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RESEARCH ARTICLE

GENETIC DIVERSITY ASSESMENT USING MOLECULAR MARKERS AND DROUGHT TOLERANCE TRAITS IN RICE ACCESSIONS

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ABSTRACT

Genetic diversity is pre-requisite for any crop improvement program as it helps in the development of superior recombinants and selection of parents with wider variability for different traits. Therefore, there is a need to diversify the genetic base of improved rice cultivars. To this end, 30 rice genotypes comprising both drought tolerant and susceptible were screened with 50 rice microsatellite markers to estimate the genetic diversity of the genotypes. A total of 123 alleles were detected using 46 primer pairs with an average of 2.7 alleles per microsatellite locus, which was sufficient to classify the rice accessions investigated. In addition, they were also screened for drought tolerance using PEG-induced stress and data of root and shoot related traits viz., Root length, Root dry weight, Shoot length, Shoot dry weight, Root shoot weight ratio, Relative shoot length, Relative root length, Relative shoot dry weight, Relative root dry weight, and Relative root-shoot weight ratio were recorded. The genetic diversity of the rice genotypes was estimated using both molecular markers as well as drought related traits. The result from the present work is expected to give more insights in selection of parents for development of drought tolerant varieties.

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INTRODUCTION

Rice (*Oryza sativa L.*) is a major food crop nourishing the world in general and Asians in particular, 90% of the world's rice is produced and consumed in Asia. Rice accounts for 23% of calorific value worldwide (Ashikari *et al*., 1999) and is grown under a wide range of agro□ecological conditions ranging from irrigated to rainfed lowland, upland, deep water and tidal conditions. 45% of the total cereal production in India is occupied by rice and is the primary food source for 60% of its population (Siddiq, 2002). Global rice demand is expected to rise from 439 million tons in 2010 to 496 million tons in 2020, to 555 million tons in 2035.This increased demand will have to be met from less land, with less water and less labour. To meet the challenge of producing excess rice from available resources, varieties / hybrids performing better under adverse conditions and with high yield potential have to be produced (Khush, 2005). Genetic diversity is pre-requisite for any crop improvement program as it helps in the development of superior recombinants (Manonmani and Fazlullah khan, 2003) and plays an important role in selection of parents having wider variability for different traits (Nayak *et al*., 2004). Rice is one of the very few crop species endowed with rich genetic diversity, more than one hundred thousand landraces and improved varieties along with a few thousand accessions of wild/weedy species are available in the gene banks. However, it still remains unknown the level of existence of useful genetic diversity, especially for complex traits like yield and adaptability to abiotic stresses. The present day breeders depend on not more than 15% of the total available variability and the remaining over 85 per cent of the genetic variation remain still untapped in the landraces and wild/weedy relatives (Wang *et al*., 2006). Analysis of genetic diversity in germplasm can facilitate in identifying diverse parental combinations to create segregating progenies with maximum genetic variability (Barrett and Kidwell, 1998) and introgression of desirable genes from diverse germplasm into the available genetic base (Thompson *et al*., 1998). Molecular marker technology serves as a powerful tool in the assessment of genetic relationships within and among species, in which differences among accessions can be revealed at the DNA level (Junjian Ni *et al*., 2002; Chakravarthi and Naravaneni, 2006). The co-dominant characteristics of the microsatellite markers and their well known map positions on the rice genome reveal high polymorphisms among different plants (Chen *et al*., 1997; Temnykh *et al*., 2000; Garcia *et al*., 2004). Droughts being the most devastating environmental stress, efforts have been all through to improve the crop productivity under water□limiting conditions. Root and shoot systems affect growth and development of seedlings. In rice, several root characteristics viz., root length, depth of rooting, root

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thickness and root to shoot ratio are considered to play an important role in resisting water deficit. Earlier studies showed varieties with longer, thicker and bigger root systems are to be drought resistant and associated, in some cases, with higher grain yield under drought (Fukai and Cooper, 1995). Drought is a major limitation for rice production in rainfed ecosystems. Evenson *et al*. (1996) estimated global rice yield lost to drought to be 18 million tonnes annually or 4 per cent of total rice production, which was valued conservatively at US$ 3.6 billion. In Asia, about 15% of rice production area experiences frequent yield loss due to drought (Widawsky and O'Toole, 1990). The genetic variation, as identified by morphological traits and molecular markers, may be useful in breeding for abiotic stress.

MATERIALS AND METHODS

Plant Material and DNA Isolation

30 genotypes were used in the present study which comprised of drought tolerant, drought susceptible varieties and NERICA (New Rice for Africa) Lines. Varieties used in the study were listed in table-1. DNA was isolated from leaf samples of 20 days old seedlings of the rice genotypes grown in petri dishes lined with moist filter paper under room temperature following the CTAB (Cetyl Tri methyl Ammonium Bromide) method (Murray and Thompson 1980). The purity and concentration of the isolated genomic DNA samples were estimated by UV-absorption spectrophotometer (Evolution-201 Spectrophotometer, Thermo Scientific) as per the procedure described by Sambrook. Quantification of DNA was done by analyzing the purified DNA on 0.8% agarose gel with lambda (λ) Hind III DNA as standard. Based on the intensity and thickness of genomic DNA bands, as compared to lambda (λ) Hind III DNA, the concentration and quality of DNA in individual samples were determined.

SSR Marker Analysis

Fifty rice microsatellite markers distributed over the 12 chromosomes were chosen for estimation of genetic diversity. The isolated DNA samples were amplified by PCR technique with 10 µl reaction containing 20 ng of DNA, 10 mM Tris–HCl, 1.5 mM MgCl2, 0.5 unit of Taq DNA polymerase, 50 µM of dNTPs and 0.1 µM each of forward and reverse primers using T100 (Bio-Rad, USA) thermal cycler. Thermal cycler reaction was performed as mentioned: an initial denaturation at 94 ºC for 10 min, the mixture was cycled 35 times at 94 ºC for 45 sec for denaturation, followed by annealing at 55–60 ºC of for 45 sec (following the TM values of the primer) and 72 ºC of extension for 60 sec and followed by a final extension at 72 ºC for 10 min. The samples run on a 3% agarose gel by using bromo-phenol blue as dye and 50-bp ladder (NEB) for an hour in 0.5× Tris–Acetic acid–EDTA (TAE) buffer. The resolved PCR bands were documented gel documentation system (Gel Doc XR+, Bio-Rad, USA) and images were stored for analysis.

