Oryza Vol. 51 No.4, 2014 (273-278)

Identification of drought tolerant and high yielding F_2 genotypes of rice under aerobic condition

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ABSTRACT

Identification of F_2 segregants for high yield and drought tolerance was envisaged by growing 250 F_2 genotypes derived from Moroberekan/IR64, japonica/indica rice cross in an augmented design under low moisture stress aerobic condition. Estimates of phenotypic coefficient of variance (PCV), genotypic coefficient of variance (GCV), heritability in broad sense (H), genetic advance as per cent mean (GAM), skewness, kurtosis and Shapiro-Wilk W test for normality were computed for eighteen drought and grain yield related traits. In all the cases except for culm angle, PCV was higher than GCV indicating the influence of environment on the characters. High H coupled with high GAM was observed for grain yield traits; Grain yield plant^{-1}, biomass yield plant^{-1} (BY), harvest index, productive tillers plant^{-1} and drought traits; leaf rolling (LR), spikelet fertility, panicle exsertion and plant vigor (PV) and hence offered good scope for selection. F_2 population has recovered maximum superior transgressive segregants for PV (73.2%), BY (65.5%), panicle length (49.8%) and test weight (49.8%). The population was skewed towards female parent (Moroberekan) for some traits and for some other traits it was skewed towards male parent (IR64) with platykurtic distribution. Chi square test fit the segregation of LR in 3 (rolled):1 (unrolled) ratio. Based on LR plasticity 64 and 45 frequencies of F_2 genotypes were classified as highly drought resistant and drought resistant. Ten F_2 genotypes were selected for high yielding and drought tolerant genotypes by direct selection.

Key words: *rice, drought, transgressive segregants, leaf rolling, heritability*

Rice is being cultivated across different range of moisture regimes *viz.*, irrigated, rainfed lowland and rainfed upland conditions. Drought is a major abiotic stress in rainfed rice ecosystem frequently affecting rice productivity in most of the rice growing countries. India has witnessed three severe drought during 2002, 2009 and 2012 which caused a significant rice yield reduction of 21.5 (2002) and 10.02 (2009) million tons (DES, 2009). Variability for drought and yield component traits is prerequisite for the identification of high yielding drought tolerant genotypes. Knowledge of heritability and genetic advance of the drought and yield related traits are also essential for efficient selection programme. In view of this, Moroberekan (drought tolerant *japonica* upland variety) was crossed with IR64 (an agronomically superior *indica* semi dwarf variety with high grain yield potential) to generate genetic variability for drought and yield traits in F_2 segregating population. Leaf rolling (LR) is the most important criteria found useful in assessing levels of drought tolerance (Chang *et al*., 1974).

The present study was conducted to analyze the genetic variability and identification of superior transgressive segregants in F_2 segregating population for drought and yield related characters under low moisture stress aerobic condition and also to find out the morphological parameters and drought related traits that are more efficient for selection under low moisture stress.

MATERIALS AND METHODS

Crossing was made between Moroberekan and IR64 during dry season 2011. True F_1 plants were confirmed with the help of polymorphic SSR marker RM242 and selfed to develop F_2 population. F_2 population of size 250 was dry sown along with their parents and checks (Azucena, drought tolerant and IR50, drought susceptible) during dry season 2012. Eighteen characters were recorded from F_2 genotypes, parents and checks (Table 1) of which, four were drought related traits viz., leaf rolling, spikelet fertility, panicle exsertion and plant vigor and remaining were morphological and yield related traits. Leaf rolling score, culm angle and panicle exsertion was recorded as per the Rice Standard Evaluation System (IRRI, 2002). Plant vigor was computed by considering six plant heights of each F_2 genotype taken at different time interval from 78 days after sowing up to stabilization of plant height by following compound growth rate analysis (Sikka and Vaidya, 1985).

F_2 individuals were sown at 25cm X 25cm and one plant per hill was maintained. The direct seeded crop was raised under aerobic condition by providing irrigation once in 3-4 days interval. The rest of cultural practices and plant protection measures were carried out as per the recommendations (http://www.aerobicrice.org).

Low moisture stress was induced for 20 days during the transition period of vegetative stage to reproductive stage by withholding irrigation between 70^{th} to 90^{th} days after sowing. Leaf rolling was recorded during noon and morning hours for three consecutive days before releasing stress for estimation of leaf rolling plasticity (Loresto and Chang, 1981; De Datta *et al*., 1988). The susceptible check IR50 was first to start the rolling symptoms in the noon and which did not unrolled at subsequent days of early morning hours. Rolling in Moroberekan and Azucena (resistant check) started at a later period of stress during the noon (with lower leaf rolling score) as the transpiration demand increased and unrolled on morning. Stress was released by providing the irrigation.

