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Molecular characterization and genetic diversity of different rice (*Oryza sativa* L.) genotypes for salt tolerance

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Abstract
The present study involved evaluation of 12 genotypes of rice under control salt and field conditions. The characters studied were days to 50% flowering, plant height (cm), panicles bearing tillers/plant, spikelets/panicle, grains/panicle, panicle length, spikelet fertility (%), test weight (g), biological yield/plant (g), harvest index (%), and grain yield/plant (g).After 7 days of salinisation, all the genotypes *viz.,* IR68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127, IR-91167-99-1-1-1-3, IR-91167-133-1-1-2-3, NUD-3, NDR-359, IR-28, FL-478, NUD-2, CSR-13, Ayyar and NDRK-2008 were salinity tolerance. After 14 days of salinisation, FL-478 were tolerant, while IR68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127, IR-91167-133-1-1-2-3, NUD-3, NUD-2 and CSR-13 were moderately tolerant genotypes and NDR-359 were susceptible genotype. After 21 days of salinisation, FL-478 were tolerant genotype, while IR-91167-99-1-1-1-3, NUD-3, NUD-2, CSR-13 and NDRK-2008 were moderately tolerant genotypes and genotypes IR68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127, IR-91167-133-1-1-2-3, NDR-359, IR-28 and Ayyar were susceptible genotype. Under salinity condition at reproductive stages of rice genotypes Ayyar followed by showed highly tolerant to salinity at reproductive stage. The genotype NUD-3, NDRK-2008 and IR-91167-99-1-1-1-3 exhibited highly susceptible to salinity at reproductive stage. The higher magnitude of positive direct effect on grain yield/plant exerted by biological yield/plant and harvest index under control condition, under saline condition biological yield/plant followed by harvest index and spikelets/panicle showed positive and direct effect on grain yield/plant. Under field condition positive and direct effect on grain yield exerted by biological yield/plant followed by harvest index, spikelets/panicle and spikelet fertility. In the present study, the 12 genotypes of rice were grouped into four different non-overlapping clusters under saline, field and control conditions. The highest intra cluster distance was recorded for cluster IV under controlled condition, cluster II under saline condition and cluster III under field condition. Under controlled condition highest inter cluster distance recorded in cluster II and cluster IV. Under saline condition, cluster II and cluster III had highest inter cluster distance. Under field condition, the highest inter cluster distance recorded in cluster I and cluster IV. RM 10772 exhibited polymorphism in 12 rice genotypes, while RM10745 exhibited Monomorphism in 12 rice genotypes.

Keywords: Cluster analysis, genetic diversity, rice genotypes, SSR marker, salt tolerance

Introduction
Rice is one of the most important cereal crops in the world. One in every three persons depends on rice for more than half of their daily food requirement (Khush and Virk, 2000). Rice (*Oryza sativa* L.) is the world's most important crop and more than half of the world's population depends on it for food (calories and protein especially in developing countries) grain. Asia is the largest producer of rice (97%) with an average productivity 3.9 tonnes per hectare. China and India account for about 50% of the world's rice area and 56% of production (Hossain and Pingali, 1998). Rice is a most important cereal crop in India and it contributes about 45% to the cereal production, 41% of the total food grain production and accounts for 20-25 per cent of the agricultural GDP.
Salinity is one of the major abiotic stresses that substantially limiting crop production and reduces the average yield of major crops by more than 50% (Abdallah *et al*., 2016) [1]. The deleterious effects of salt stress on agricultural yield are significant, mainly because crops exhibit slower growth rates, reduced tillering and, over months, reproductive development is affected (Munns *et al*., 2008) [13].

Salinity primarily imposes on plants an osmotic stress and secondarily ion toxicity stress. Excess salt in the soil may adversely affect plant growth either through osmotic inhibition of water uptake by roots or specific ion effects. Specific ion effects may cause direct toxicity or alternatively, the insolubility or competitive absorption of ions may affect plant nutritional balances (Silva *et al*., 2008) [17]. Excess Na + in plant cells directly damages membrane systems and organelles, resulting in plant growth reduction and abnormal development prior to plant death (Quintero *et al*., 2007; Siringam *et al*., 2011) [15, 18]. If excessive amount of salt enters into plant, the concentration of salt will eventually rise to a toxic level in older transpiring leaves causing premature senescence and reduced photosynthetic leaf area of a plant to a level that cannot sustain growth (Rad *et al*., 2012a). Photoinhibition coupled with salinity stress causes serious damage to many cellular and physiological processes including photosynthesis, nutrient uptake, water absorption, root growth, spikelet formation, fertilization of florets, and cellular metabolism, which all obviously lead to yield reduction (Darwish *et al*., 2009) [6]. High salt concentration may lead to plant death and no yield.

Molecular markers have proven useful in both basic and applied research, such as DNA fingerprinting, varietal identification and diversity analysis, phylogenetic analysis, marker assisted breeding and map based cloning of genes in rice (Wu and Tanksley, 1993; Xu 2002) [19, 20]. Rice genome sequencing and comparison of sequence databases of *indica* and *japonica* rice genomes have provided an almost unlimited number of DNA markers such as SSR for high-resolution genetic analysis.

Molecular markers have several advantages over the traditional phenotypic markers. They are 100 percent heritable, not environmentally affected and available in abundant numbers, which increases power of discrimination. A significant progress has been made towards the development and use of molecular marker technology in rice breeding programme. First advancement in molecular markers was the development of Restriction Fragment Length Polymorphism (RFLP) markers (Botstein *et al*., 1980 & Beckmann and Soller, 1986) [4, 3]. Most applications of molecular markers in plants have so far utilized RFLPs and several high genetic maps have been developed for many important crop species (Paterson *et al*., 1991) [14].

Screening of germplasms at seedling stage is readily acceptable as it is based on a simple criterion of selection, it provides rapid screening difficult at vegetative and reproductive stage (Gregorio *et al.*, 1997) [9]. Screening under controlled condition has the benefit of reduced environment effects and the hydroponic system is free of difficulties associated with soil related stress factors. The conventional methods of plant selection for salt tolerance are not easy because of the large effects of the environment and low narrow sense heritability of salt tolerance. This hinders the development of an accurate, rapid and reliable screening technique. The aim of the present study was to screen rice germplasms under salinized and non-salinized conditions and to evaluate microsatellite markers for the identification of salt tolerant genotypes.

