlated with tolerance of the rice (*O. sativa* L.) plant to salinity stress (Bohra and Dorffling, 1993; IRRRI, 1996, Lee et al., 2003). Therefore seedling survival and K and Na concentrations at seedling stage under control and stressed conditions, was measured in hydroponic systems as follows. Seeds of the LRs (20 per cultivar, 10 each for the control and stressed conditions) were germinated in 3 separate sets on netted, floating styrofoam in a completely randomized design.

The size of the float was 28 × 32 × 1.25 cm with a hundred holes. The germinated seedlings were allowed to grow in 10 liters normal Yoshida culture solution/per float until the seedlings reached the 4-leaf stage (14–18 days from germination). The ECe of the culture solution was increased to 6 dS/m for the next two days and then to 12 dS/m until 90% of the leaves of the sensitive control were damaged (23–27 days after stress application). This was done by replacing the culture solution with Yoshida solution containing, NaCl (Ponnamperuma, 1977). The pH of the non-aerated Yoshida culture solution was adjusted to 5.0 every day and changed every five

days. BRRIdhan 29 and Pokkali were used as the sensitive and tolerant controls respectively. Data for percent survival and total leaf area affected was recorded according to the Standard Evaluation System of Rice at IRRI (IRRI, 1996; Gregorio et al., 1997). The percent reduction in shoot and root length and dry weight compared to controls were recorded for each seedling. For the measurement of sodium and potassium concentration in shoot and root at 0 dS/m and 12 dS/m, plants were washed in flowing tap water for 30 sec and 10 oven-dried plants from each replicate were pooled, ground and analyzed by a flame photometer (Jenway, UK) after 48 h of extraction with 1N HCl following the procedure described by Yoshida et al. (1976). Concentrations were expressed as percent of dry weight. Sodium to potassium ratio was also determined. All the data were analyzed statistically and means were separated using DMRT when a significant F-value was obtained. Regression analysis was carried out, by assuming the Leaf Na/K ratio or Na concentration as the independent variable and leaf area affected, and percent reduction in shoot height or dry weight as dependent variables. The screening was done in a screen house, in March–April, July–August and October to November, 2002, so that variation due to changes in humidity would not affect the outcome of the screening tests (Asch et al., 1995). The temperatures and relative humidity in the screen-house varied between 30–37 °C and 50–80%, respectively.

Field observation

For the *O. sativa* L. landraces, 20 seedlings (25 day-old) of each cultivar were transplanted (July to November, 2002) in a 3 m × 2 row plot at the BRRIdhan research fields, in Gazipur, Dhaka. Fertilizer applied to the soil during transplanting was half of that normally provided for MVs. Amounts of urea, TSP, MP and Gypsum were respectively, 80, 50, 60 and 35 kg/ha. The concentrations of N, P, K and S in Kg/ha were therefore, 36.3, 10.1, 30.3 and 6.36, respectively. Soil ECe value was 2 dS/m. Individual plants were 15 cm apart within rows and there was a distance of 25 cm between rows. Five plants from each row were selected randomly for measurement of morphological characteristics. Variation among the 33 *O. sativa* L. cultivars, including Pokkali and BRRIdhan 40, in plant height, total tiller number, panicles per plant, panicle length, flag leaf length, spikelets per panicle, percent fertility, 1000 grain weight and grain yield per plant were evaluated using Duncan's multiple range

test. Only data for 4 yield components as well as days required to maturity after seeding has been reported in the results section.

Analysis of ionic composition of soil from collection sites

Soil at a depth of 10 cm from 3 locations in each harvested field where the farmer had grown a particular landrace was collected. Soil samples were collected from Satkhira and Khulna in the first week of December, when the soil salinity is low because this period coincides with the end of the monsoon season. Soil samples from Feni and Noakhali were collected in April, where the soil salinity may be moderate in certain regions at this time of the year (Panaullah, 1993). The 3 samples from each field were dried separately, powdered and then mixed in equal proportions before analysis. Soil pH was measured in a 1:2.5 soil-water ratio and for ECe, water extract in a 1:1 ratio was used. Na, K, Ca, Mg, P, S, B and Zn were extracted and analyzed at the Regional Laboratory of the Soil Resources Development Institute (SRDI) in Comilla. Extraction of Na and K was according to the method of Helmke and Sparks (1996); Ca and Mg according to Suarez (1996); Cu and Zn according to Reed and Martens (1996); Fe according to Loeppert and Inskeep (1996); Mn according to Gambrell (1996); B according to Keren (1996); P according to Kuo (1996) and S according to Tabatabai (1996). All the ions were analyzed by atomic absorption spectrometry, except S and B, which were analyzed colorimetrically (Page et al., 1982).

Results

A total of 196 reproducible polymorphic bands or alleles were identified using 60 microsatellite primers after amplification of the DNA from the 46 rice genotypes. The 60 microsatellite primers were evenly distributed over the 12 rice chromosomes (Table 2). In 66% cases, agarose gel electrophoresis was adequate in detecting polymorphism showing 2–4 alleles across the various rice varieties (Figure 1). Two alleles were observed for 18% of the primers, 3 alleles for 42% and 4 for 8%. When RM180 was used, 7 alleles were observed in agarose gels similar to the reported range of sizes from 104 to 200 bp (Temnykh et al., 2000). Therefore for 32% of the primers, amplified bands were monomorphic or only a few varieties showed

amplification at a second level. In these cases, polyacrylamide gel electrophoresis was used to resolve amplified DNA resulting in bands of 3–7 different molecular sizes for the different primers (Figure 2). Genetic diversity or Heterozygosity was found to be 0.57 for the landraces (LRs) of the coastal region, while that of modern varieties (MVs) was 0.46 and salt tolerant modern varieties (SMVs) was 0.40.

Genetic relatedness between the 31 LRs as well as the traditional standards, Pokkali and Nona Bokra based on their shared SSR alleles was computed using Jaccard's coefficient followed by clustering into a dendrogram (Figure 3a). Six groups are apparent, at the coefficient value of 0.366. Three are named SW1, SW2 and SW3, all of which contain photoperiod sensitive landraces from the highly saline southwest coastal regions of Satkhira and Khulna. Two more groups, called MNE1 and MNE2 have photoperiod insensitive landraces from the mildly saline coast of the mid northeast, Noakhali, as well as non-saline Faridpur and Habigonj, further north from the above coastal region. The 6th group of the dendrogram consists of landraces from all the different coastal regions as well as Nona Bokra and is called the Het group. The internationally well-known standard for saline tolerant rice, Pokkali as well as Gunshi, a landrace grown in the mildly saline Barisal, did not group with any of the 30 other landraces. Comparison of the matrix of similarity generated with the cophenetic matrix of the dendrogram showed a good fit ($r = 0.79$), indicating the reliability of the groups within the tree diagram (Figure 3a).

When the genetic similarity of all 46 cultivars, including the 31 LRs, 8 SMVs, 5 MVs as well as Pokkali and Nona Bokra, was computed as above and subjected to clustering using the Jaccard's coefficient, all the MVs and SMVs were found within the Het group (Figure 3b), except that IR65195-3B-13-2-3, Hida and Pokkali became grouped separately and IR29 and IR36 did not group with any other cultivar.