Data Analysis

Only the clear and unambiguous bands of SSR markers were scored. The sizes of the amplified fragments were estimated with the help of Alpha image software by Gel documentation system using 100 bp DNA ladders (NEB) as size standard. Markers were scored for the presence (1) or absence (0) of the corresponding band among the genotypes. To measure the informativeness of the markers, the polymorphism information content (PIC) for each SSR marker was calculated according to the formula (Botstein *et al*., 1980): $PIC=1-\Sigma Pi^2 - \Sigma\Sigma Pi^2\ Pj^2$ where 'i' is the total number of alleles detected for SSR marker and 'Pi' is the frequency of the i^{th} allele in the set of hundred genotypes investigated and j = i+1. Genetic diversity among 30 rice varieties, was estimated by NTSYS-pc (version 2.02J) similarity was calculated by Jaccard's coefficient and dendogram was constructed by Sequential Agglomerative Hierarchical Non-overlapping (SAHN) based unweighted Pair Group Method with Arithmetic means (UPGMA) to infer genetic relationships. A neighbour-joining tree with bootstrap values was constructed utilizing unweighted pair group method with arithmetic averages algorithm with the help of DARwin (version 6.0.014).

Table 1. List of genotypes used in the study

S.No	Name of the Variety	S.No	Name of the Variety
1	Annada	16	Nagina-22
2	Azucena	17	NERICA LINE-7
3	BPT-5204 (SambaMahsuri)	18	NERICA LINE-16
4	CSR-30 (Yamini)	19	NERICA LINE-22
5	INRC- 10192	20	NERICA LINE-24
6	Lalnakanda	21	NERICA LINE-32
7	MTU- 1001 (Vijetha)	22	NERICA LINE-34
8	MTU-1010 (Cotton Dora Sannalu)	23	NERICA LINE-42
9	MTU- 7029 (Swarna)	24	NERICA LINE-44
10	AC-37566 (*Oryza glaberimma*)	25	NERICA LINE-45
11	Sabita	26	NERICA LINE-46
12	Solunpiket	27	NERICA LINE-48
13	Swarna sub-1	28	NERICA LINE-50
14	Tellahamsa	29	NERICA LINE-52
15	Vandana	30	NERICA LINE-60

Drought response under greenhouse conditions

30 rice genotypes sown on moistened filter paper in petri dishes were maintained at 30°C for 48h in an incubator for germination. The germinated seeds were maintained at room temperature (27°C ± 1°C) for three days. The seedlings were transferred to Yoshida's nutrient solution (Yoshida *et al*., 1976) under greenhouse conditions and allowed to grow. After three days, the seedlings of each of the line were evenly distributed into four Petri dishes, two of which for water deficit (stress) treatment and two for control. Water deficit was created by using half-strength Yoshida's nutrient solution containing 15 per cent PEG-6000 (W/W) with osmotic potential (OP) of -2.36 to -2.95 bars at 25-30°C, while the control was continued with normal nutrient solution. Five days later, OP level of the stress treatment was increased to -4.04/-4.91 bars by replacing 15 per cent with 20 per cent PEG 6000 to the nutrient solution and maintained for two weeks. Though this is considered to be the critical concentration for early seedling stage screening of rice for drought tolerance (Blum *et al*., 1989 and Gloria *et al*., 2002), the seedlings were subjected to still higher stress by raising further the OP level to - 6.15/-7.35 bars (PEG 6000 at 25% of nutrient solution) for four days. The nutrient solution was replenished once a week in control; while for keeping the water potential stable, the solution containing PEG was changed on alternate days in the stress treatment. The pH of both the treatments was adjusted to 5.0 by adding 1 mol/l HCl or NaOH solution every 24 hours. Response to the treatment was observed 29 days after sowing (DAS). On removing from nutrient solution, the seedlings were thoroughly washed with distilled water and blotted with tissue paper to remove excess water before observing for root and shoot length.

Table 2. Description of the drought tolerance related traits measured under greenhouse conditions

Trait code	Trait full Name and (Units)	Description
SL (C/S)	Shoot length (cm)	Length from node/collar region to the tip of the shoot
RL (C/S)	Root length (cm)	Length of the seminal root (Seminal root is the longest root at early seedling stage)
SDW (C/S)	Shoot dry weight (mg)	Weight of shoot after Oven-drying at 80ºC for 24 h.
RDW (C/S)	Root dry weight (mg)	Weight of roots after Oven-drying at 80ºC for 24 h
RS (C/S)	Root shoot weight ratio	Ratio of root dry weight to the shoot dry weight
SL (R)	Relative shoot length	
RL (R)	Relative root length	
SDW (R)	Relative shoot dry weight	Ratio of the parameter under stress to the control
RDW (R)	Relative root dry weight	
RS (R)	Relative root shoot weight ratio	

C- Control: Normal half strength Yoshida's nutrient solution
S- Stress: PEG-6000 contained half strength Yoshida's nutrient solution (Yoshida *et al.*, 1976).

Figure 1. Measurement of root and shoot length of rice genotypes

The same root and shoot samples were used for measuring root and shoot dry weights, after drying them at 70°C for 48h (Gregorio *et al*., 2002 and Ali *et al*., 2006). May be due to hot weather conditions three genotypes (MTU-1010, Tellahamsa and Sabita) were not germinated, hence data was recorded for the twenty seven genotypes. For the study of secondary traits related to drought tolerance, observations were made on five seedlings per replication and averaged following standard procedures as detailed in Table 2.

Statistical procedure

The data with respect to the above characters was subjected to the following statistical tools for the analysis of diversity.

Analysis of variance

Analysis of variance was computed based on Randomized Block design for each of the character separately as per standard statistical procedure (Panse and Sukhatme, 1978). The significance was tested by referring to the values of 'F' table (Fischer and Yates, 1967).

$Y_{ij} = \mu + g_i + r_j + e_{ij}$

Where,

Y_{ij} = Phenotypic observation of 'i^{th}' genotype and 'j^{th}' replocation
μ = General mean
g_i = True effect if 'i^{th}' genotype
r_j = True effect of 'j^{th}' replication
e_{ij} = Random error association with 'i^{th}' genotype and 'j^{th}' replication.