Materials and methods

The experimental site was "Student Instructional Farm" and "Net house" of Department of PMB&GE of N.D. University of Agriculture and Technology, Kumarganj, Faizabad.

Experimental materials

A total of 12 rice genotypes were used in the study *viz*., IR-68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127, IR-91167-99-1-1-1-3, IR-91167-133-1-1-2-3, NUD-3, NDR-359, IR-29, FL-478, NUD-2, CSR-13, Ayyar, NDRK-2008.

Screening of rice genotypes for salinity at seedling stage

Screening of rice genotypes at seedling stage (7, 14, and 21 days interval) was carried out in hydroponics system in lab condition in Department of Plant Molecular Biology and Genetic Engineering. Determining the level of salinity tolerance during seedling stages is difficult. Plant height, root length, tillering ability and biomass decrease when affected by salinity. Salinity also reduces panicle length, number of tillers and spikelet per panicle, fertility and panicle weight, thus reducing grain yield (Akbar and Ponnamperuma, 1972) [2].

Preparation of stock solutions

Proper preparation of stock solutions was done to avoid nutrient deficiencies and mineral toxicities, not attributed to salinity stress. It is advisable to prepare fresh stock solutions every two months. The amounts of solution depend on the number of test entries screened during a two-month period. The required amounts of each element for a two-month period, for preparation of 4 litre stock solutions are given in table 3.2. For the macronutrient stock solutions, weighed the required amount of reagent and transferred to a 1000 ml beaker and do initial mixing with about 750 ml distilled water. Mixed the solution in 2 litre volumetric flask, then added distilled water and made up volume to 2 litre. Mixed the solution properly for 15 min using a glass rod, then transfered to stock solution bottle. Preparation of micronutrient stock solution is critical because most nutrient deficiencies and other toxicities could be traced to improper preparation. Thus in micronutrient preparation considerable attention was given. Each reagent of the micronutrient solution listed in table 1 was dissolved separately. Only ferric chloride was dissolved in 100 ml distilled water. Mixed all solutions together by using 2.0 liter capacity volumetric flask. Added the ferric chloride solution to the mixture just before citric acid and stired the mixture for 15 min. Finally, 100 ml sulfuric acid was added to the mixture and volume was made up to 2.0 litre and stored in a dark glass bottle. The final colour of the solution was yellowish brown. All stock solutions was properly labeled and kept in separate.

Table 1: Preparation of stock solution

Element	Reagent (AR grade)	Preparation (g/4 litre solution)	Preparation (g/1 litre solution)
Macronutrient			
N	Ammonium nitrate (NH_4NO_2)	365.6	91.40
P	Sodium phosphate monobasic monohydrate ($NaH_2PO_4H_2O$)	147.4	36.85
K	Potassium sulfate (K_2SO_4)	285.6	71.40
Ca	Calcium chloride dehydrate ($CaCl_2$ $2H_2O$)	469.4	117.35
Mg	Magnesium sulfate 7-hydrate ($MgSO_4$ $7H_2O$)	1296.0	324.0
Micronutrient: Dissolved each reagent separately and mix in 2 litter distilled water then added 200ml H_2SO_4 and make up volume to 4litre			

Mn	Manganese chloride 4-hydrate (MnCl 13-4 H_2O)	8.00	2.0
Mo	Ammonium molybdate 4-hydrate [$(NH_4)_3Mo/O24\ 4H_2O$]	0.295	0.073
Zn	Zinc sulfate 7 hydrate ($ZnSO_4\ 7H_2O$)	0.110	0.027
B	Boric acid (H_2SO_4)	3.736	0.934
Cu	Cupric sulfate 5 hydrate ($CuSO_4\ 5H_2O$)	0.124	0.031
Fe	Ferric chloride 6 hydrate ($FeCl_2\ 6H_2O$)	30.800	7.7
	Citric acid monohydrate	47.600	11.9

***Source*:** Adapted from Yoshida *et al.*, (1976) [21]
Note: For easy handling and storage, hydrate reagents are preferred

Handling of seedlings and salinization
Test seeds have to be heat-treated for 5 days in a convection oven set at 50^0C to break seed dormancy. Proper breaking of the seed dormancy is very essential in this screening technique. Delay in germination of some entries will likely make these entries more sensitive to salt. Seedling vigor has great advantage at this point since salinization occur at very early seedling stage. After breaking the dormancy, surface sterilize seeds with fungicide and rinse well with distilled water. Placed sterilized seeds in petri dishes with moistened filter papers and incubated at 30 ºC for 48 h to germinate. Sowed two pregerminated seeds per hole on the Styrofoam seedling float. The radicle should be inserted through the nylon mesh). Suspend the Styrofoam seedling float on the tray filled with distilled water. There are adequate nutrients in the endosperm for the seedlings to grow normally for 3-4 days. After 3 days, when seedlings are well established, replaced the distilled water with salinized nutrient solution. Initial salinity is at EC = 6 dSm^1. Three days later, increased salinity to EC=12 $dSm^1$by adding NaCl to the nutrient solution. Renew the solution every 8 days and maintain the pH at 5.0 daily.

Table 2: Standard Evaluation Score (SES) of visual salt injury at seedling stages

Score	Observation	Tolerance
1	Normal growth no symptoms on leaves	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
5	Growth severely retarded, most leaves rolled, only few were elongating	Moderately tolerant
7	Complete cessation of growth, most leaves dry, some plants dying	Susceptible
9	Almost all plants dead or dying	Highly susceptible

Screening of rice genotypes at reproductive stage
Reproductive stage salinization was maintained in the net house of Department of Plant Molecular Biology and Genetic Engineering. Three pots (25 cm height and 25 cm diameter) were maintained for each genotype and soil was fertilized with 50:25:25 NPK mg kg^{-1} of soil is used and salinization started from 21 days to maturity stage with NaCl and EC=10 dSm^1, pH=5.0. The observation were recorded on day to 50% flowering, plant height (cm), panicle bearing tillers/plant, panicle length (cm), spikelets/panicle, grains/panicle, spikelet fertility (%), test weight (g), biological yield/plant(g), harvest index (%), grain yield/plant.