Seedlings of 6 landraces scored well in comparison to Pokkali under 12 dS/m saline stress in hydroponic systems. These had high survival percent, low percent leaf area affected, low reduction in shoot height, low Na concentration and low Na/K ratios in shoots (Table 3). These cultivars are Ashfal, Benapole, Jamainaru, Lakshmikajol, Patnai Balam and Horkuch, all farmer popular landraces of the Khulna region (Tables 3 and 4). All 6 cluster very closely with Pokkali in the dendrogram generated from the similarity matrix of scores from the above seedling

screening tests using the Pearson Correlation coefficient (Figure 4). Capsule, Morichshail and Kajalshail also performed moderately well in above stress tests at the seedling stage. Capsule grouped closely with Pokkali, while Morichshail and Kajalshail fuse at a slightly higher level. All the landraces popular in the southwest districts cluster with Pokkali when seedling stress scores were considered. In addition, Binnatoa, Kajalshail and Gunshi also group with Pokkali. Binnatoa and Kajalshail are grown in the mid-northeast. Gunshi grows in the mildly salt stressed Barisal in the mideast. All the cultivars of the mid northeast cluster with salt sensitive MV, BRRIdhan 29. Chinikanai, which is popular in the southwest also grouped with BRRIdhan 29. Dakshail and Mohini, also popular in the southwest form a group of their own with Khaiyaboro, a cultivar grown in non-saline areas away from the coast.

Morphological observations of the 31 traditional landraces in the field, in normal unstressed soil, show these to be of typical, moderate to tall plant-type, with spread-out tillers (Table 4). Jatai Balam, Patnai Balam, Morichshail and Kachra show good yields when compared to the SMV control, BRRIdhan 40 (Table 4). Gheegoj, Jamainaru, Lakshmikajol, Mohini, Hoglapata and Horkuch also show reasonable yields (Table 4).

Considering the seedling screening under saline stress as well as the morphological observation of the mature plants in non-stressed condition, it can be concluded that Jamainaru, Lakshmikajol, Patnai Balam, Horkuch and Morichshail may prove to be good donors for salt tolerance traits. Ashfal has a very good tolerance score but moderate yield. Kajalshail and Raniselute gave moderate tolerance scores but have only average yields. These 8 landraces may have good potential as donors for salt tolerance traits. All of them, except for Kajalshail are grown popularly in the saline southwest district of Khulna, while Kajalshail is popular in mildly saline coastal areas in Noakhali (Table 5).

Ionic composition of the soil from the fields where the landraces had been grown, was analyzed. Results for one field per landrace has only been reported (Table 5). The composition of the soil samples collected from the southwest, as well as, the northeast was generally similar in that calcium and magnesium concentrations were very high, iron high, phosphorous and zinc low, and levels of sulphate and boron toxically high. Differences included low potassium in the

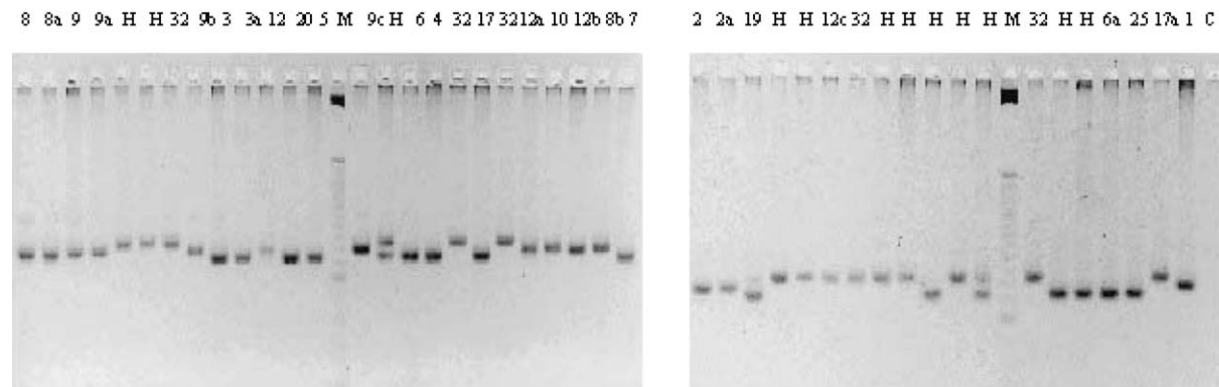


Figure 1. Amplified DNA of traditional rice cultivars using microsatellite primer RM17 after resolution in 4% agarose gel. The lanes are numbered according to the serial followed in Table 3. Lanes which are not numbered show amplified DNA from modern varieties (H). Lane M and C are 25 bp DNA ladder and PCR control respectively. a, b, c denote different accessions of the same landrace.

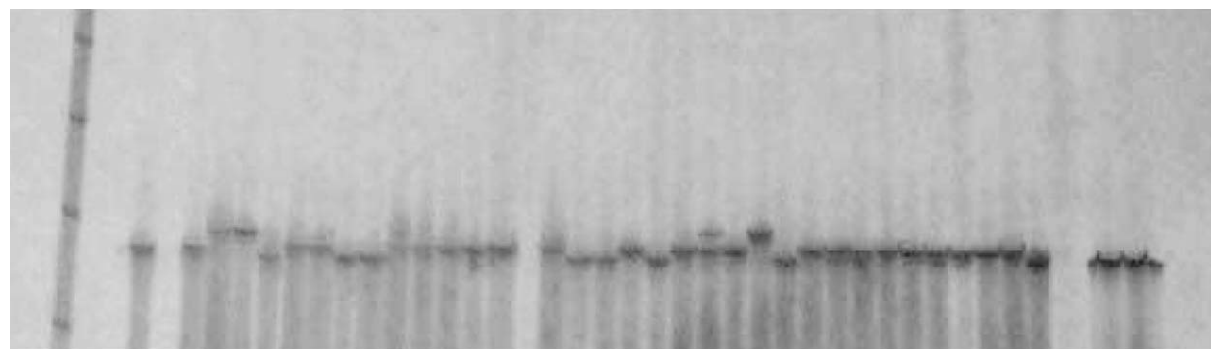


Figure 2. Amplified DNA of traditional rice cultivars from the southwest region using microsatellite primer RM124 after resolution in 6% polyacrylamide gel. The lanes are numbered according to the serial followed in Table 3. Lane G, M and C show gap, 25 bp DNA ladder and PCR control respectively. Lane NB denotes Nona Bokra.

mid-northeast and high in the southwest and higher level of sulphate toxicity in the former areas.

Discussion

Thirty-one landraces of the coastal saline region were genotyped and partially characterized, twenty of which were collected directly from the farmer's fields. Farmer's preference for these landraces, despite introduction of modern varieties (Bose and Hossain, 2003) suggests greater adaptability of these landraces in the variable coastal environment, which includes unpredictable rainfall, variable water stagnation in low lands, depending upon the season, sea-water intrusion due to upstream withdrawal of water, again depending upon the amount of rainfall in the upper riparian areas in our neighboring country. The landraces are also adapted to the ion toxicities as well as deficiencies

of the coastal soils. Investigation of the relatedness of the landraces based on their SSR alleles, separated cultivars growing in the southwest (SW1, SW2 and SW3) from those in the mid-northeast (MNE1 and MNE2). Due to lower levels of saline stress in the mid-northeast, farmers grow Aus or dry season photoperiod insensitive varieties, which are seeded in April. These are the ones forming the groups MNE1 and MNE2. Therefore genetic variation relates to adaptability of the cultivars in distinct coastal regions. The southwest coastal soil has a higher amount of Ca, K and Fe compared to the mid-northeast, while its sulfur toxicity is less (Table 5). Its P and organic content is higher and drainage poor due to prevalence of clayey soil, in contrast to poor organic matter and clay loam soil in the mid-northeast (Karim and Iqbal, 2001). Pokkali and Gunshi did not group with any of the landraces. Therefore, these two have distinct genetic identity.