Genotypic and Phenotypic coefficients of variance

The genotypic and phenotypic coefficients of variation were calculated according to the formula given by Falconer (1981).

$$\text{Genotypic coefficient of variation} = \frac{\text{Genotypic standard deviation}}{\text{Mean}} \times 100$$

$$\text{Phenotypic coefficient of variation} = \frac{\text{Phenotypic standard deviation}}{\text{Mean}} \times 100$$

Categorization of the range of variation was effected as proposed by Sivasubramanian and Madhavamenon (1973).

<10%:low
10-20%:moderate
>20%:high

Heritability and genetic advance

Heritability in the broad sense refers to the proportion of genotypic variance to the total observed variance in the total population. Heritability (h^2) in the broad sense was calculated according to the formula given by Allard (1960).

$$h^2 = \frac{\sigma^2_g}{\sigma^2_p}$$

Where,
h^2 = heritability in broad sense
σ^2_g = genotypic variance
σ^2_p = phenotypic variance (σ^2_g) + (σ^2_e)
σ^2_e = environmental variance

As suggested by Johnson *et al*. (1955) (h^2) estimates were categorized as:

Low:0-30%
Medium:30-60%
High:above 60%

Genetic advance refers to the expected gain or improvement in the next generation by selecting superior individuals under certain amount of selection pressure. From the heritability estimates the genetic advance was estimated by the following formula given by Burton (1952).

$$GA = K. h^2 (b). \sigma_p$$

Where,
GA = expected genetic advance
K = Selection differential, the value of which is 2.06 at 5% selection intensity
σ_p = phenotypic standard deviation
h^2 (b) = heritability in broad sense

In order to visualize the relative utility of genetic advance among the characters, genetic advance as per cent for mean was computed.

$$\text{Genetic advance as per cent of mean} = \frac{GA}{\text{Grand mean}} \times 100$$

The range of genetic advance as per cent of mean was classified as suggested by Johnson *et al.* (1955).

Low:less than 10%
Moderate:10-20%
High:more than 20%

Estimation of genetic divergence

A measure of group distance based on multiple characters was given by Mahalanobis (1936) using D^2 statistic. With the help of this, genetic divergence between genotypes was estimated.

D^2 values between i^{th} and j^{th} genotypes for 'p' characters were calculated as:

$$D^2_{ij} = \sum_{t=1}^{p} (\bar{Y}_{it} - \bar{Y}_{jt})^2$$

where
Y_{it} = Uncorrelated mean values of i_{th} genotype for 't' character,
Y_{jt} = Uncorrelated mean values of j_{th} genotype for 't' character,
D^2_{ij} = D^2 between i_{th} and j_{th} genotype.

Computation of D^2 values

For a given combination of 'i' and 'j' genotype, the mean deviation i.e., $Y_{it} - Y_{jt}$ for t = 1,2,.....P variables were computed and D^2 values were calculated as sum of square deviations:

$$D^2_{ij} = \sum_{t=1} (Y_{it} - Y_{jt})^2$$

where
Y_{it} = Uncorrelated mean values of i_{th} genotype for't' character,
Y_{jt} = Uncorrelated mean values of j_{th} genotype for't' character,
D^2_{ij} = D^2 between i_{th} and j_{th} genotype.

Testing of significance of D^2 values

The D^2 values obtained for a pair of population is taken as the calculated values of X^2 and is tested against the tabulated value of X^2 for P degrees of freedom, where P is the number of characters considered.

Grouping of genotypes into various clusters

Grouping of populations into different clusters was done using Tocher's method as described by Rao (1952). The criterion used in clustering by this method is that any two variables belonging to same cluster should atleast on an average show a similar D^2 value than those belonging to different clusters. For this purpose, D^2 values of all combinations of each genotype were arranged in an increasing order of magnitude in a tabular form as described by Singh and Chaudary (1979). To start with two populations having the smallest distance from each other was considered to which a third population having a smallest D^2 value from the first two populations was added. Similarly, next nearest fourth populations was considered and this procedure was continued. At certain stage where it was felt that after adding a particular population there was abrupt increase in the average D^2 value, that population was not considered for including in that cluster. The group of the first cluster was then omitted and the rest was treated in a similar way. This process was continued till all the genotypes were included in to one or other cluster.

Average intra cluster distance

For the measurement of intra cluster distance, the formula used was $\Sigma D^2_i/n$

Where
ΣD^2_i = Sum of distances between all possible combinations (n) of the populations included in a cluster
n = Number of clusters

Average inter cluster distance

Clusters were taken one by one and their distances from other clusters were calculated. The distance between two clusters was the sum of D^2 values between the numbers of one cluster to each of the number of other cluster divided by the product of the number of genotypes in both the clusters under consideration. The square root of the average D^2 value gave the genetic distance between the clusters. Based on D^2 values (inter cluster distance) the scale given by Rao (1952) for rating of the distance was adopted and the cluster diagram was prepared.

$$\text{Average inters cluster distance} = \frac{D^2}{n_1 \times n_2}$$

n_1 and n_2 are number of genotypes of two clusters

Category'D' values

Closely relatedBelow 22
Moderately divergentBetween 22 and 30
Highly divergentAbove 30

RESULTS AND DISCUSSION

Microsatellite marker analysis

In order to estimate genetic diversity, 50 rice microsatellite (RM) markers distributed evenly on all 12 chromosomes were chosen to screen the 30 rice genotypes. Forty six of them were found to show polymorphism (92%), while four markers were monomorphic. The average number of alleles per locus was 2.7, with a range of 2 to 5, which was significantly lower than the average number of alleles reported by Zhu *et al.* (2004) (4.37) and Spada *et al.* (2004) (7.2) and higher than those obtained by Chuan-Guang and Gui-Quan (2010). This variation in results could be attributed to the utilization of fewer SSR loci and the number of genotypes used in the present study than the previous workers. In another study, Yu *et al.* (2003) used 193 rice accessions collected from 26 countries with 101 SSR primer pairs and detected an average allele number of 6.3 per locus, which was also higher than the value reported here. The PIC value ranged from a minimum of 0.53 (RM17611) to maximum of 0.88 (RM8254), with an average of 0.77. The average PIC values, in the present study (0.77) is more than the previous studies of Giarrocco *et al*. (2007) (0.69) and Jayamani *et al*. (2007) (0.667) as we have used hypervariable microsatellite markers. Previously Narshimulu *et al*. (2011) demonstrated that the microsatellite markers of hypervariable nature would be more polymorphic than the non-hypervariable markers.