Methods for field trail
Rice varieties, under normal condition were transplanted in three replications with three meter row length in each replication with three rows each variety in 20 cm row to row and 15 cm plant to plant spacing in randomized block design (RBD). Observations were recorded on randomly selected five plants from each variety in each replication at maturity. These plants were harvested and threshed separately. The data were recorded on following characters:

1. **Days to 50% flowering:** This was recorded as number of days from the date of sowing to the emergence of 50% panicles.
2. **Plant height (cm):** It was measured in centimeter from the ground level to the tip of main panicle excluding awn at the time of maturity.
3. **Panicle bearing tillers/plants:** The total number of panicle bearing tillers were counted from 5 randomly selected plants and average were taken for each variety.
4. **Panicle length (cm):** It was measured in centimeters from the tip of main panicle at the time of maturity.
5. **Number of spikelets per panicle:** It was determined by counting total number of filled and unfilled grains in each replication.
6. **Number of grain per panicle:** It was determined by counting total number of filled grains from main panicle of five plants in each replication.
7. **Spikelet fertility (%):** Spikelet fertility was calculated by the following formula

$$\text{Spikelets fertility (\%)} = \frac{\text{Number of grains per panicle}}{\text{Number of spikelets per panicle}} \text{X } 100$$

8. **Test weight (g):** Test weight was recorded by taking weight of 1000 matured dried seeds of each genotype in each replication with the help of electronic balance.
9. **Biological yield/plant (g):** All above ground plant parts were cut and sun dried and the biological yield was recorded with the help of top pan balance.
10. **Harvest index (%):** The recovery of grains in total dry matter considered as harvest index was expressed in percentage. It was calculated by the followed formula:

$$\text{Harvest Index (\%)} = \frac{\text{Grain Yield}}{\text{Biological yield}} \text{X } 100$$

11. **Grain yield/plant (g):** For determining this trait grains were threshed from the sample dried in sun and then weighted to determine grain yield**.**

Statistical analysis
The experimental data were compiled by taking mean values over randomly selected plant from three replications and subjected to the following statistical analysis:
1. Estimation of genetic divergence analysis,

Estimation of genetic divergence (D^2)
The genetic divergence of 12 genotypes of rice was worked out using Mahalanobis (1936) [12] D^2 statistics (Rao, 1952) [16].

The 11 characters under saline, control and field condition in rice were included for this analysis:

The calculation of D^2 values involved following steps

1. A set of uncorrelated linear combinations (y's) was obtained by pivotal condensation of the common dispersion matrix (Rao, 1952) [16] of a set of correlated variables (x's). The common dispersion matrix was arranged with the help of error of mean squares and mean sum of products.
2. Using the relationship between y's and x's the mean values of different genotypes for different characters (X_1 to X_{10}) were transformed into the mean values of a set of uncorrelated linear combinations (Y_1 to Y_{10}).
3. The D^2 values between i^{th} and j^{th} genotypes for k^{th} characters is calculated as:

$D^2_{ij} = K (Y_{it}-Y_{jt})^2$
Where, t = 1
The K components were calculated separately and added to get D^2_{ij}.

4. The 'K' components of 'D^2_{ij}' for each combination were ranked in descending order of magnitude.
5. These ranks were added up for each component D^2_{ij} over all combinations of i and j the rank totals were obtained.

Group constellation

The D^2values were arranged in an increasing order of magnitude. The grouping of the strains into different clusters was done using Tocher's method (Rao, 1952) [16]. The two most closely associated groups were chosen and third groups were found which had the smaller average D^2 value from the first two. Similarly, the fourth was chosen to have the smallest average D^2 from the first three and so on. The D^2 value did not fit in with the former group and was, therefore, taken as another cluster.

Intra and inter-cluster distance

The inter-cluster D^2 was calculated as the sum of n (n-1)/2 genotypes within a cluster divided by total number of combinations. All possible D^2 values between the groups of two clusters were added and then divided by n_1 X n_2 for computing inter-cluster distance.

Where, n_1 and n_2 = the number of genotypes in two clusters.

Cluster mean

The cluster mean for the particular character is the summation of mean values of the strains included in a cluster divided by number of strain in the cluster.

DNA extraction, purification and quantification

DNA isolation and quantification: Taken leaves samples ground in liquid nitrogen using sterilized mortar pestle. The powdered mycelium was transferred to a 2 ml Eppendorf tube containing 880 µl of extraction buffer (2% CTAB buffer, 4M NaCl, 0.5M EDTA, 1M Tris-Cl, 0.02% β-Mercaptoethanol). After incubation at 65°C for 1-hour, equal volume of phenol: chloroform: isoamylalcohol (25:24:1) was added and centrifuged @ 12000 rpm for 10 minutes. After proper mixing, transfer the clear supernatant to an Eppendorf tube, an equal volume of chloroform: isoamylalcohol (24:1) was added followed by mixing and centrifuged @ 12000 rpm for 10 minutes.

Then, add chilled absolute alcohol to the supernatant, mix well and keep at -20 °C for 2 hrs. After centrifugation, DNA pellet was washed with 70% ethanol, air dried and resuspended in 100µl TE buffer. The gel electrophoresis and Nanodrop Spectrophotometer were used to determine the quality and quantity of fungal DNA. After quantification, the DNA samples were diluted to a concentration of 30-50 ng/µl for use in PCR reaction

PCR amplification and gel elution: The PCR reaction was performed in 25 µl reaction volume with, 0.5 µM of primers, 10 mM dNTP, 1.5 mM MgCl2, 50 ng of template DNA, 1X *Taq* buffer and 1U of DNA Taq polymerase. The PCR was performed with following parameters: initial denaturation at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 45 sec, annealing at 55 °C for 45 sec, extension at 72 °C for 45 sec followed by a final extension at 72 °C for 10 min. The amplified PCR products were analyzed in gel electrophoresis and documented under UV using gel documentation system

Table 3: Sequence of SSR primer-pairs provided clear amplification in rice genotypes