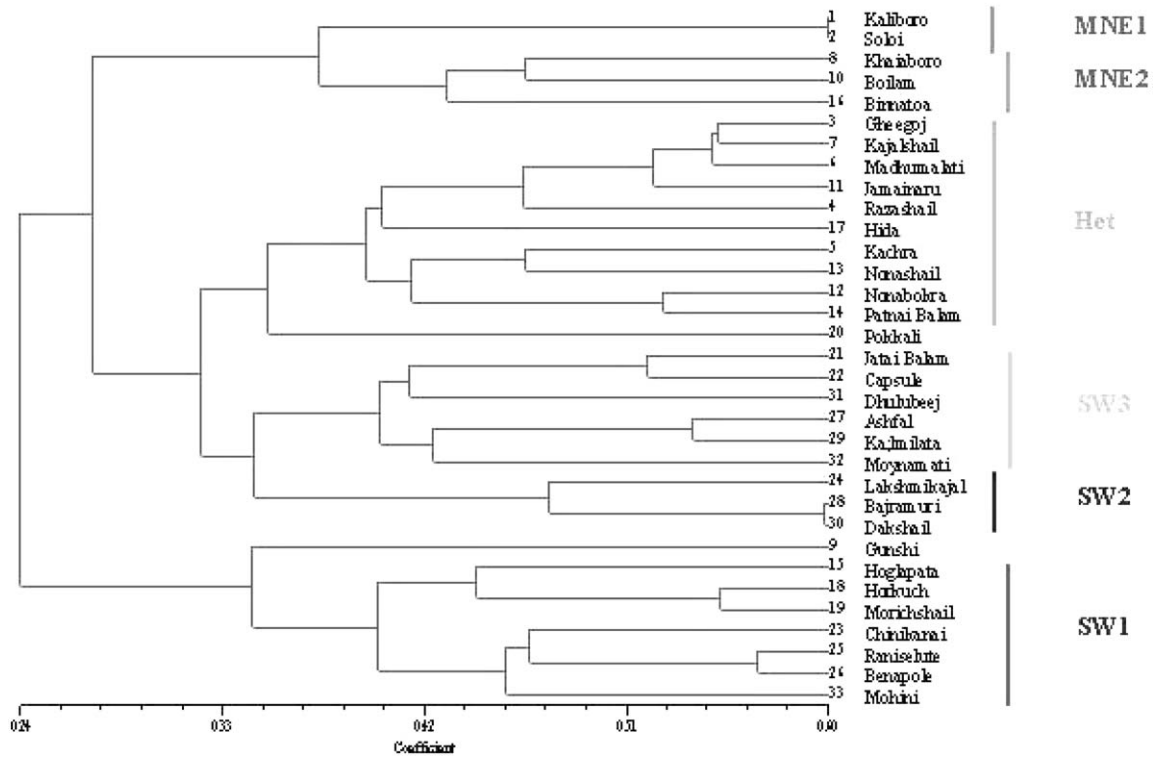


Figure 3a. Dendrogram of 31 traditional landraces along with Nona Bokra and Pokkali.

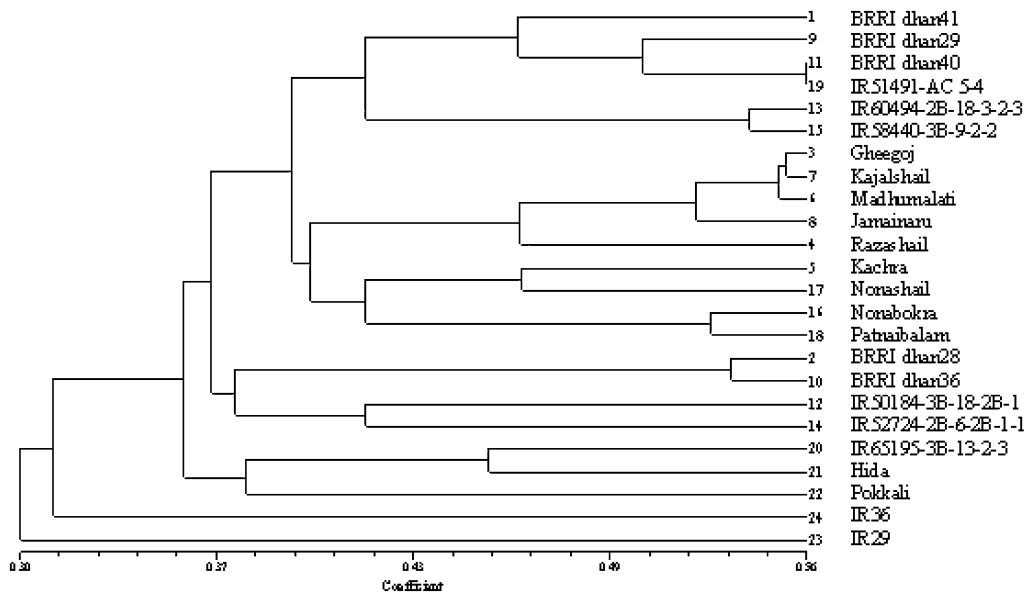


Figure 3b. Dendrogram of modern varieties and salt tolerant modern varieties along with cultivars of the heterogeneous group (Het).