Table 3. Details and PIC values of the primers used in the study

S.No.	Primer name	Chr no.	Motif	No.of Alleles	Pic value	S.no	Primer name	Chr no.	Motif	No.of Alleles	Pic value
1	RM10000	1	AGAT(15)	3	0.77	26	RM7446	5	AAAT(22)	2	0.7
2	RM10615	1	AAG(31)	3	0.86	27	RM6917	6	AAT(25)	2	0.67
3	RM11096	1	AGAT(9)	2	0.84	28	RM8226	6	AAG(14)	5	0.69
4	RM8126	1	AAT(38)	2	0.68	29	RM20030	6	ACAT(13)	3	0.71
5	RM6292	1	AGC(12)	2	0.81	30	RM20429	6	AAT(31)	2	0.88
6	RM12353	2	AAT(26)	3	0.75	31	RM439	6	AAT(13)	2	0.71
7	RM6640	2	ACT(14)	2	0.79	32	RM21700	7	ACAT(18)	2	0.86
8	RM6641	2	ACT(14)	2	0.73	33	RM21941	7	AAT(25)	3	0.75
9	RM12941	2	AGAT(12)	2	0.79	34	RM22554	8	AAT(20)	2	0.8
10	RM8254	2	AAG(27)	3	0.88	35	RM22688	8	AAT(28)	3	0.75
11	RM6318	2	AAG(12)	2	0.81	36	RM22925	8	AGAT(11)	3	0.78
12	RM14208	2	AAC(22)	3	0.72	37	RM22969	8	ACG(7)	2	0.76
13	RM14270	3	AT(46)	2	0.84	38	RM23017	8	AAT(18)	3	0.84
14	RM14728	3	AAG(11)	3	0.77	39	RM23036	8	AGAT(15)	3	0.82
15	RM14735	3	AT(42)	3	0.79	40	RM23362	8	AAG(19)	2	0.73
16	RM15303	3	AAG(15)	2	0.76	41	RM23574	8	AAT(30)	4	0.76
17	RM6987	3	AAG(22)	5	0.86	42	RM23741	9	AT(36)	2	0.85
18	RM16266	4	AAT(19)	4	0.74	43	RM24015	9	AGAT(9)	3	0.63
19	RM16410	4	CCG(7)	3	0.87	44	RM24044	9	AAG(11)	4	0.64
20	RM7051	4	AATC(7)	2	0.77	45	RM25262	10	AAT(38)	3	0.78
21	RM16910	4	AG(12)	3	0.8	46	RM8207	10	AAG(23)	3	0.79
22	RM17611	4	ATC(13)	3	0.53	47	RM26190	11	AGAT(13)	2	0.84
23	RM5844	5	AAT(22)	2	0.67	48	RM26632	11	AAAG(9)	4	0.79
24	RM18362	5	AT(45)	3	0.73	49	RM27840	12	(AAT)37	3	0.77
25	RM18639	5	AAT(17)	4	0.72	50	RM28279	12	AATC(8)	3	0.77

Assessment of genetic relatedness of rice genotypes

The UPGMA cluster analysis based phylogeny tree (Figure 2) grouped all 27 genotypes into two clusters. MTU-1010 a drought susceptible high yielding variety fell into cluster A and all the other varieties in cluster B. Cluster B is sub divide into three sub clusters, the drought susceptible varieties formed a separate cluster indicating the potentiality of molecular markers especially microsatellite markers for estimating the genetic relatedness of rice varieties according to their donor characters. Nerica Lines, derivatives of a cross between *Oryza sativa* and *Oryza glaberrima.* have clustered along with Drought tolerant varieties and Swarna, a drought susceptible variety clustered separately.

The submergence tolerant varieties unsurprisingly Swarna Sub1 and Sabita were formed a sub cluster with drought tolerant varieties providing the evidence that the mechanisms governing the both the traits are almost similar. Previously also many reports can be found reporting that grouping of drought and salt tolerant varieties into one group. For instance, Reddy *et al.* (2009) reported grouping of CSR30, a salt tolerant variety, Sabita, a mega variety for deep water rice and pokkali, known for salt tolerance grouped together. The dendrogram (Figure 3) constructed by using DARwin is divided into four clusters (Figure-). Cluster I comprised of drought tolerant varieties NL-7, NL-46, Azucena, NL-45, NL-32, NL-16, Solunpiket and NL-52. Cluster II had Swarna sub-1, NL-48, Sabita, NL-24, N-22, NL-60, NL-44, Annada and Vandana. Clusters III comprised of NL-42, NL-22, NL-34, INRC - 10192, Lalnakanda, Yamini and NL-50 and cluster IV had MTU-1010, Tellahamsa, BPT-5204, Swarna, *O. glaberimma* and MTU-1001.

Analysis of variance

Analysis of variance showed significant differences for all the characters under study. The results of analysis of variance are given in Table 4

Mean performance, Genetic variability, Heritability and Genetic advance

The mean performances of 15 root and shoot characters for 27 genotypes are presented in Table 5.