S. No.	Primer	Sequence	Tm (^{0}C)	Range of amplified allele (bp)
1	RM10772	GCACACCATGCAAATCAATGC(F) CAGAAACCTCATCTCCACCTTCC(R)	56	150-180
2	RM10745	TAGCGAATTGACACACCGAGTACG(F) ACTTCACCGTCGGCAACATGG(R)	55	150-220

Results

Salinity screening at seedling stage

The rice genotypes were screened in the lab condition at pH 5.0 and EC 12 dSm^{-1} in Yoshida (1976) [21] solution. The rice genotypes scored for salinity tolerance at seedling stage based on Standard Evaluation System (SES), 1996 at 7, 14 and 21 days after salinization (Table 4.1). The data revealed that IR68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127, IR-91167-99-1-1-1-3, IR-91167-133-1-1-2-3, NUD-3, NDR-359, IR-28, FL-478, NUD-2, CSR-13, Ayyar, and NDRK-2008 rice genotypes exhibited salinity tolerant with score of 3 at 7 days. At 14 days after salinization genotypes, FL-478 were found tolerant, while IR68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127, IR-91167-133-1-1-2-3, NUD-3, NUD-2 and CSR-13 were recorded moderately tolerant and genotype NDR-359 showed susceptibility to salinity. At 21 days after salinization, FL-478 were found tolerant, while IR-91167-99-1-1-1-3, NUD-3, NUD-2, CSR-13 and NDRK-2008 showed moderately tolerant to salinity and genotypes IR68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127, IR-91167-133-1-1-2-3, NDR-359, IR-28 and Ayyar showed susceptible to salinity (Table 4).

Table 4: Salinity score at seedling stage in lab condition

S. No.	Varieties	Salinity Score		
		7 Days	14 Days	21 Days
1	IR-68144-2B-2-2-3-1-120	3	5	7
2	IR-68144-2B-2-2-3-1-127	3	5	7
3	IR-91167-99-1-1-1-3	3	5	5
4	IR-91167-133-1-1-2-3	3	5	7
5	NUD-3	3	5	5
6	NDR-359	3	7	7
7	IR-29	3	5	7
8	FL-478	3	3	3
9	NUD-2	3	5	5
10	CSR-13	3	5	5
11	Ayyar	3	5	7
12	NDRK-2008	3	5	5

Salinity screening at reproductive stage

Rice genotypes IR-68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127, IR-91167-99-1-1-1-3, IR-91167-133-1-1-2-3, NUD-3, NDR-359, IR-29, FL-478, NUD-2, CSR-13, Ayyar, NDRK-2008 were screened in a randomized complete block design with three replications in net house of PMB & GE department during 2017 with EC=12 dS m^{-1} and pH=5 The pots size was 25 cm height and 25 cm diameter to provide good condition.

A total of twelve (variety 12 × replication 3 for each) experimental pots were prepared for both salinity and controlled conditions. Pots filled with fertilized soil, 50:25:25 N, P, K mg kg^{-1} was used and three seedlings in each pot was transplanted and maintained further for study. The control pot was irrigated with tap water and the experimental plant was irrigated with tap water with NaCl treatment. The salinization was started from 21 days to maturity stage of rice genotypes with NaCl at EC=12 dS m^{-1} and pH=5.

On the basis of grain yield per plant under salinity condition at reproductive stages of rice genotypes Ayyar (20.75) followed by FL-478 (15.77) showed superior mean performance and exhibited highly tolerant to salinity at reproductive stage. The genotype NUD-3, NDRK-2008 and IR-91167-99-1-1-1-3 exhibited highly susceptible to salinity at reproductive stage.

Genetic divergence analysis

The study of genetic divergence among the 12 rice genotypes was carried out by using Mahalanobis D^2 statistics as described by Rao (1952) [16].

The clustering pattern for 12 genotypes under controlled condition was grouped into four non-overlapping clusters (Table 5a). Cluster II having highest number of genotypes namely, IR-68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127, IR-91167-99-1-1-1-3, IR-91167-133-1-1-2-3 and NDR-359. Cluster I having three genotypes namely, NUD-3, IR-29 and NDRK-2008. Cluster III and IV having two genotypes in each namely, FL-478 and NUD-2 in cluster III and CSR-13, Ayyar in cluster IV.

The clustering pattern for 12 genotypes under saline condition was grouped into four non-overlapping clusters (Table 5b). Cluster I having four genotypes namely, IR-68144-2B-2-2-3-1-127, IR-91167-99-1-1-1-3, IR-91167-133-1-1-2-3 and NDRK-2008. Cluster II also having four genotypes namely, NDR-359, NUD-2, CSR-13 and Ayyar. Cluster III also having two genotypes namely, IR-68144-2B-2-2-3-1-120 and FL-478. Cluster IV also having two genotypes namely, NUD-3 and IR-29.

The clustering pattern for 12 genotypes under field condition was grouped into four non-overlapping clusters (Table 5c). Cluster IV having highest number of genotypes namely, NUD-3, CSR-13, Ayaar and NDRK-2008. Cluster III having three genotype namely, IR-68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127 and IR-91167-133-1-1-2-3. Cluster I having three genotypes namely, IR-91167-99-1-1-1-3, FL-478 and NUD-2. Cluster II having two genotypes namely, NDR-359 and IR-29.

Table 5(a): Clustering pattern of rice genotype on the basis of D^2 analysis for 11 characters in control condition

Cluster	No. Genotypes	Name of varieties
Cluster I	3	NUD-3, IR-29, NDRK-2008
Cluster II	5	IR-68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127, IR-91167-99-1-1-1-3, IR-91167-133-1-1-2-3, NDR-359
Cluster III	2	FL-478, NUD-2
Cluster IV	2	CSR-13, Ayyar

Table 5(b): Clustering pattern of rice genotype on the basis on D^2 analysis for 11 characters in saline condition

Cluster	No. Genotypes	Name of varieties
Cluster I	4	IR-68144-2B-2-2-3-1-127, IR-91167-99-1-1-1-3, IR-91167-133-1-1-2-3, NDRK-2008
Cluster II	4	NDR-359, NUD-2, CSR-13, Ayyar
Cluster III	2	IR-68144-2B-2-2-3-1-120, FL-478
Cluster IV	2	NUD-3, IR-29