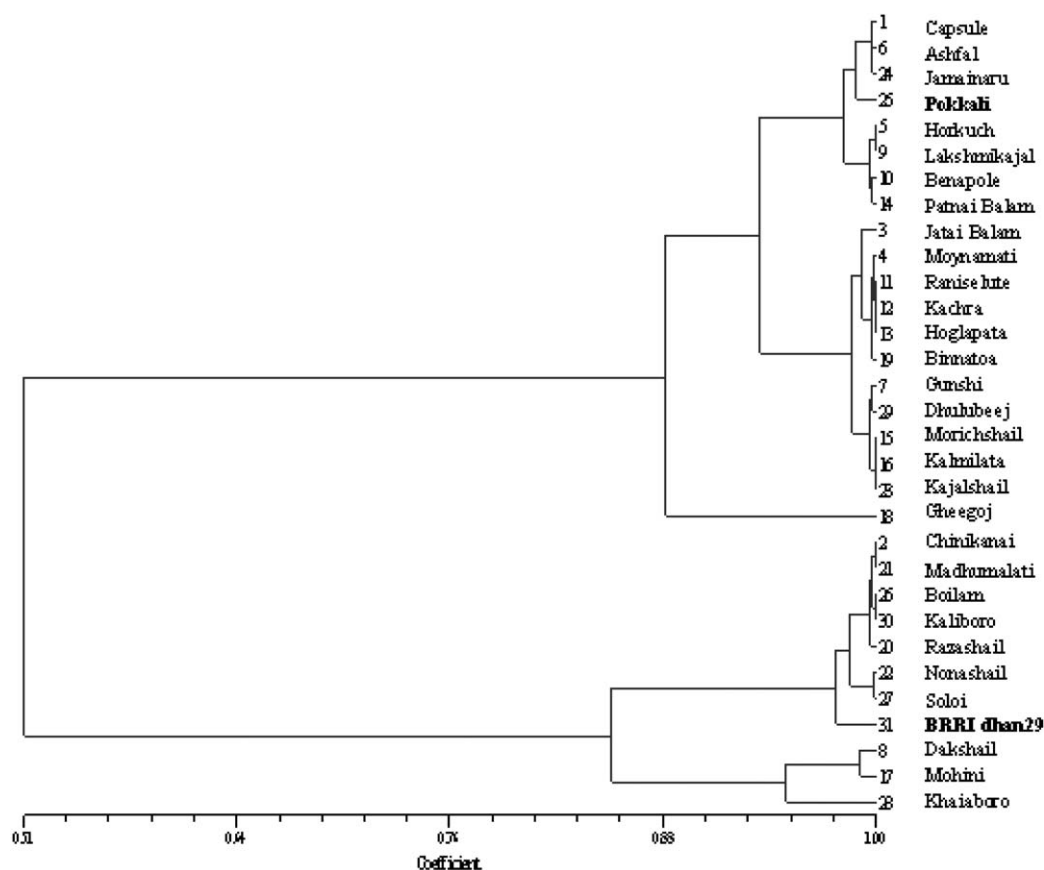


Figure 4. Dendrogram from the data of salinity screening of 29 landraces along with Pokkali and BRRIdhan29.

Three landraces from the southwest, 4 from the mid-northeast, 2 from non-coastal areas as well as Nona Bokra cluster together in a group named Het (heterogeneous). All the total 46 fingerprinted cultivars, including the modern varieties (MVs) as well as the salt tolerant modern varieties, clustered within the Het group (cophenetic correlation $r = 0.8$), with 3 exceptions. IR36 and IR29 showed independent lineage, and IR65195-3B-13-2-3, Hida and Pokkali grouped separately, although Hida was part of the Het in the dendrogram of the landraces only. Hida is actually an lowland variety, grown in Rajshahi in the North, which was mixed in as a blind sample from the BRRIdgermplasm bank. That is why screening tests as well as field observations were not undertaken. IR65195-3B-13-2-3 however has both Pokkali and Nona Bokra as salt tolerance donors, since TCCP266-B-B-B-10-3-1 is a somaclonal mutant of Pokkali (Gregorio et al., 2002). With the exception of two landraces, Kajalshail and Nonashail, all the cultivars in the Het group, are a part of international collections, since they have

their corresponding IRGC or IRTP numbers (Table 4). All the landraces of the Het group are also popularly grown in many areas of the coast. Proportion of Razashail, is 1.2% of the total planted monsoon rice, including modern varieties (Bose and Hossain, 2003). Patnai Balam is next forming 0.4%, Kajalshail 0.3%, while Gheegoj and Hida are grown in 0.2% of total monsoon-rice planting area (Bose and Hossain, 2003).

Evaluation of the physiological response under seedling saline stress identified 6 landraces whose performance was not significantly different from Pokkali, the benchmark, or reference cultivar for assessment of tolerance. These six cultivars grouped with Pokkali in a dendrogram based on computation of similarity based on the above physiological response, using the Pearson's product moment coefficient. All the cultivars that grouped with Pokkali had low Na/K scores (0.81–1.05) while that of Pokkali was 1.03. Low Na/K ratios at the seedling stage are associated with tolerance due to Na exclusion as well as partitioning of Na into older leaves (Flowers and Yeo, 1995; Moradi

Table 3. Percent survival, area leaf affected, shoot and root reduction in height, shoot sodium and potassium concentration and ratio in hydroponically-grown 45-day-old seedlings of 31 landraces, 27 days after saline stress application at 12 dS/m

Serial	Variety name	Percent survival	Percent leaf area affected	Percent shoot length reduction	Percent leaf Na at 12 dS/m	Percent leaf K at 12 dS/m	Shoot Na/K ratio at 12 dS/m
1*	Binnatoa	60.0 c-i	50.0 e-j	9.5 abc	1.98 a	1.35 bc	1.47 a-d
2*	Boilam 3538	20.0 kl	80.0 m-q	25.5 ijk	2.78 a-h	1.28 c	2.00 a-d
3	Gheegoj 2413	33.3 jk	70.0 k-o	21.0 f-j	3.03 a-i	1.98 abc	2.06 a-d
4	Kajalshail 612	66.7 b-g	40.0 b-g	9.5 abc	2.96 a-i	2.20 abc	1.4 a-d
5	Madhumalati 614	15.0 kl	85.0 opq	21.8 g-j	3.24 c-i	1.94 abc	1.69 a-d
6	Nonashail	8.3 l	86.7 opq	33.5 l	3.27 d-i	1.73 abc	2.52 de
7	Gunshi 3869	63.3 b-h	40.0 b-g	16.5 c-h	3.06 a-i	2.28 abc	1.35 a-d
8*	Kaliboro 1281	18.33 kl	81.7 n-q	24.7 ijk	3.56 ghi	1.63 abc	1.81 a-d
9*	Soloi 1713	10.0 l	91.7 pq	31.2 kl	3.29 d-i	1.577 abc	2.16 bcd
10*	Khaiaboro 4539	40.0 ij	76.7 l-p	28.1 f-j	3.46 e-i	1.887 abc	1.79 a-d
11	Hida	—	—	—	—	—	—
12	Razashail 1061	20.0 kl	83.3 n-q	20.0 e-j	3.50 f-i	1.77 abc	2.28 cd
13	Ashfal	80.0 abc	26.7 abc	6.9 ab	2.39 a-e	2.67 a	0.91 ab
14	Benapole	76.7 a-d	30.0 a-d	4.7 a	2.49 a-g	2.47 abc	1.03 abc
15	Bajramuri	—	—	—	—	—	—
16	Dakshail	46.7 g-j	56.7 g-k	8.3 abc	3.11 b-i	1.82 abc	1.85 a-d
17	Hoglapata	56.7 d-i	43.3 c-h	10.3 a-d	3.03 a-i	2.01 abc	1.66 a-d
18	Horkuch	70.0 a-f	33.3 a-e	7.4 ab	2.59 a-h	2.61 ab	1.05 abc
19	Jamainaru 2039	80.0 abc	23.3 ab	9.0 abc	2.17 abc	2.68 a	0.81 a
20	Kachra 973	60.0 c-i	46.7 d-i	11.4 a-d	3.08 b-i	2.16 abc	1.5 a-d
21	Kalmilata	63.3 b-h	40.0 b-g	10.7 a-d	2.71 a-h	2.37 abc	1.17 abc
22	Lakshmikajol	73.3 a-e	36.7 b-f	7.5 ab	2.81 a-h	2.45 abc	1.18 abc
23	Mohini	43.33 hij	63.3 i-m	13.02 a-f	3.41 e-i	1.633 abc	2.25 cd
24	Morichshail	63.3 b-h	40.0 b-g	10.5 a-d	2.97 a-i	2.26 abc	1.4 a-d
25	Patnai Balam	70.0 a-f	30.0 a-d	8.3 abc	2.387 a-e	2.66 a	0.89 ab
26	Raniselute	50.0 f-j	40.0 b-g	9.2 abc	3.32 e-i	2.04 abc	1.82 a-d
27	Capsule	83.3 ab	26.7 abc	13.3 b-f	2.20 a-d	2.56 abc	0.86 a
28	Chinikanai	16.7 kl	86.7 opq	24.3 h-k	2.83 a-i	1.83 abc	1.65 a-d
29	Dhulubeej	60.0 c-i	40.0 b-g	13.0 a-f	2.15 ab	1.81 abc	1.19 abc
30	Jatai Balam	53.3 e-j	50.0 e-j	12.5 a-e	2.47 a-f	2.51 abc	1.12 abc
31	Moynamoti	60.0 c-i	43.3 c-h	11.0 a-d	3.07 b-i	2.15 abc	1.48 a-d
32	Pokkali	90.0 a	16.0 a	7.8 ab	2.19 abc	2.2 abc	1.03 abc
33	BRRIdhan 29	5.7 l	96.0 q	14.39 b-g	3.90 i	1.48 abc	3.40 ef