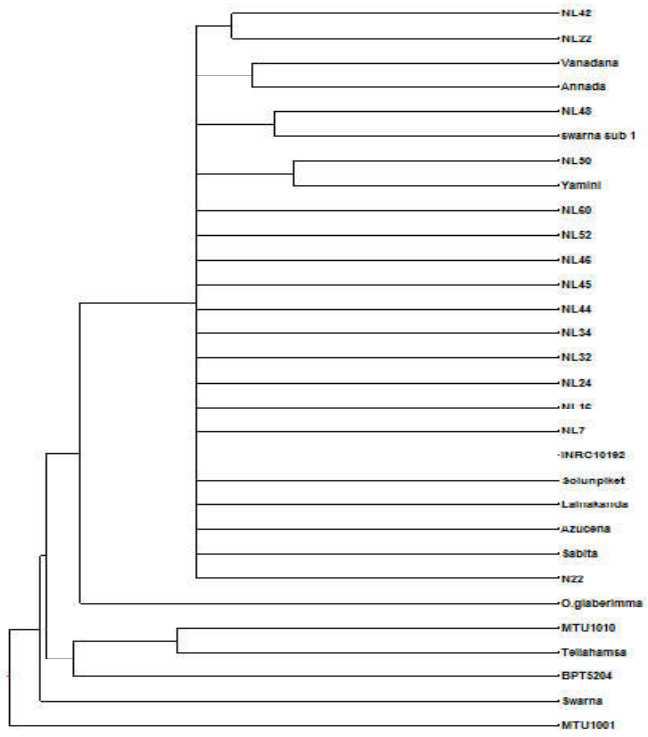

Figure 2. UPGMA tree for 30 Rice genotypes used in the study

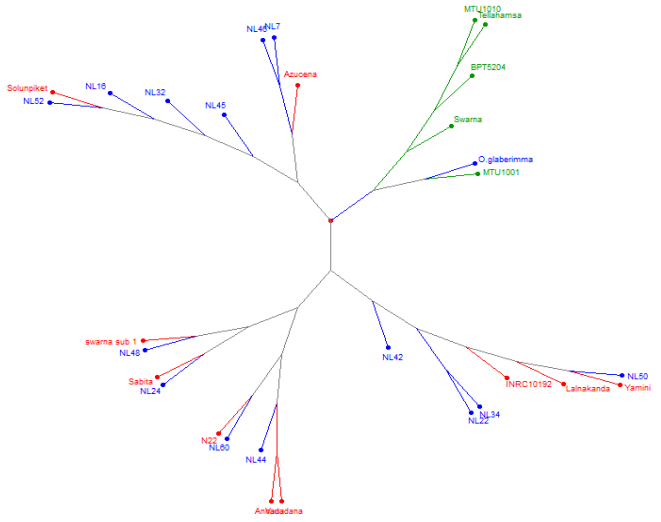

Figure 3. Genetic relationship among 48 rice varieties estimated using Unbiased Neighbour-Joining dendrogram

The genotypic and phenotypic coefficients of variation, heritability, genetic advance and genetic advance per cent of mean were estimated for 27 genotypes including checks. Results of these are presented in Table 6.

Root length (cm)

Root length under control conditions ranged from 4.16 cm (BPT-5204) to 9.04 cm (Lalnakanda) with a mean of 6.23 cm. The genotypic and phenotypic coefficients of variation were high i.e., 22.98 and 23.09 respectively. The heritability estimate for this trait (0.991) and genetic advance per cent of mean (47.131) were high while genetic advance (2.953) was moderate. Root length under stress conditions ranged from 3.03 cm (MTU-1001) to 7.50 cm (swarna sub-1) with a mean of 4.64 cm. The genotypic and phenotypic coefficients of variation were high i.e., 24.80 and 25.01respectively. The heritability estimate for this trait (0.983) and genetic advance per cent of mean (50.664) were high while genetic advance (2.351) was moderate. Relative root length ranges from 0.30 cm (Lalnakanda) and 1.00 cm (NL-50) with a mean of 0.77cm. The genotypic and phenotypic coefficients of variation were high *i.e.,* 24.25 and 24.58 respectively. The heritability estimate (0.973) and genetic advance per cent of mean (49.296) were high and the genetic advance (0.380) was low.

Shoot length (cm)

Shoot length under control ranges from 3.59 cm (NL-50) to 6.92 cm (Lalnakanda) with a mean of 5.12cm. The genotypic and phenotypic coefficients of variation were low *i.e.,* 18.60 and 18.45 respectively. The heritability estimate for the trait (0.984) was high and the genetic advance per cent (1.932) was moderate and the genetic advance per cent of mean (37.703) was low. Shoot length under stress condition ranges from 2.65 cm (NL-22) to 5.65 cm (Azucena) with a mean value of 3.85 cm. The genotypic and phenotypic coefficients of variation were low *i.e.,* 17.32 and 17.04 respectively. The heritability estimate for the trait (0.967) and genetic advance per cent (1.343) were moderate while genetic advance as per cent of mean (34.522) was low. Relative shoot length ranges from 0.41 cm (Lalnakanda) to 0.93 cm (Azucena) with a mean of 0.77cm. The genotypic and phenotypic coefficients of variation were low *i.e.,* 15.599 and 16.170 respectively. The heritability estimate for this character (0.931), genetic advance (0.237) and genetic advance as per cent of mean (31.000) were low.

Root dry weight (mg)

Under control, dry weight of root ranges from 9.55 mg (BPT-5204) to 20.80 mg (Lalnakanda) with a mean of 14.20 mg. The genotypic and phenotypic coefficients of variation were moderate *i.e.,* 23.67 and 23.50 respectively. The heritability estimate for the trait (0.986) and genetic advance per cent (6.826) was high, while the genetic advance as per cent of mean (48.075) was moderate. Under stress, dry weight of root ranges from 7.15 mg (NL-22) to 16.27 mg (Swarna sub-1) with a mean of 10.43 mg. The genotypic and phenotypic coefficients of variation were high *i.e.,* 24.57 and 24.99 respectively. The genetic advance per cent (5.912) and genetic advance per cent of mean (49.770) were high while the heritability estimate for the trait (0.967) was moderate. Relative root dry weight ranges from 0.29 mg (Lalnakanda) to 1.01 mg (NL-50) with a mean value of 0.76 mg. The genotypic and phenotypic coefficients of variation were high *i.e.,* 25.270 and 25.807 respectively. The heritability for this trait (0.959) and genetic advance (0.389) were moderate while genetic advance per cent of mean (50.973) was low.

Shoot dry weight (mg)

Under control, dry weight of shoot ranges from 10.81 mg (NL-50) to 22.22 mg (Lalnakanda) with a mean of 15.66 mg. The genotypic and phenotypic coefficients of variation were moderate *i.e.,* 21.097 and 21.309 respectively. The heritability estimate for this character (0.980) and the genetic advance per cent (6.738) were low while the genetic advance per cent of mean (43.027) was moderate. Under stress, dry weight of shoot ranges from 8.24 mg (Lalnakanda) to 16.91 mg (Azucena) with a mean of 11.76 mg. The genotypic and phenotypic coefficients of variation were low *i.e.,* 16.81 and 17.19 respectively. The heritability estimate for this character (0.956) was moderate

while the genetic advance as per cent of mean (33.855) was high. Relative shoot dry weight ranges from 0.37 mg (Lalnakanda) to 1.24 mg (NL-50) with a mean value of 0.77 mg. The genotypic and phenotypic coefficients of variation were low *i.e.,* 20.624 and 21.180 respectively. The heritability for this trait (0.948) and genetic advance (0.320) were low and genetic advance as per cent of mean (41.064) was moderate.