Table 5(c): Clustering pattern of rice genotype on the basis on D^2 analysis for 11 characters in field condition

Cluster	No. Genotypes	Name of varieties
Cluster I	3	IR-91167-99-1-1-1-3, FL-478, NUD-2
Cluster II	2	NDR-359, IR-29
Cluster III	3	IR-68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127, IR-91167-133-1-1-2-3
Cluster IV	4	NUD-3, CSR-13, Ayyar, NDRK-2008

Cluster distance

The estimates of intra and inter-cluster distances represented by D^2 under control, salt and field conditions are given in table 6 (a), 6 (b) and 6(c) respectively. The intra cluster distance under controlled condition ranged from 89.89 (cluster III) to 289.04 (cluster IV). The highest intra cluster distance was recorded for cluster IV (289.04) and followed by cluster II (220.83). The highest inter cluster distance recorded in cluster II and cluster IV (654.72) followed by cluster III

and cluster IV (448.34). The intra cluster distance under saline condition ranged from 79.56 (cluster I) to 171.44 (cluster II). The highest intra cluster distance was recorded for cluster II (171.44) and followed by cluster IV (152.68). The highest inter cluster distance recorded in cluster II and cluster III (683.78) followed by cluster I and cluster III (589.24). The intra cluster distance under field condition ranged from 0.00 (cluster IV) to 284.42 (cluster III). The highest intra cluster distance was recorded for cluster III followed by cluster II (234.84). The highest inter cluster distance recorded in cluster I and cluster IV (3249.81) followed by cluster II and cluster IV (1649.68)

Table 6(a): Estimation of average inter cluster D^2 value under controlled conditions

	1 Cluster	2 Cluster	3 Cluster	4 Cluster
1 Cluster	152.08	276.33	389.19	401.40
2 Cluster		220.83	360.34	654.72
3 Cluster			89.89	448.34
4 Cluster				289.04

Table 6(b): Estimation of average inter cluster D^2 value under saline conditions

	1 Cluster	2 Cluster	3 Cluster	4 Cluster
1 Cluster	79.56	216.54	589.24	495.17
2 Cluster		171.44	683.78	481.93
3 Cluster			134.72	385.89
4 Cluster				152.68

Table 6(c): Estimation of average inter cluster D^2 value under field conditions

	1 Cluster	2 Cluster	3 Cluster	4 Cluster
1 Cluster	172.02	476.97	1547.84	3249.81
2 Cluster		234.84	744.71	1694.68
3 Cluster			284.42	757.16
4 Cluster				0.00

Table 6.1(a): Cluster mean of 12 rice germplasm under controlled condition

Characters	Days to 50% flowering	Plant height (cm)	Panicle bearing tiller/ plant	Panicle length (cm)	Spikelets /panicle	Grains / panicle	Spikelet fertility (%)	Test weight (g)	Biological yield/plant(g)	Harvest index (%)	Grain yield/plant(g)
Cluster I	97.00	79.86	8.56	21.27	128.49	121.66	94.68	21.40	34.12	47.50	16.18
Cluster II	85.17	81.66	6.13	22.73	116.20	109.35	93.95	20.42	31.72	47.30	15.04
Cluster III	90.33	79.43	7.96	21.68	89.66	82.23	91.63	21.95	43.80	40.29	17.89
Cluster IV	99.22	92.34	5.99	23.02	115.08	104.06	90.17	27.17	36.37	43.77	15.86

Table 6.1(b): Cluster mean of 12 rice germplasm under saline condition

Characters	Days to 50% flowering	Plant height (cm)	Panicle bearing tiller/ plant	Panicle length (cm)	Spikelets /panicle	Grains / panicle	Spikelet fertility (%)	Test weight (g)	Biological yield/plant(g)	Harvest index (%)	Grain yield/plant (g)
Cluster I	104.00	69.39	5.97	19.29	121.98	112.42	92.25	18.52	29.06	39.95	11.59
Cluster II	101.89	83.80	4.34	20.55	107.78	98.95	92.12	23.79	31.94	37.16	11.75
Cluster III	85.17	72.21	4.87	20.40	110.84	101.65	90.78	17.68	26.99	42.93	11.61
Cluster IV	87.00	77.15	5.74	19.38	80.23	74.65	93.05	18.94	37.94	38.15	14.72

Table 6.1(c): Cluster mean of 12 rice germplasm under field condition

Characters	Days to 50% flowering	Plant height (cm)	Panicle bearing tiller/ plant	Panicle length (cm)	Spikelets/ panicle	Grains / panicle	Spikelet fertility (%)	Test weight (g)	Biological yield/plant(g)	Harvest index (%)	Grain yield/plant(g)
Cluster I	95.78	72.64	13.51	23.74	139.01	127.85	92.05	22.04	30.52	44.86	13.79
Cluster II	102.93	81.81	10.21	22.69	105.81	98.09	92.83	24.54	40.99	42.04	17.29
Cluster III	103.78	98.64	9.39	25.07	140.76	127.07	90.28	27.91	35.98	44.75	16.07
Cluster IV	106.33	92.44	6.87	21.11	86.44	79.26	91.70	33.09	43.58	45.33	19.76

Cluster Mean

Estimates of cluster mean under controlled, saline and field conditions are given in table 4.7.2(a), 4.7.2(b) and 4.7.2 (c), respectively

1. In control condition, the highest cluster mean for days to 50% flowering was recorded in case of cluster IV (99.22) followed by cluster I (97.00). The lowest cluster mean for days to 50% flowering was found in case of cluster II (85.17). In salinity condition, the highest cluster mean for days to 50% flowering was recorded in case of cluster I (104.00) followed by cluster II (101.89). The lowest cluster mean for days to 50% flowering was found in case of cluster III (85.17). In field condition, the highest cluster mean for days to 50% flowering was recorded in case of cluster IV (106.33) followed by cluster III (103.78). The lowest cluster mean for days to 50% flowering was found in case of cluster I (95.78).
2. In control condition, the highest cluster mean for plant height was recorded in case of cluster IV (92.34) followed by cluster II(81.66). The lowest cluster mean

for plant height was found in case of cluster III (79.43). In salinity condition, the highest cluster mean for plant height was recorded in case of cluster II (83.80) followed by cluster IV (77.15). The lowest cluster mean for plant height was found in case of cluster I (69.39). In field condition, the highest cluster mean for plant height was recorded in case of cluster III (98.64) followed by cluster IV (92.44). The lowest cluster mean for plant height was found in case of cluster I (72.64).