Cultivars marked with an asterisk are photoperiod insensitive. Means followed by a common letter are not significantly different at the 5% level by DMRT.

et al., 2003). The capacity of plants to maintain low Na/K ratio has been correlated with their capacity for salt tolerance as reviewed by Maathuis and Amtmann (1999) and Tester and Davenport (2003). Under IRRI's standard system of evaluation (IRRI, 1996; Gregorio et al., 1997), all 6 landraces scored between 3–5, which was graded according to the percent leaf area affected after about a 25-day period of salt stress and when the age of the seedlings was 45 days. Un-

der these conditions, the score of the tolerant check, Pokkali, was less than 3 and that of the sensitive check BRRIdhan 29, around 9. We found a significant dependence of the percent leaf area affected and tolerance score on Na/K ratio (coefficient of determination $r^2 = 0.69$ and 0.63 , respectively) for all the landraces after regression analysis. Sodium concentrations were expressed as percent of dry weight and these were found to be higher than the reported val-

Table 4. Location, SSR group identification, days to maturity and mean of yield components of 31 traditional rice cultivars grown from August to November in normal soil at the BRRIdhan field station

Sl no.	Cultivar name	Origin	IRGC entry with same name	Days to mat.	Plant height	Panicles /plant	Spikelets /panicle	Percent fertile grains	1000 grain weight (gm)
1*	Binnatoa	Noakhali, MNE2		90	119 I	7.0 ghi	38.51 m	81.64 ab	22.9 ef
2*	Boilam 3538	Noakhali, MNE2	37389	-		-	-		-
3	Gheegoj 2413	Noakhali, Het	IRTP 13300	116	132 efg	12.8 ab	101.73 d-i	77.01 a-e	27.48 bcd
4	Kajalshail 612	Noakhali, Het		117	122 hi	15.0 a	73.13 jkl	68.12 c-g	27.91 a-d
5	Madhumalati 614	Noakhali, Het	46274	118	138 cde	13.0 ab	106.32 c-h	62.52 f-I	20.56 g
6	Nonashail	Noakhali, Het		111	122 hi	11.2 b-f	63.51 l	68.0 c-g	28.43 abc
7	Gunshi 3869	Barisal	37093	-	-	-	-		-
8*	Kaliboro 1281	Faridpur ¹ , MNE1	43880	-	-	-	-		-
9*	Soloi 1713	Faridpur ¹ , MNE1	37598	-	-	-	-		-
10*	Khaiaboro 4539	Habigonj ¹ , MNE2	IRTP 4216	-	-	-	-		-
11	Hida	Rajshahi, Het	26471	-	-	-	-		-
12	Razashail 1061	Narail ¹ , Het	37577	95	119 i	8.0 f-I	79.88 i-l	67.19 f-i	15.74 h
13	Ashfal	Khulna, SW2		121	139 bcd	7.0 ghi	117.36 b-e	73.1 a-g	26.64 cd
14	Benapole	Khulna, SW1		117	140 a-d	9.2 d-h	89.22 g-k	51.36 ijk	24.03 e
15	Bajramuri	Khulna, SW3		-	-	-	-		-
16	Dakshail	Khulna, SW3		118	139 b-e	6.6 hi	126.96 bc	48.58 jk	16.79 h
17	Hoglapata	Khulna, SW1		127	145 abc	10.4 b-f	92.31 e-j	81.78 ab	28.07 a-d
18	Horkuch	Khulna, SW1		119	145 abc	11.2 b-f	82.1 h-l	74.69 a-f	28.36 abc
19	Jamainaru 2039	Khulna, Het	37116	121	135 def	12.2 a-d	114.65 b-f	71.12 b-g	28.85 abc
20	Kachra 973	Khulna, Het	31821	121	144 abc	12.6 abc	75.76 jkl	86.53 a	29.47 ab
21	Kalmilata	Khulna, SW2		121	141 a-d	10.4 b-f	91.33 f-j	77.97 a-d	26.76 cd
22	Lakshmikajal	Khulna, SW3		121	141 a-d	11.4 b-e	97.9 d-j	77.32 a-e	26.07 d
23	Mohini	Khulna, SW1		120	128 fgh	10.2 b-g	78.284 i-l	83.17 ab	30.02 a
24	Morichshail	Khulna, SW1		121	144 abc	10.6 b-f	114.51 b-f	82.16 ab	28.19 a-d
25	Patnai Balam	Khulna, Het	31914	127	133 def	9.4 c-h	134.24 ab	84.32 ab	22.87 ef
26	Raniselute	Khulna, SW1		127	144 abc	10.4 b-f	92.67 e-j	81.49 ab	26.68 cd
27	Capsule	Satkira, SW2		105	121 hi	9.8 b-h	65.45 kl	53.48 h-k	22.15 efg
28	Chinikanai	Satkira, SW1	49041	112	141 a-d	5.8 I	130.85 abc	72.76 b-g	12.40 I
29	Dhulubeej	Satkira, SW2		-	-	-	-		-
30	Jatai Balam	Satkira, SW2		117	123 hi	12.8 ab	121.1 bcd	81.02 abc	28.4 abc
31	Moynamoti	Satkira, SW2		106	111 j	10.6 b-f	89.18 g-k	64.69 e-h	21.35 fg
32	Pokkali	Sri Lanka	IRTP 10169	104	147 a	6.6 hi	151.42 a	54.59 hij	27.67 bcd
33	BRRIdhan 40	SMV		123	110 j	11.0 b-f	148.3 a	81.0 abc	24.0 e

Cultivars marked with an asterisk * are photoperiod insensitive. Means followed by a common letter are not significantly different at the 5% level by DMRT. SMV: Salt tolerant modern variety. SW1, SW2, SW3, MNE1, MNE2 and Het refer to the groups in the dendrogram in Figure 3a.

¹Non-saline coastal areas.

ues for Pokkali (Lee et al., 2003), so that our Na/K ratios were proportionately higher for all cultivars. The higher concentration of Na can be explained by the fact that the sensitive control BRRIdhan 29 needs 5–7 days longer to score 9 compared to IR29, the sensitive standard used by IRR. Since BRRIdhan 29 is a farmer-accepted cultivar in the non-saline coastal region, we chose it as the sensitive check.