Volumetric soil moisture content (%), which is the ratio between the volume of water present and the total volume of the sample, was recorded at three sites in each of the three plots at two days interval by using Theta Probe soil moisture sensor (Delta-T devices, 1999) during the period of low moisture stress. The soil moisture level was observed at three different depths 0-6cm, 6-12cm and 12-18cm (Table 2). Soil moisture content was observed to decline gradually in each depth as moisture stress period advanced (Fig.1).

Table 1. Mean performance of parents and checks used in the study for eighteen quantitative characters under investigation

	Mean ± SE			
Characters	Moroberekan	IR64	Azucena	IR50
PHT	99.33 ±2.6	62.64 ± 0.85	124.43 ± 0.51	72.96 ± 0.81
LW	12.05 ± 0.26	8.64 ±0 .09	13.56 ± 0.61	9.64 ± 0.10
CA	1 ± 0	3 ± 0	1 ± 0	1±0
TN	7.78 ± 3.18	36 ± 2.24	10.86 ± 0.3	27.39 ± 1.44
PTN	4 ± 1.26	21.3 ± 1.31	6.14 ± 0.86	23.29 ± 1.96
DF	134.00 ± 1.83	119.70 ± 091	124.29 ± 0.12	116.11 ± 0.82
DM	164.08 ± 1.36	150.07 ± 1.14	149.00 ± 0.08	151.68 ± 1.00
PL	24.48 ± 1.39	21.11 ± 0.56	28.53 ± 1.03	22.78 ± 0.53
PW	4.41 ±0 .653	2.42 ± 0.16	4.95 ± 0.44	2.23 ± 0.16
TW	2.91 ± 0.61	2.58 ± 0.06	3.37 ± 0.21	1.27 ± 0.06
NOS	135.77 ± 6.39	90.2 ± 6.32	154.29± 17.76	110.40 ± 6.69
BY	48.27 ± 7.45	57.15 ± 3.54	73.42± 10.97	55.33 ± 3.26
YLD	16.80 ± 1.75	47.70 ± 2.15	18.40 ± 3.03	43.27 ± 2.22
HI	0.24 ± 0.01	0.46 ± 1.36	0.26 ± 0.04	0.44 ± 0.01
LR	1 ± 0	7.89 ± 0.35	4.33 ± 0.42	8.33 ± 0.33
PEN	-1.51 ± 1.46	-0.63 ± 0.70	5.93 ± 1.19	-3.47 ± 0.48
SF	71.29 ± 6.75	89.36± 1.95	77.13 ± 6.52	73.21 ± 3.47
PV	8.69 ± 3.88	11.77 ± 0.70	12.25 ± 1.2	10.51 ± 0.67

Abbreviations: PHT: Plant height (cm), LW: leaf width (mm), CA: Culm angle (score), TN: Total tillers $plant^{-1}$, PTN: Productive tillers $plant^{-1}$, DF: Days to Flowering (days), DM: Days to maturity (days), PL: Main panicle length (cm), PW: Main panicle weight (g), TW: Test weight (g), NOS: Number of spikelets $panicle^{-1}$, BY: Biomass yield $plant^{-1}$ (g), YLD: Grain yield $plant^{-1}$ (g), HI: Harvest index, LR: Leaf rolling Score, PEN: Panicle exsertion (± cm), SF: Spikelet fertility (%),PV: Plant vigour (%)

Meteorological observations *viz*. maximum air temperature (°C) at 7.00 hrs, pan evapotranspiration (mm), relative humidity (%), sunshine hours and maximum soil temperature (°C) at 15 cm depth were recorded during low moisture stress period from AICRP on Agro-meteorology, UAS, GKVK Bangalore (Fig. 2). During this period no rainfall was noticed.

The genotypic and phenotypic coefficients of variation were computed as suggested by Burton and Devane (1953) and for heritability (h^2) and genetic advance (GA) the method outlined by Hanson *et al*. (1956) and Johnson *et al*. (1955), respectively, was employed. The test of normality of the F_2 population was carried out by performing ''Shapiro-Wilks'' W test (Shapiro *et al*. 1968) using a STATISTICA statistical package. The skewness and kurtosis of the population was estimated using the SPSS statistical package. The genetics of leaf rolling was tested by using *chi* square test.