3. In control condition, the highest cluster mean for panicle bearing tillers per plant was recorded in case of cluster I (8.56) followed by cluster III (7.96). The lowest cluster mean for panicle bearing tillers per plant was found in case of cluster IV (5.99). In salinity condition, the highest cluster mean for panicle bearing tillers per plant was recorded in case of cluster I (5.97) followed by cluster IV (5.74). The lowest cluster mean for panicle bearing tillers per plant was found in case of cluster II (4.34). In field condition, the highest cluster mean for panicle bearing tillers per plant was recorded in case of cluster I (13.51) followed by cluster II (10.21). The lowest cluster mean for panicle bearing tillers per plant was found in case of cluster IV (6.87).
4. In control condition, the highest cluster mean for panicle length was recorded in case of cluster IV (23.02) followed by cluster II (22.73). The lowest cluster mean for panicle length was found in case of cluster I (21.27). In salinity condition, the highest cluster mean for panicle length was recorded in case of cluster II (20.55) followed by cluster III (20.40). The lowest cluster mean for panicle length was found in case of cluster I (19.29). In field condition, the highest cluster mean for panicle length was recorded in case of cluster III (25.07) followed by cluster I (23.74). The lowest cluster mean for panicle length was found in case of cluster IV (21.11).
5. In control condition, the highest cluster mean for spikelets per panicle was recorded in case of cluster I (128.49) followed by cluster II (116.20). The lowest cluster mean for spikelets per panicle was found in case of cluster III (89.66). In salinity condition, the highest cluster mean for spikelets per panicle was recorded in case of cluster I (121.98) followed by cluster III (110.84). The lowest cluster mean for spikelets per panicle was found in case of cluster IV (80.23). In field condition, the highest cluster mean for spikelets per panicle was recorded in case of cluster III (140.76) followed by cluster I (139.01). The lowest cluster mean for spikelets per panicle was found in case of cluster IV (86.44).
6. In Control condition, the highest cluster mean for grains per panicle was recorded in case of cluster I (121.66) followed by cluster II (109.35). The lowest cluster mean for grains per panicle was found in case of cluster II (82.23). In salinity condition, the highest cluster mean for grains per panicle was recorded in case of cluster I (112.42) followed by cluster III (101.65). The lowest cluster mean for grains per panicle was found in case of cluster IV (74.65). In field condition, the highest cluster mean for grains per panicle was recorded in case of cluster I (127.85) followed by cluster III (127.07). The lowest cluster mean for grains per panicle was found in case of cluster IV (79.26).
7. In control condition, the highest cluster mean for spikelets fertility was recorded in case of cluster I (94.68) followed by cluster II (93.95). The lowest cluster mean for spikelets fertility was found in case of cluster IV (90.17). In salinity condition, the highest cluster mean for spikelets fertility was recorded in case of cluster IV (93.05) followed by cluster I (92.25). The lowest cluster mean for spikelets fertility was found in case of cluster III (90.78). In field condition, the highest cluster mean for spikelets fertility was recorded in case of cluster II (92.83) followed by cluster I (92.05). The lowest cluster mean for spikelets fertility was found in case of cluster III (90.28).
8. In Control condition, the highest cluster mean for test weight was recorded in case of cluster IV (27.17) followed by cluster III (21.95). The lowest cluster mean for test weight was found in case of cluster II (20.42). In salinity condition, the highest cluster mean for test weight was recorded in case of cluster II (23.79) followed by cluster IV (18.94). The lowest cluster mean for test weight was found in case of cluster III (17.68). In field condition, the highest cluster mean for test weight was recorded in case of cluster IV (33.09) followed by cluster III (27.91). The lowest cluster mean for test weight was found in case of cluster I (22.04).
9. In Control condition, the highest cluster mean for biological yield per plant was recorded in case of cluster III (43.80) followed by cluster IV (36.37). The lowest cluster mean for biological yield per plant was found in case of cluster II (31.72). In salinity condition, the highest cluster mean for biological yield per plant was recorded in case of cluster IV (37.94) followed by cluster II (31.94). The lowest cluster mean for biological yield per plant was found in case of cluster III (26.99). In field condition, the highest cluster mean for biological yield per plant was recorded in case of cluster IV (43.58) followed by cluster II (40.99). The lowest cluster mean for biological yield per plant was found in case of cluster I (30.52).
10. In control condition, the highest cluster mean for harvest index was recorded in case of cluster I (47.50) followed by cluster II (47.30). The lowest cluster mean for harvest index was found in case of cluster III (40.29). In salinity condition, the highest cluster mean for harvest index was recorded in case of cluster III (42.93) followed by cluster I (39.95). The lowest cluster mean for harvest index was found in case of cluster II (37.16). In field condition, the highest cluster mean for harvest index was recorded in case of cluster IV (45.33) followed by cluster I (44.86). The lowest cluster mean for harvest index was found in case of cluster II (42.04).
11. In Control condition, the highest cluster mean for grain yield per plant was recorded in case of cluster III (17.89) followed by cluster I (16.18). The lowest cluster mean for grain yield per plant was found in case of cluster II (15.04). In salinity condition, the highest cluster mean for grain yield per plant was recorded in case of cluster IV (14.72) followed by cluster II (15.75). The lowest cluster mean for grain yield per plant was found in case of cluster I (11.59). In field condition, the highest cluster mean for grain yield per plant was recorded in case of cluster IV (19.76) followed by cluster II (17.29). The lowest cluster mean for grain yield per plant was found in case of cluster I (13.79).