Field observation of the morphology of the landraces in unstressed soil at the BRRIdhan field station, identified plants with acceptable plant type and yield parameters. The morphological observations could not be made with replicated plots due to lack of seeds, particularly the ones collected from the germplasm bank at BRRIdhan. Since the size of the manually prepared field, where the landraces were planted was small, about

Table 5. Ionic composition, electrical conductivity and pH in the 1st week of April, 2002, of soil in area of Landrace collection

Region of plantation	Landrace	pH	ECe dS/m	Ca	Mg	K	Na	P	S	B	Fe	Zn
				meq/100 g soil						$\mu\text{g/g}$ soil		
Sonagazi, Feni	Razashail Binnatoa*	4.6	5.87	4.2	4.1	0.19	2.7	2.50	157	0.71	29	2.5
Char Jubilee, Noakhali	Gheegoj	7.1	0.66	6.9	4.6	0.28	2.0	2.90	156	0.42	12	1.46
Char Laurance, Noakhali	Kajalshail	5.5	5.62	9.9	5.7	0.16	3.4	2.70	299	0.83	30	3.32
Char Laurance, Noakhali	Boilam*	5.6	3.14	10.0	5.5	0.15	3.4	1.81	259	0.86	24	2.44
Char Jabbar, Lakshmipur	Kajalshail	5.5	1.32	7.0	6.0	0.31	2.5	3.40	163	0.68	32	1.72
Char Jabbar, Lakshmipur	Gheegoj	5.8	1.90	8.4	7.3	0.30	3.2	2.34	186	0.53	19	3.68
Bigordana, Deluti Union, Paikgacha, Khulna	Jatai Balam	5.3	1.74	7.8	6.4	0.54	3.3	1.17	83	0.87	68	1.46
As above	Benapole	5.2	2.81	10.8	6.2	0.75	4.0	26.90	142	0.97	109	2.06
As above	Patnai Balam, Lakshmikajol	4.7	0.83	9.4	6.3	0.42	2.9	1.23	134	1.24	121	3.26
Sener Ber, Deluti Union, Paikgacha, Khluna	Morichshail	5.2	0.68	6.6	6.5	0.70	3.4	2.32	87	0.85	102	1.82
As above	Bajramuri, Dakshail, Hoglapata, Kalmilata	7.6	3.39	27.8	9.5	0.87	5.3	19.19	145	1.27	28	2.06
As above	Kachra, Mohini, Raniselute	6.9	0.83	13.2	5.9	0.58	3.3	5.70	79	0.67	32	1.44
Sreefaltala, Soladana Union, Paikgacha, Khulna	Ashfal	7.8	2.98	35.4	9.6	0.86	4.3	9.65	145	0.87	17	1.26
As above	Horkuch	7.9	2.4	33.1	9.2	0.93	5.2	9.50	112	0.91	18	1.58
Hazrakhal, Mariali Union, Ashashuni, Satkhira	Dhulubeez	4.9	1.24	5.8	5.5	0.60	3.6	1.94	103	0.86	52	1.86
As above	Moynamoti	4.7	4.14	6.3	6.0	0.85	5.6	2.31	177	1.26	62	5.58
As above	Patnai Balam	5.4	2.07	7.1	7.0	0.90	4.1	4.36	120	0.75	61	3.3
As above	Jatai Balam	6.9	6.04	6.7	6.3	1.10	5.2	2.74	89	0.47	10	1.10
As above	Chinikanai	4.4	5.79	6.4	6.1	0.50	3.0	2.70	100	0.52	87	2.08
As above	Capsule	4.5	3.72	4.8	4.5	0.76	4.0	1.08	107	0.64	61	2.64

*These landraces grown in the Aus season, from April to July.

23 m², variability in field conditions was deemed to be insignificant.

Combining good tolerance scores with good plant-type, identified the following 8 landraces to be potential donors for salt tolerance traits in breeding programs, even though the variability among the landraces was too low to allow clustering. These are Jamainaru, Lakshmikajal, Patnai Balam, Horkuch, Morichshail, Ashfal, Raniselute and Kajalshail. The former 7 are all landraces from the southwest Khulna region, whereas Kajalshail is popularly grown in the mid northeast Noakhali region. All of these cultivars

however, need a 10-day longer period for maturity compared to Pokkali, which may become even longer due to saline stress. If saline stress occurs in the first 60 days and is then removed, then the number of panicles per square meter is directly affected which is one of the components of yield (Bennett and Khush, 2003). Therefore landraces that show good performance at the seedling stage are expected to contribute more towards this component of yield. Na/K ratio, have been also shown to be negatively correlated with yield in the fully-grown plant by Mishra and coworkers (2001). In mature plants, it has been reported that tolerant cul-

tivars were able to efficiently exclude sodium from the reproductive parts (Khatun et al., 1995; Moradi et al., 2003). Therefore any genotypes showing the ability to exclude Na at the seedling stage are expected to do so when more mature. Asch et al. (2000) have shown that salinity-induced yield loss can be predicted with a high degree of confidence through Na/K ratio of seedlings when 60 days old. Yield component such as 1000-grain weight has been shown to be the least affected by salinity stress (Moradi et al., 2003). Therefore landraces with good scores for this yield component in unstressed soil and having good scores for tolerance under seedling stress are expected to be good donors for salt tolerance traits.

In breeding programs for production of salt tolerant rice, the potential donor LRs, which grouped into Het can be crossed with SMVs within or outside the Het group (Figures 3a and b), depending on the objective of an area-specific improved cultivar or one with broader adaptability. The LRs from the SW1, 2 or 3 groups could be crossed with SMVs like IR50184-3B-18-2B-1 which has Pokkali and SR26B as well as good yields or IR60494-2B-18-3-2-3 which has Nona Bokra and Pokkali. The above 8 landraces were also found to be Heterozygous to Pokkali with respect to the rice microsatellite marker from chromosome 1, RM140. This marker has been found to be linked to the major QTL for salt tolerance and low Na/K ratio in Pokkali (Elahi et al., 2003). Other markers are being tested for heterozygosity, within the linked region.

The MNE group contained varieties that are grown in the northeastern coast as well as in non-saline, Narail and Faridpur, in east and central Bangladesh respectively, and Comilla and Habiganj, in the north-east. Seeds for all these landraces were from the BRRI germplasm bank and were from a collection of 1973 provided as blind samples, until after the fingerprinting was completed. It turns out that cultivars with the same name are grown in the non-saline areas above as well as the northeastern coastal areas (Bose and Hossain, 2003). The common factors about these landraces are that they grow in areas, which are non-saline and where the soil has low to medium phosphorus and potassium content. We determined the heterozygosity between landraces with the same name but grown in different locations (results not shown). In all cases, the heterozygosity was less than 0.1, which was not enough to separate them, when a dendrogram was attempted.

Farmer popularity of the cultivars that performed well were noted from unpublished results of scien-

tists from IRRI and Bangladesh (Bose and Hossain, 2003). These data were collected from the department of extension (DAE) survey under the Ministry of Agriculture in 1996/97. Jamainaru was grown in 3.3% area in Bagerhat and was amongst the top 10 rice varieties grown in this area. The seeds that we used were from a 1973 BRRI gene bank collection, where the area of collection was noted as Khulna. The DAE survey showed the area under Patnai Balam cultivation in Satkhira to be 7.6% and in Khulna 3.6%, also ranking among the top 10 rice varieties to be grown. Our collections of Patnai Balam were also from farmers in Khulna as well as Satkhira. Raniselute and Morichshail, both collected from Khulna were cited to be cultivated only in 1.5% and 1% area of Khulna, respectively. Our Horkuch collection was from Khulna. However the DAE survey showed that it was grown only in 0.4% area in Satkhira. Although we collected Lakshmikajal from Khulna, the DAE survey observation was that it is grown only in 3.6% area of Lakshimpur in the northeast coast, again representing one of the top 20 rice of this area. Discrepancies between location of cultivation area may indicate shifting farmer preferences over different years. Landraces, which remain popular over years may therefore indicate their greater adaptability.