Table 4. ANOVA for root and shoot characters in rice genotypes

S.No.	Character	Mean sum of squares			
		Replications (d.f.=2)	Treatments (d.f.=26)	Error (d.f.=52)	Total (d.f.=80)
1	Root length (C) (cm)	0.0200	167.2**	1.01	168.23
2	Shoot length (C) (cm)	0.0700	70.09**	0.75	70.86
3	Root dry weight (C) (mg)	0.2800	873.15**	8.45	881.88
4	Shoot dry weight (C) (mg)	1.6900	857.16**	11.46	870.31
5	Root shoot weight ratio (C)	0.0040	3.348**	0.108	3.460
6	Root length (S) (cm)	0.0020	103.902**	1.165	105.069
7	Shoot length (S) (cm)	0.0700	34.67**	0.77	35.51
8	Root dry weight (S) (mg)	0.0800	518.40**	11.77	530.25
9	Shoot dry weight (S) (mg)	0.7300	309.49**	9.44	319.66
10	Root shoot weight ratio (S)	0.004	2.767**	0.156	2.927
11	Shoot length (R) (cm)	0.0004	1.1396**	0.0552	1.1952
12	Root length (R) (cm)	0.0002	2.7544**	0.0498	2.8044
13	Shoot dry weight (R) (mg)	0.00002	2.02415**	0.07237	2.0965
14	Root dry weight (R) (mg)	0.0004	2.939**	0.0829	3.0223
15	Root shoot weight ratio (R)	0.003	4.775**	0.320	5.098

** Significant at 1%
C – Control, S- Stress, R- Relative

Root shoot weight ratio

Root shoot weight ratio under control ranges from 0.64 (NL-45) to 1.50 (Solunpiket) with a mean of 0.93. The genotypic and phenotypic coefficients of variation were moderate *i.e.,* 22.189 and 22.730 respectively. The observed heritability for this trait (0.953), genetic advance (0.413) and genetic advance as per cent of mean (44.624) were moderate. Root shoot weight ratio under stress ranges from 0.61 (NL-42) to 1.32 (Swarna sub-1) with a mean of 0.89. The genotypic and phenotypic coefficients of variation were moderate *i.e.,* 20.784 and 21.671 respectively. The observed heritability for this trait (0.920) and genetic advance (0.367) were low while genetic advance per cent of mean (41.064) was moderate.

Relative root shoot weight ratio

Relative root shoot weight ratio ranges from 0.44 (Solunpiket) to 1.53 (*O. glaberimma*) with a mean value of 1.00. The observed heritability for this character (0.906) was low whereas genetic advance (0.477) and genetic advance as per cent of mean (47.747) were moderate. Information on coefficient of variation is useful in measuring the range of variability present in the characters. Heritability and genetic advance are important selection parameters. Genetic coefficient of variation (GCV) along with heritable estimates would provide a better picture of the amount of genetic advance to be expected by phenotypic selection (Burton, 1952). It is suggested that genetic gain should be considered in conjunction with heritability estimates (Johnson *et al*., 1955). Heritability estimates along with genetic advance are normally more helpful in predicting the gain under selection than heritability estimates alone.

Genetic divergence

The quantitative assessment of genetic divergence was made by adopting Mahalanobis D^2 statistic for root and shoot characters. Genetic divergence was estimated for 27 genotypes and the results obtained from the study are presented below.

Mahalanobis generalized distance (D^2)

In order to assess the genetic diversity among the rice genotypes, D^2 statistic was used following the procedure given by Rao (1952).

Since all the 15 characters were correlated, they were transformed into uncorrelated linear combination through Tocher cluster method in Figure 4.

Grouping of genotypes into various clusters

The rice genotypes were grouped into eight clusters based on D^2 values such that the genotypes belonging to same cluster had an average smaller D^2 values than other. The distribution of genotypes into eight clusters is shown in the Table 7 Out of eight clusters, cluster I was the largest comprising of nine genotypes followed by cluster II with six genotypes, cluster III and IV with four genotypes and cluster V, VI, VII, VIII with one genotype each. The clusters V, VI, VII and VIII were represented by single genotype indicating high degree heterogeneity among the genotypes.

Average inter and intra cluster distances

The average intra inter cluster D^2 values are represented in Table 8. Intra cluster D^2 values ranged from zero (cluster V, VI, VII, VIII) to 11.97 (cluster III). Maximum intra cluster distance was observed in cluster III (11.97), followed by cluster IV (10.71), cluster II (9.89) and cluster I (7.97), indicating that some genetic divergence still existed among the genotypes. From the inter cluster D^2 values of the eight clusters, it can be seen that highest divergence occurred between cluster IV and V (28.44) followed by cluster III and VII (24.65), cluster IV and VI (23.82), cluster III and VIII (23.64), cluster III and V (23.18), cluster V and VII (22.60) suggesting that crosses involving varieties from these clusters would give wider and desirable combinations while the lowest was noticed between cluster I and VI (11.96), followed by cluster I and V (12.01), cluster V and VI (12.70), cluster II and IV (15.84), cluster I and II (14.34). It is assumed that maximum amount of heterosis will be manifested in cross combinations involving the parents belonging to most divergent clusters. The greater the distance between the two clusters, the wider the genetic diversity between the genotypes.