Cluster contribution

The highest per cent contribution in genetic divergence in rice genotypes under controlled condition was recorded by test weight (27.27) followed by spikelets/panicle (24.24), days to

50% flowering and harvest index (12.12), spikelet fertility and biological yield/plant (9.09). Under saline condition the maximum per cent contribution in genetic divergence observed by days to 50% flowering (48.48) followed by spikelets/panicle (15.15), biological yield/plant, grain yield/plant (12.12) and test weight (10.61). The highest per cent contribution in genetic divergence in rice genotypes under field condition was recorded by test weight (69.70) followed by spikelets /panicle (13.64), harvest index (9.09) and grains/panicle (6.06).

Table 7(a): Contribution under controlled condition

Source	Contribution%	Time Ranked 1st
Days to 50% flowering	12.12	8.00
Plant height	1.52	1.00
Panicle bearing tillers/plant	0.01	0.00
Panicle length (cm)	0.01	0.00
Spikelets/panicle	24.24	16.00
Grains/panicle	0.01	0.00
Spikelet fertility (%)	9.09	6.00
Test weight (g)	27.27	18.00
Biological yield/plant(g)	9.09	6.00
Harvest index (%)	12.12	8.00
Grain yield/plant(g)	4.55	3.00

Table 7(b): Contribution under saline condition

Source	Contribution%	Time Ranked 1st
Days to 50% flowering	48.48	32.00
Plant height	1.52	1.00
Panicle bearing tillers/plant	0.01	0.00
Panicle length (cm)	0.01	0.00
Spikelets/panicle	15.15	10.00
Grains/panicle	0.01	0.00
Spikelet fertility (%)	0.01	0.00
Test weight (g)	10.61	7.00
Biological yield/plant(g)	12.12	8.00
Harvest index (%)	0.01	0.00
Grain yield/plant(g)	12.12	8.00

Table 7(c): Contribution under field condition

Source	Contribution%	Time Ranked 1st
Days to 50% flowering	0.01	0.00
Plant height	1.52	1.00
Panicle bearing tillers/plant	0.01	0.00
Panicle length (cm)	0.01	0.00
Spikelets/panicle	13.64	9.00
Grains/panicle	6.06	4.00
Spikelet fertility (%)	0.01	0.00
Test weight (g)	69.70	46.00
Biological yield/plant(g)	0.01	0.00
Harvest index(%)	9.09	6.00
Grain yield/plant(g)	0.01	0.00

Molecular analysis for rice genotype

Fig 1 showed PCR amplification with primer RM 10772. (Fig-1) showed that bands exhibited by IR-68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127, IR-91167-99-1-1-1-3, IR-91167-133-1-1-2-3, NUD-3, NDR-359, IR-29 and FL-478 are in range of 150-160bp. NUD-2, CSR-13, Ayyar and NDRK-2008 showed dissimilar banding pattern and it had size 120bp, 100bp, 80bp and 70bp, respectively. Thus primer RM 10745 was polymorphic. Fig 2 showed PCR amplification with primer RM 10745. (Fig-2) showed that bands recorded All the band seen in IR-68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127, IR-91167-99-1-1-1-3, IR-91167-133-1-1-2-3, NUD-3, NDR-359, IR-29, FL-478, NUD-2, CSR-13, Ayyar and NDRK-2008 are in range of 210-220bp. Thus primer RM 10745 was monomorphic.

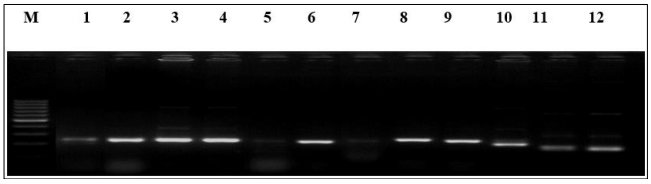

Fig 1: SSR RM10772

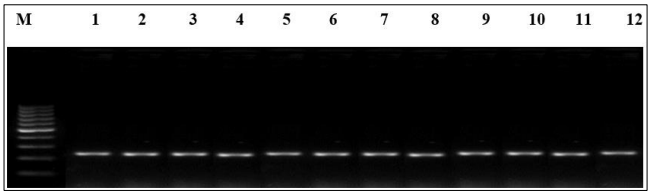

Fig 2: SSR RM-10745

M- Marker

1. IR-68144-2B-2-2-3-1-120, 2. IR-68144-2B-2-2-3-1-127, 3. IR-91167-99-1-1-1-3, 4. IR-91167-133-1-1-2-3, 5. NUD-3, 6. NDR-359, 7. IR-29, 8. FL-478, 9. NUD-2, 10. CSR-13, 11. Ayyar, 12. NDRK-2008

Discussion

The characters studied were days to 50% flowering, plant height (cm), panicles bearing tillers/plant, spikelets/panicle, grains/panicle, panicle length (cm), spikelet fertility (%), test weight (g), biological yield/plant (g), harvest index (%), and grain yield/plant (g). The nature of associations among different characters was studied by using genetic divergence by D^2 statistics as suggested by Mahalanobis, 1936 [12] and stability analysis by Eberhart and Russell model. The salinity scoring for 12 rice genotypes was done at pH 5.0 and EC 12 dSm^{-1}, under controlled environmental condition at seedling and reproductive stages using a visual score 1 to 9 of SES, 1996 (Table 4.1). The data revealed that IR68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127, IR-91167-99-1-1-1-3, IR-91167-133-1-1-2-3, NUD-3, NDR-359, IR-28, FL-478, NUD-2, CSR-13, Ayyar and NDRK-2008 rice genotypes exhibited salinity tolerance with score of 1 and 3 at 7 days. At 14 days of salinization, FL-478 were found tolerant, while IR68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127, IR-91167-133-1-1-2-3, NUD-3, NUD-2 and CSR-13 were recorded moderately tolerant and NDR-359 were found susceptible. At 21 days after salinization, FL-478 were found tolerant, while IR-91167-99-1-1-1-3, NUD-3, NUD-2, CSR-13 and NDRK-2008 showed moderately tolerant to salinity and genotypes IR68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127, IR-91167-133-1-1-2-3, NDR-359, IR-28 and Ayyar were found susceptible. The similar kind of work was also recorded by Yoshida *et al.*, (2002). On the basis of grain yield per plant under salinity condition at reproductive stages of rice genotypes Ayyar followed by showed highly tolerant to salinity at reproductive stage. The genotype NUD-3, NDRK-2008 and IR-91167-99-1-1-1-3 exhibited highly susceptible to salinity at reproductive stage.