We have checked the names of the LRs against the gene bank collections at IRRI, listed in the site www.cgiar.org/irri/iris. Any entry was recorded and noted in Table 4. Jamainaru, grouped within the Het family, is mentioned as a photoperiod insensitive variety. We however found Jamainaru to be responsive to daylength hours and therefore, photoperiod sensitive. We have fingerprinted a LR collected from the BRRI gene bank called Patnai; however we collected a LR called Patnai Balam from farmers in Satkhira. Incidentally both these LRs have the IRGC entries 31913 and 31914, respectively. On fingerprinting, we found that the Heterozygosity between these two is less than 0.1, which indicated that these cannot be separated as different cultivars. We have included only the fingerprinting data of Patnaibalam in the dendrogram in Figure 3a.

A combination of agarose as well as denaturing polyacrylamide was used to resolve microsatellite regions of the different rice cultivars from the coastal areas of Bangladesh. Using this approach, which was time-effective, adequate heterozygosity was found as reported by other authors as well (Nagaraju et al., 2002). Although the heterozygosity is greater when

AFLP (Fuentes et al., 1999) or RAPD (Parsons et al., 1997) markers are used, one can be sure that all the regions of the 12 rice chromosomes have been represented with microsatellites. Moreover genetic distance between two reference varieties like Pokkali and Nona Bokra (Singh et al., 1999) was similar to what we found in our approach as described in the discussion above. Seven cultivars, which are grown exclusively in the highly saline zones and one for the moderately saline mid-northeast, were identified with superior salinity tolerance levels at the seedling stage as well as having good agronomic properties. These landraces will be taken up for further study and their yield parameters under moderate saline stress determined. The fingerprinting results of these cultivars also indicate linkages between these and other modern varieties, including some salt tolerant modern varieties. Hence this work will assist the breeders in selection of salt tolerant donors, which are different from the traditional ones, Pokkali and Nona Bokra.

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References

- Asch F, Dorffling K and Dingkuhn M 1995 Response of rice varieties to soil salinity and air humidity: A possible involvement of root-borne ABA. *Plant Soil* 177, 11–19.
- Asch F, Dingkuhn M, Dorffling K and Miezian K 2000 leaf K/Na ratio predicts salinity induced yield loss in irrigated rice. *Euphytica* 113, 109–118.
- Bashar M K and Sarkar H C 1997 Rice genetic resources conservation and utilization in Bangladesh. *In* Proceedings of a national workshop on plant genetic resources, 26–29 August, BARC, Dhaka. Eds. Hossain M G, Arora R K and Mathur P N. pp. 66–70. Bangladesh Agri. Res. Council, Dhaka, Bangladesh.
- Bennett J and Khush G S 2003 Enhancing salt tolerance in crops through molecular breeding: A new strategy. *J. Crop Prod.* 7, 11–65.
- Bohra J S and Dorffling K 1993 Potassium nutrition of rice (*Oryza sativa* L.) varieties under NaCl salinity. *Plant Soil* 152, 299–303.
- Bose M L and Hossain M 2003 Compiled database on area under seasonal rice varieties in Bangladesh 1996/97. Unpublished data collected from Dept. of Agri. Extension, Ministry of Agri., People's Republic of the Govt. of Bangladesh, Dhaka, Bangladesh.
- Bose M L, Isa M A, Bayes A, Sen B and Hossain M 2001 Impact of modern rice varieties on food security and cultivar diversity. *In* Rice Research for Food Security and Poverty Alleviation. Proceedings of the Intl. Rice Res. Conference, 31 March – April 3, 2000. Eds. Peng S and Hardy B, pp. 617–630. Intl. Rice Res. Inst., Los Banos, Philippines.
- BRRI 2003 *In* 'Cultivation of Modern Rice' Bangladesh Rice Res. Inst. publication in Bangla language, pp. 10–18.
- Dice L R 1945 Measures of the amount of ecologic association between species. *Ecology* 26, 297–302.
- Doyle J J and Doyle J L 1990 Isolation of plant DNA from fresh tissue. *Focus* 12, 13–15.
- Elahi C M F, Seraj Z I, Rasul N M, Das K C, Tarique AA, Biswas K, Salam M A, Gomosta A R, Tumimbang E, Adorada D, Gregorio G and Bennett J 2003 Breeding rice for salinity tolerance using the Pokkali allele: finding a linked marker. *In* *In vitro* culture, transformation and molecular markers for crop improvement. Ed. Islam A S. Oxford and IBH, New Delhi. In press.
- Evenson R E and Gollin D 1997 Genetic resources, international organizations and improvement in rice varieties. *Econ. Dev. Cult. Change* 45, 471–500.
- Fuentes J L, Escobar F, Alvarez A, Gallego G, Duque M C, Ferrer M, Deus J E and Tohme J M 1999 Analyses of genetic diversity in Cuban rice varieties using isozyme, RAPD and AFLP markers. *Euphytica* 109, 107–115.
- Flowers T J and Yeo A R 1995 Breeding for salinity resistance in crop plants: where next? *Aust. J. Plant Physiol.* 22, 875–884.
- Gambrell R P 1996 Manganese. *In* Methods of Soil Analysis. Part 3. Chemical Methods. Ed. Sparks D L. pp. 677–678. Soil Science Society of America, Inc. American Society of Agronomy, Inc. Madison, Wisconsin, USA.
- Gregorio G B, Senadhira D and Mendoza R D 1997 Screening rice for salinity tolerance. *In* IRRI discussion paper series no. 22. Intl. Rice Res. Inst., Los Banos, Philippines, pp. 30.
- Gregorio G B, Senadhira D, Mendoza R D, Manigbas N L, Roxas J P and Guerta C Q 2002 Progress in breeding for salinity tolerance and associated abiotic stresses in rice. *Field Crop Res.* 76, 91–101.
- Guevarra E, Loresto G C and Jackson M T 2001 Use of conserved rice germplasm. *Plant Genet. Resour. Newslett.* 124, 51–56.
- Helmke P A and Sparks D L 1996 Lithium, Sodium, Potassium, Rubidium and Cesium *In* Methods of Soil Analysis. Part 3. Chemical Methods. Ed. Sparks D L. pp. 559–560. Soil Science Society of America, Inc. American Society of Agronomy, Inc. Madison, Wisconsin, USA.
- Hoisington D, Khairallah M, Reeves T, Ribaut J-M, Skovmand B, Taba S and Warburton M 1999 Plant genetic resources: What can they contribute toward increased crop productivity? *P. Natl. Acad. Sci. USA* 96, 5937–5943.
- Hossain M 1998 Rice research, technological progress, and the impact on the rural economy: the Bangladesh case. *In* Impact of Rice Research. Eds. Pingali P and Hossain M. pp. 311–342. Thailand Dev. Res. Inst. and Intl. Rice Res. Inst., Los Banos, Philippines.