Table 6. Mean performance of genotypes of rice for root and shoot characters

Character	Root length (cm) (C)	Root length (cm) (S)	Shoot length (cm) (C)	Shoot length (cm) (S)	Root dry weight (mg) (C)	Root dry weight (mg) (S)	Shoot dry weight (mg) (C)	Shoot dry weight (mg) (S)	Relative shoot length (cm)	Relative root length (cm)	Root shoot weight ratio (C)	Root shoot weight ratio (S)	Relative shoot dry weight (gm)	Relative root dry weight (gm)	Relative root shoot weight ratio
Annada	8.88	6.33	5.53	4.48	20.42	14.33	17.04	13.44	0.81	0.71	1.20	1.07	0.79	0.70	0.89
Azucena	8.29	6.49	6.07	5.65	19.06	14.40	18.30	16.91	0.93	0.78	1.04	0.85	0.92	0.76	0.82
BPT-5204	4.16	3.73	5.73	4.47	9.55	8.67	17.61	13.43	0.78	0.90	0.54	0.65	0.76	0.91	1.19
CSR-30	6.15	5.75	6.77	4.63	14.13	13.14	20.45	13.86	0.68	0.94	0.69	0.95	0.68	0.93	1.37
INRC-10192	7.86	5.07	5.18	3.81	18.07	11.31	15.72	11.65	0.74	0.65	1.15	0.97	0.74	0.63	0.85
Vandana	6.94	4.44	5.49	4.52	15.96	9.93	16.54	13.44	0.82	0.64	0.97	0.74	0.81	0.62	0.77
Swarna sub-1	7.74	7.50	5.32	4.20	17.80	16.27	15.94	12.33	0.79	0.97	1.12	1.32	0.77	0.91	1.18
Lalnakanda	9.04	2.73	6.92	2.84	20.80	6.04	22.22	8.24	0.41	0.30	0.94	0.73	0.37	0.29	0.78
Solunpiket	7.29	3.16	4.60	3.83	16.76	7.17	11.17	10.81	0.83	0.43	1.50	0.66	0.97	0.43	0.44
MTU-7029	8.75	3.04	6.05	3.86	20.11	7.20	18.96	11.57	0.64	0.35	1.06	0.62	0.61	0.36	0.59
MTU-1001	6.53	3.03	5.47	3.12	15.11	7.16	16.82	9.53	0.57	0.46	0.90	0.75	0.57	0.47	0.84
N-22	5.62	4.47	5.42	3.56	12.75	11.12	16.37	10.78	0.66	0.79	0.78	1.03	0.66	0.87	1.33
AC-37556	5.79	5.25	6.13	3.80	12.93	12.17	18.46	11.42	0.62	0.91	0.70	1.07	0.62	0.94	1.53
NL-7	6.81	5.82	5.27	3.55	15.27	13.14	15.65	10.49	0.68	0.86	0.98	1.25	0.67	0.86	1.28
NL-16	5.53	4.66	3.79	3.44	12.49	10.89	11.47	10.34	0.91	0.84	1.09	1.05	0.90	0.87	0.97
NL-22	4.75	3.13	3.65	2.65	10.92	7.15	11.06	7.68	0.73	0.66	0.99	0.93	0.69	0.65	0.94
NL-24	5.51	4.79	4.41	3.93	12.63	11.16	13.27	11.74	0.89	0.87	0.95	0.95	0.88	0.88	1.00
NL-32	4.45	3.85	3.80	3.18	10.78	8.97	11.62	9.95	0.84	0.86	0.93	0.90	0.86	0.83	0.98
NL-34	4.61	4.10	3.71	3.03	12.48	9.96	11.30	9.10	0.82	0.89	1.11	1.10	0.81	0.80	0.99
NL-42	5.49	4.78	4.35	3.82	12.45	11.12	13.06	11.79	0.88	0.87	0.95	0.94	0.90	0.89	0.99
NL-44	5.17	4.73	4.61	3.79	11.99	10.98	13.62	11.37	0.82	0.92	0.88	0.97	0.84	0.92	1.10
NL-45	7.05	5.71	6.41	4.95	13.87	12.90	21.79	14.52	0.77	0.81	0.64	0.89	0.67	0.93	1.40
NL-46	5.49	4.62	5.19	4.11	10.34	8.35	15.95	12.39	0.79	0.84	0.65	0.67	0.78	0.81	1.04
NL-48	4.88	4.13	4.77	3.38	11.03	9.58	14.61	13.29	0.71	0.85	0.76	0.72	0.91	0.87	0.95
NL-50	4.25	4.24	3.59	3.44	10.01	10.06	10.81	13.33	0.96	1.00	0.93	0.75	1.24	1.01	0.81
NL-52	5.79	5.13	5.41	4.09	13.09	7.56	18.86	12.45	0.76	0.89	0.69	0.61	0.66	0.58	0.87
NL-60	5.44	4.61	4.73	3.91	12.55	10.91	14.16	11.64	0.83	0.85	0.89	0.94	0.82	0.87	1.06
Mean	6.23	4.64	5.12	3.85	14.20	10.43	15.66	11.76	0.77	0.77	0.93	0.89	0.77	0.76	1.00
C.V.	2.23	3.23	2.35	3.14	2.84	4.56	3.00	3.62	4.26	4.01	4.92	6.13	4.82	5.23	7.86
S.E.	0.08	0.09	0.07	0.07	0.23	0.27	0.27	0.25	0.02	0.02	0.03	0.03	0.02	0.02	0.04
C.D. 5%	0.23	0.25	0.20	0.20	0.66	0.78	0.77	0.70	0.05	0.05	0.07	0.09	0.06	0.06	0.13
C.D. 1%	0.30	0.33	0.26	0.27	0.88	1.04	1.02	0.93	0.07	0.07	0.10	0.12	0.08	0.09	0.17

C- Control, S- Stress

Table 6. Estimates of variability, heritability and genetic advance in rice genotypes

Characters	Phenotypic variance	Genotypic variance	PCV (%)	GCV (%)	Heritability in broad sence (h2) (%)	Genetic advance% (at 5%)	Gen. Adv as % of mean (5%)
Root length (C) (cm)	2.093	2.074	23.094	22.986	0.991	2.953	47.131
Root length (S) (cm)	1.347	1.325	25.011	24.802	0.983	2.351	50.664
Shoot length (C) (cm)	0.908	0.894	18.600	18.451	0.984	1.932	37.703
Shoot length (S) (cm)	0.454	0.440	17.328	17.041	0.967	1.343	34.522
Root dry weight (C) (mg)	11.303	11.140	23.678	23.507	0.986	6.826	48.075
Root dry weight (S) (mg)	6.797	6.571	24.993	24.573	0.967	5.192	49.770
Shoot dry weight (C) (mg)	11.136	10.916	21.309	21.097	0.980	6.738	43.027
Shoot dry weight (S) (mg)	4.089	3.907	17.199	16.812	0.956	3.981	33.855
Relative shoot length (cm)	0.015	0.014	16.170	15.599	0.931	0.237	31.000
Relative root length (cm)	0.036	0.035	24.585	24.255	0.973	0.380	49.296
Root shoot weight ratio (C)	0.044	0.042	22.730	22.189	0.953	0.413	44.624
Root shoot weight ratio (S)	0.037	0.034	21.671	20.784	0.920	0.367	41.064
Relative shoot dry weight (gm)	0.027	0.025	21.180	20.624	0.948	0.320	41.371
Relative root dry weight (gm)	0.039	0.037	25.807	25.270	0.959	0.389	50.973
Relative root shoot weight ratio	0.065	0.059	25.592	24.355	0.906	0.477	47.747