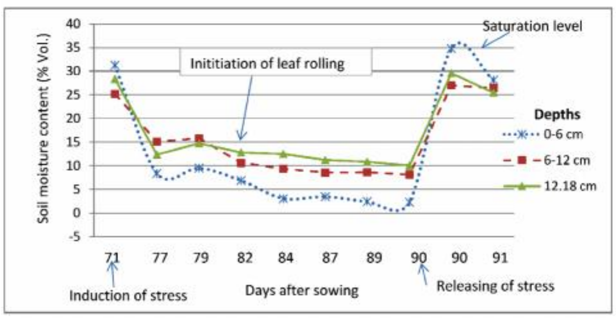

Fig. 1 Gradual decrease in soil moisture level during low moisture stress period from 70th to 90th days after sowing at 0-6cm, 6-12cm and 12-18cm depths. Initiation of leaf rolling was observed on 12th day of stress.

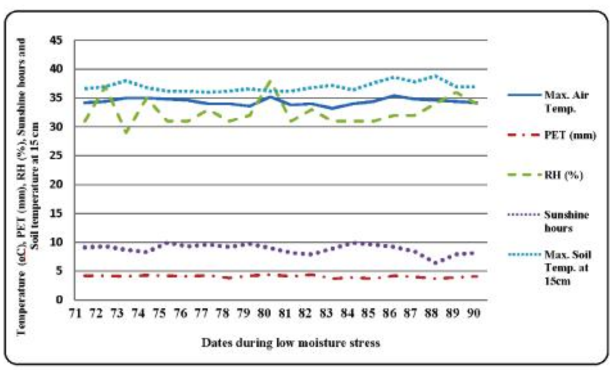

Fig. 2 Meteorological observations during low moisture stress period from 70th to 90th days after sowing during dry season 2012 at UAS, GKVK Bangalore.

RESULTS AND DISCUSSIONS

Since F_2 is a highly segregating population exhibited a wide range of variability for most of the quantitative characters (Table 4). Grain yield plant^{-1} (6.67) has shown maximum range among the characters under study which indicates there is ample chance of getting extreme types. Genetic parameters of variation for drought and yield related traits are given in the Table 3. Grain yield plant^{-1}, biomass yield plant^{-1}, leaf rolling, total and productive tillers plant^{-1} and single panicle weight exhibited greater variance indicating an ample scope for the selection. Hittalmani *et al*. 2003 have reported that, DH lines from IR64xAzucena rice cross have shown wide variation for ten quantitative traits in nine locations of Asia, similar result has been observed for most of the traits under study. PCV was observed to be higher than respective GCV for all the characters except culm angle under study suggesting that these traits are influenced by environment. Grain yield plant^{-1} and other grain yield contributing traits viz., biomass yield plant^{-1}, harvest index, total and productive tillers plant^{-1}, panicle weight, number spikelets panicle^{-1} (Girish *et al.*, 2006; Venkataramana and Hittalmani, 1999) and culm angle and drought related traits viz., leaf rolling, spikelet fertility, plant vigor and panicle exsertion (Aravind, 2011) were exhibited larger GCV indicating that observed variation was due to genetic influence.

The variability observed in the quantitative traits is the sum total of hereditary effects of concerned genes

Table 2. Mean soil moisture content (% volume basis) recorded during low soil moisture stress period under aerobic condition during dry season-2012.

Depth	21/3/2012	27/3/2012	29/3/2012	1/4/2012	3/4/2012	6/4/2012	8/4/2012	9/4/2012	9/4/2012*	10/4/2012
0-6 cm	31.24	8.4	9.5	6.9	3.07	3.47	2.43	2.27	34.77	28.13
6-12 cm	25.24	15.07	15.8	10.63	9.37	8.57	8.63	8.1	27	26.4
12.18 cm	28.37	12.37	14.73	12.8	12.5	11.23	10.83	10.03	29.57	25.47

*soil moisture content at saturation level after releasing stress.