- IRRI, 1996 Standard evaluation system manual. 4th edition. Intl. Rice Res. Inst., Los Banos, Philippines, 52 pp.
- IRRI, Medium term plan 2002-4. www.irri.org.
- Jaccard P 1908 Nouvelles recherches sur la distribution florale. B. Soc. Vaud. Sci. Nat., 44, 223–270.
- Jackson M T 1999 Managing the world's largest collection of rice genetic resources. In Proceedings of the Intl. Symposium on Rice Germplasm Evaluation and Enhancement. Eds. J N Rutger, J F Robinson, R H Dilday. pp. 22–28. Arkansas Agr. Expt. Station Special Report, Arkansas, USA.
- Karim Z and Iqbal A 2001 Impact of land degradation in Bangladesh. Changing Scenario in Agricultural Land Use. Compilation of data from the geographical information system (GIS) project. Bangladesh Agri. Res. Council publication. 106 pp.
- Keren R 1996 Boron. In Methods of Soil Analysis. Part 3. Chemical methods. Ed. Sparks D L. pp. 610–613. Soil Science Society of America, Inc. American Society of Agronomy, Inc. Madison, Wisconsin, USA.
- Khatun S, Rizzo C A and Flowers T J 1995 Genotypic variation in the effect of salinity on fertility in rice. Plant Soil 173, 239–250.
- Kuo S 1996 Phosphorous. In Methods of Soil Analysis. Part 3. Chemical methods. Ed. Sparks D L. pp. 894–895. Soil Science Society of America, Inc. American Society of Agronomy, Inc. Madison, Wisconsin, USA.
- Lee K-S, Choi W-Y, Ko J-C, Kim T-S and Gregorio G B 2003 Salinity tolerance of japonica and indica (*Oryza sativa* L.) at the seedling stage. Planta 216, 1043–1046.
- Loeppert R H and Inskeep W P 1996 Iron. In Methods of Soil Analysis. Part 3. Chemical Methods. Ed. Sparks D L. pp. 654–657. Soil Science Society of America, Inc. American Society of Agronomy, Inc. Madison, Wisconsin, USA.
- Ludbrook J 2002 Statistical techniques for comparing measures and methods of measurement: A critical review. Clin. Exp. Pharmacol. P. 29, 527–536.
- Mantel N A 1967 The detection of disease clustering and a generalized regression approach. Cancer Res. 27, 209–220.
- Maathuis F J M and Amtmann A 1999 K nutrition and Na toxicity: the basis of cellular K/Na ratios. Ann. Bot. London 84, 123–133.
- McCouch S R, Chen X and Panaud O 1997 Microsatellite mapping and applications of SSLP's in rice genetics and breeding. Plant Mol. Biol. 35, 89–99.
- Mishra B, Singh R K and Senadhira D 2001 Recent advances and future strategies for breeding salt tolerant rice varieties. In Rice Research for Food Security and Poverty Alleviation. Eds. Peng S and Hardy B. pp. 275–284. International Rice Research Institute, Los Banos, Philippines.
- Moradi F, Ismail A M, Gregorio G B and Egdane J A 2003 Salinity tolerance of rice during reproductive development and association with tolerance at the seedling level. Ind. J. Plant Physiol. In Press.
- Nagaraju J, Kathirvel M, Kumar R R, Siddiq E A and Hasnain S E 2002 Genetic analysis of traditional and evolved Basmati and non-basmati rice varieties by using fluorescence-based ISSR-PCR and SSR markers. P. Natl. Acad. Sci. USA 99, 5836–5841.
- Nei M 1973 Analysis of gene diversity in subdivided populations. P. Natl. Acad. Sci. USA 70, 3321–3323.
- Page A L, Miller R H and Keemy D R 1982 Methods of soil analysis. Part 2. Chemical and Microbiological Properties. 2nd Edition, American Society of Agronomy, Soil Science Society of America, Madison USA. pp. 1159.
- Panaud O, Chen X and McCouch S R 1996 Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa* L.). Mol. Gen. Genet. 252, 597–607.
- Panaullah G M 1993 Soil salinity and associated problems in connection with crop production in the coastal regions of Bangladesh. In Proceedings of the Workshop on Coastal Salinity and Crop Production in Bangladesh. pp. 1–30. Bangladesh Rice Research Institute Publication, Dhaka, Bangladesh.
- Parsons B J, Newbury H J, Jackson M T and Ford-Lloyd B V 1997 Contrasting genetic diversity relationships are revealed in rice (*Oryza sativa* L.) using different marker types. Mol. Breeding 3, 115–125.
- Pearson K 1896 Mathematical contributions to the theory of evolution: III Regression, heredity and panmixia. Phil. Trans. Royal Soc. 187, 253–318.
- Ponnamperuma F N 1977 Screening Rice for Tolerance to Mineral Stress. IRRI Research Paper Series no. 6. 21 pp.
- Reed S T and Martens D C 1996 Copper and Zinc. In Methods of Soil Analysis. Part 3. Chemical Methods. pp. 707–708. Ed. Sparks D L. Soil Science Society of America, Inc. American Society of Agronomy, Inc. Madison, Wisconsin, USA.
- Rohlf F J 2000 NTSYSpc. Numerical taxonomy and Multivariate Analysis System. Version 2.11 f. Exeter software. www.exetersoftware.com.
- Senadhira D, Zapata-Arias F J, Gregorio G B, Alejar M S, de la Cruz H C, Padolina T F and Galvez A M 2002 Development of the first salt tolerant rice cultivar through indica/indica anther culture. Field Crop Res. 76, 103–110.
- Singh K N, Nandi R, Shanmugasundaram P, Sadasivan S, Huang N, Brar D S and Khush G S 1999 High resolution DNA fingerprinting of Indian rice (*Oryza sativa* L.) varieties by amplified fragment length polymorphism. Genet. Resour. Crop Eval. 46, 427–433.
- Sneath P H A and Sokal R R 1973 Numerical Taxonomy. Freeman, San Francisco. 573 pp.
- Suarez D L 1996 Beryllium, Magnesium, Calcium, Strontium and Barium. In Methods of Soil Analysis. Part 3. Chemical Methods. Ed. Sparks D L. pp. 583–584. Soil Science Society of America, Inc. American Society of Agronomy, Inc. Madison, Wisconsin, USA.
- Tabatabai M A 1996 Sulfur. In Methods of Soil Analysis. Part 3. Chemical Methods. Ed. Sparks D L. pp. 950–951. Soil Science Society of America, Inc. American Society of Agronomy, Inc. Madison, Wisconsin, USA.
- Tanksley S D and McCouch S R 1997 Seed banks and molecular maps: Unlocking genetic potential from the wild. Science 277, 1063–1066.
- Temnykh S, Park W D, Ayers N, Cartinhour S, Hauck N, Lipovich L, Cho Y G, Ishii T and McCouch S R 2000 Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). Theor. Appl. Genet. 100, 697–712.
- Tester M and Davenport R 2003 Na tolerance and Na transport in higher plants. Ann. Bot-London 91, 503–527.
- Thanh N D, Zheng H G, Dong N V, Trinh L N, Ali M L and Nguyen H T 1999 Genetic variation in root morphology and microsatellite DNA loci in upland rice (*Oryza sativa* L.) from Vietnam. Euphytica 105, 43–51.
- Yoshida S, Forno D A, Cock J H and Gomez K A 1976 Laboratory manual for physiological studies of rice. 3rd edition. International Rice Research Institute, Los Banos, Philippines. 83 pp.

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