C-Control, S-Stress

Table 7. Clustering pattern among rice genotypes

Cluster No.	No. of genotypes	Name of the genotypes
I	9	NL-24, NL-42, NL-45, NL-60, NL-16, N-22, NL-32, NL-22, NL-34.
II	6	Yamini, *O. Glaberimma*(AC-37556), Vandana, NL-7,INRC-10192, NL-46.
III	4	Lalnakanda, Swarna, Vijetha, Solunpiket.
IV	4	Annada, Azucena, Swarna sub-1, NL-44.
V	1	NL-48
VI	1	Samba masuri
VII	1	NL-52
VIII	1	NL-50

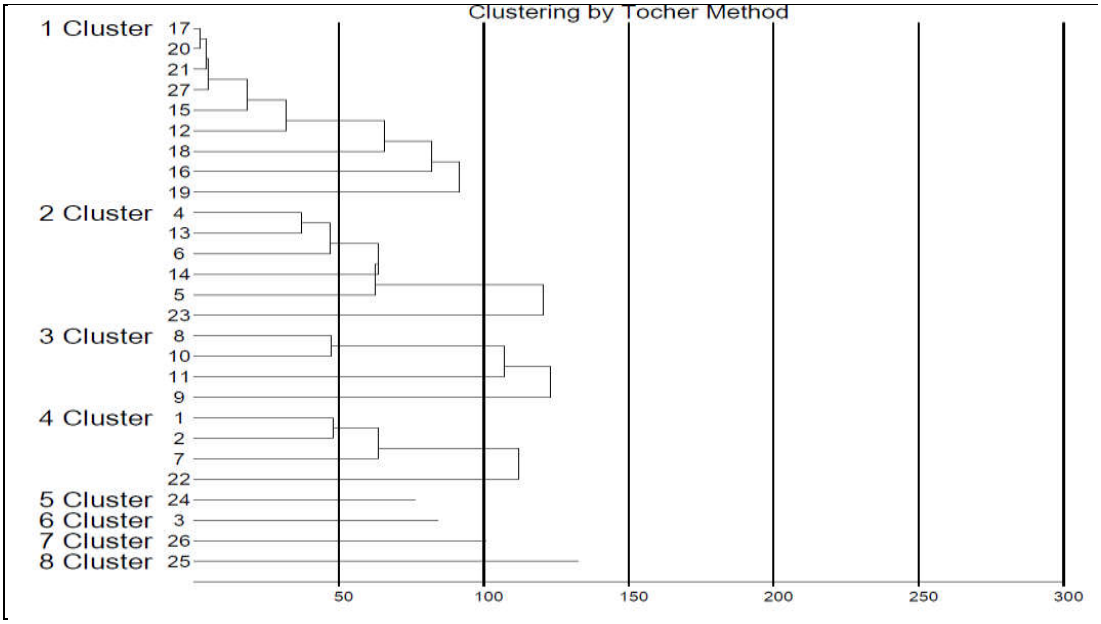

Figure 4. Clustering pattern of genotypes in rice by Tocher method

Table 8. Intra (diagonal) and inter- cluster average of D^2 values of rice genotypes

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII
Cluster I	7.92	14.34	18.67	22.38	12.01	11.96	19.08	15.46
Cluster II		9.89	16.77	13.84	19.68	15.30	14.27	16.42
Cluster III			11.97	22.32	23.18	19.05	24.65	23.64
Cluster IV				10.71	28.44	23.82	16.24	19.96
Cluster V					0.00	12.70	22.60	22.09
Cluster VI						0.00	19.34	20.30
Cluster VII							0.00	19.70
Cluster VIII								0.00

Table 9. Cluster means for root and shoot characters in rice genotypes (cluster analysis)

	Root length (C) (cm)	Root length (S) (cm)	Shoot length (C) (cm)	Shoot length (S) (cm)	Root dry weight (C) (mg)	Root dry weight (S) (mg)	Shoot dry weight (C) (mg)	Shoot dry weight (S) (mg)
Cluster. I	5.18	4.35	4.27	3.48	12.12	10.25	12.88	10.49
Cluster. II	6.51	5.16	5.67	4.07	14.45	11.34	17.13	12.21
Cluster. III	7.90	2.99	5.76	3.41	18.20	6.89	17.29	10.04
Cluster. IV	7.99	6.51	5.83	4.82	17.79	14.48	18.27	14.30
Cluster. V	4.25	4.13	4.77	3.38	11.03	9.58	14.61	13.29
Cluster. VI	4.16	3.73	5.73	4.47	9.55	8.67	17.61	13.43
Cluster. VII	5.79	5.13	5.41	4.09	13.09	7.56	18.86	12.45
Cluster. VIII	5.79	4.24	3.59	4.44	10.01	10.06	10.81	13.33

C -Control, S- stress

Cluster means of the characters

The cluster means for each of the 8 characters are presented in Table 9. From the data it can be seen that considerable differences existed for all the characters under study. The data indicated that the cluster mean for root length under control was high in cluster IV (7.99) and low in cluster VI (4.16). The root length under stress was higher in cluster IV (6.51) and low in cluster III (2.99). The cluster mean for shoot length under control was high in cluster IV (5.83) and low in cluster VIII (3.59). Cluster V (3.38) recorded low stress shoot length than cluster IV (4.87). The root dry weight under control was high in cluster III (18.20) and low in cluster VI (9.55). Root dry weight under stress was high in cluster IV (14.48) and low in cluster III (6.89). Shoot dry weight under control recorded high in cluster VII (18.86) and low in cluster VIII (10.81). Cluster

IV (14.30) recorded high value of shoot dry weight under stress and low in cluster III (10.04).

Conclusion

The results of the present study gives useful information regarding the selection of suitable parents in breeding of drought tolerant rice cultivars besides being useful in mapping of important QTLs related to drought tolerant traits. The molecular markers linked to the QTLs could be of value in selection of lines with drought tolerance. Using varieties tolerant to different stresses in breeding programmes can bring out useful transgressive segregants with increased tolerance to more than one stress. The results reported here have significant implications for rice breeding and basic studies as well. Closely related or moderately distant parents can be chosen based on the overall grouping reported here and depending on the breeding objectives.

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