The importance of genetic divergence in plant breeding has been emphasized by several scientists (Griffing and Lindstrom, 1954) [10]; Hawkas (1981) [11]. Mahalanobis D^2 statistic has been utilized by several workers for the assessment of genetic divergence in different crops. In the present study, the 12 genotypes of rice were grouped into four

different non-overlapping clusters under control, saline and field conditions. This showed that genotypes in different clusters are genetically variable, which may provide basis for consideration in hybridization programmes. Under controlled condition, cluster II having highest number of genotypes and highest intra cluster distance was recorded for cluster IV followed by cluster II. The highest inter cluster distance recorded in cluster II and cluster IV. Test weight showed maximum cluster contribution followed by spikelets/panicle and days to 50% flowering. Under saline condition, cluster I having highest number of genotypes and highest intra cluster distance was recorded for cluster II followed by cluster IV. The highest inter cluster distance recorded in cluster II and cluster III. Maximum per cent contribution in genetic divergence observed in days to 50% flowering showed highest cluster contribution followed by spikelets/panicle, biological yield/plant and grain yield/plant. Under field condition, cluster IV having highest number of genotypes and highest intra cluster distance was recorded for cluster III followed by cluster II. The highest inter cluster distance recorded in cluster I and cluster IV. Test weight showed highest cluster contribution followed by spikelets/panicle, and harvest index. Chand *et al*. (2005) [5] and Devi *et al.* (2006) [7] also reported similar result in their studies.

Development of salt tolerant rice variety is very difficult through conventional breeding method. Application of molecular (DNA markers) techniques along with conventional approach is only option for improvement of abiotic stresses. For molecular analysis genomic DNA was isolated from each genotypes. The isolated DNA were scanned with the SSR markers to find out the DNA markers associated with salt tolerance. The genotypes were screened with SSR markers, RM 10772 and RM 10745. After screening with RM 10772, the genotypes IR-68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127, IR-91167-99-1-1-1-3, IR-91167-133-1-1-2-3, NUD-3, NDR-359, IR-29 and FL-478 showed similar banding pattern, while NUD-2, CSR-13, Ayyar and NDRK-2008 showed dissimilar banding pattern. Thus present study primer RM 10745 was polymorphic. After screening with RM 10745, all the genotypes *viz.* IR-68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127, IR-91167-99-1-1-1-3, IR-91167-133-1-1-2-3, NUD-3, NDR-359, IR-29, FL-478, NUD-2, CSR-13, Ayyar and NDRK-2008 shows similar banding pattern. Thus present study primer RM 10745 was monomorphic.

References

1. Abdallah MMS, Abdelgawad ZA, ElBassiouny HMS. Alleviation of the adverse effects of salinity stress using trehalose in two rice varieties. South African Journal of Botany 2016;103:275-282.
2. Akbar M, Ponnamperuma FN. Saline soils of south and south east Asia as potential land. IRRI Los Banos Laguna, Philippines 1972.
3. Beckmann JS, Soller M. Restriction fragment length polymorphisms in plant genetic improvement. Plant Mol. and Cell Bio 1986;3:197-250.
4. Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American J Human Genet 1980;32:314-333.
5. Chand SP, Roy SK, Mandal GS, Mahto PD, Panda S, Sarkar G *et al*. Genetic variability and character association in rainfed lowland aman paddy (*O. sativa* L.) Environment and Ecology 2005;22(2):430-434.
6. Darwish E, Testerink C, Khalil M, El-Shihy O, Munnik T. Phospholipid signaling responses in salt-stressed rice leaves. Plant and Cell Physiology 2009;50:986-997.
7. Devi SL, Dass A, Pandey MK, Kole CR. Depiction of genetic diversity in rice. Crop Res 2006;32(3):459-491.
8. Eberhart SA, Russell WA. Stability parameters for comparing varieties. Crop Sci 1966;6:36-40.
9. Gregario GB. Tagging salinity tolerance genes in rice using amplified fragment length polymorphism (AFLP), Ph.D. Thesis University of Philippines Los Banos Laguna Philippines 1997.
10. Griffing B, Lindstrom EW. A study of the combining ability of corn hybrids having varying proportions of corn belt and non-corn belt germplasm. Agron. J 1954;46:545-552.
11. Hawkes JG. Germplasm collection, preservation and use. In plant breeding II, eds. K.J. Frey, Iowa State. Univ. Press., Iowa 1981, 57-84.
12. Mahalanobis. D^2 and metroglyph analysis in mid duration genotypes of rice. Agric. Sci. J 1936;56(3):151-156.
13. Munns R, Tester M. Mechanisms of salinity tolerance. Annual Review of Plant Biology 2008;59:651-681.
14. Paterson AH, Tanksley SD, Sorells ME. DNA markers in plant improvement, Adv. in Agron 1991;46:39-90.
15. Quintero JM, Fournier JM, Benlloch M. Na + accumulation in shoot is related to water transport in K + -starved sunflower plants but not in plants with a normal K + status. Journal of Plant Physiology 2007;164:60-67.
16. Rao CR. Advanced statistical method in biometrical research. Edn. John wiley and sons, New York 1952, 390.
17. Silva C, Martinez V, Carvajal M. Osmotic versus toxic effects of NaCl on pepper plants. Biologia Plantarum 2008;52(1):72-79.
18. Siringam K, Juntawong N, Cha-um S, Kirdmanee C. Salt stress induced ion accumulation, ion homeostasis, membrane injury and sugar contents in saltsensitive rice (*Oryza sativa* L. spp. indica) roots under is oosmotic conditions. African Journal of Biotechnology 2011;10:1340-1346.
19. Wu KS, Tanksley SD. Abundance, polymorphism and genetic mapping of microsatellites in rice. Mol. Gen. Genet 1993;241:225-235.
20. Xu Y. Global view of QTL: rice as a model In: quantitative genetics, genomics and plant breeding (ed Kang, M.S.), Walling ford (UK): CAB International 2002, 109-134.
21. Yoshida S, Forno AD, Cock JH, Gomez KA. Laboratory manual for physiological studies of rice. The International Rice Res. Institute, Los Banos, Philippines 1976.