Table 4. Estimates of genetic parameters for eighteen quantitative traits in F_2 population of Moroberekan/IR64 cross evaluated under aerobic condition during dry season 2012

Character	Min. range	Max.	Standardized	Mean	PCV	GCV	h^2_{bs}(%)	GAM (%)	Skewness	Kurtosis
PHT	54	120	0.71	92.6	14.48	11.2	59.86	17.86	-0.13	-0.441
LW	6.4	25.2	1.9	9.87	16.2	13.82	72.76	22.28	3.504	32.439
CA	1	7	2.98	2.02	62.9	62.9	100	129.6	0.965	0.314
TN	5	58	2.53	20.9	44.77	23.49	27.52	25.38	1.033	1.563
PTN	2	41	2.89	13.5	55.93	37	43.73	50.41	0.959	0.984
DF	97	144	0.39	121	6.57	4.57	48.45	6.56	-0.28	0.63
DM	113	168	0.37	149	6.03	4.76	62.23	7.73	-0.58	0.844
PL	14.5	32	0.72	24.3	12.34	5.65	20.92	5.32	-0.09	0.408
PW	0.5	8.82	2.54	3.28	45.5	16.08	12.48	11.7	0.553	0.618
TW	1.03	4.5	1.19	2.91	19.31	14.95	59.91	23.84	-0.1	0.289
NOS	30	328	2.46	121	35.38	21.68	80.21	58.24	0.631	1.901
BY	9.42	371.44	4.2	86.2	63.49	54.93	74.86	97.91	1.576	3.91
YLD	0.77	166.09	6.67	24.8	83.71	74.15	78.45	135.3	2.076	8.956
HI	0.01	0.56	2.55	0.25	46.95	41.52	78.21	75.64	13.62	203.363
LR	0	9	1.9	4.73	62.02	59.48	91.99	117.5	0.119	-1.253
PEN	1	1	1.5	5.32	29.85	20.54	47.36	29.12	-0.19	-0.558
SF	11.39	100	1.2	73.8	26.98	24.56	82.88	46.07	-0.96	0.123
PV	3.39	48.57	3.11	14.5	31.63	23.81	56.7	36.93	1.534	11.245

Abbreviations for the traits are the same as in Table 1
PCV=Phenotypic Coefficient of Variation, GCV=Genotypic Coefficient of Variation, h^2_{bs}=Broad Sense Heritability (%), GAM=Genetic Advance as Percentage of Mean (%).

as well as environmental influence, which is partitioned into heritable and non-heritable components by using appropriate genetic parameters such as heritability and genetic advance. This information is required for the plant breeder for achieving the desired level of crop improvement. Higher value of heritability coupled with high genetic advance was exhibited in leaf width, total and productive tillers plant^{-1}, test weight, spikelet fertility, biomass yield plant^{-1}, grain yield plant^{-1}, harvest index, number of spikelets panicle^{-1}, plant vigor, leaf rolling, panicle exertion and culm angle. Since h^2 is an estimate of the heritable portion of variation, a higher h^2value in quantitative characters is useful as they provide the base of selection on the phenotypic performance. The same trend has been reported for total and productive tillers plant^{-1}, test weight, biomass yield plant^{-1}, grain yield plant^{-1}, harvest index, number of spikelets panicle^{-1} (Girish *et al*., 2006), panicle exertion (Venkataramana and Hittalmani, 1999), leaf rolling (Zulqarnain *et al*., 2012). This indicates the expression of these traits is due to additive gene effect. Genetic gain expected to be high from selection of these traits, therefore these traits could be improved by mass selection and other methods based on progeny testing. Higher estimate of $h^2$and moderate GA were observed for plant height and days to maturity this was in confirmation with findings of Venkataramana and Hittalmani (1999) and Mulugetta *et al.* (2012). Days to flowering had moderate h^2 and low GA. Whereas panicle weight and panicle length had low h^2 and low GA.

If the W statistic is significant, then the hypothesis that the respective distribution is normal should be rejected. The results of ''Shapiro-Wilks W test'' indicates that the F_2 population did not show a normal distribution for all the traits except panicle length and test weight (Table 5).

F_2 population was negatively skewed (towards IR64 variety) for plant height, days to flowering, days to maturity, panicle length, panicle exsertion, test weight and spikelet fertility (Table 4) and positively skewed (towards IR64 variety) for Harvest index, grain yield plant^{-1} and tiller number. In genetic terms, the recombinants with genome fraction from IR64 for these traits were predominant in the population as short growth, earliness in flowering and maturity, low panicle exsertion, low test weight, low spikelet fertility, shorter panicle, higher harvest index, grain yield plant^{-1} and tiller number. Whereas, leaf width, panicle weight, biomass yield plant^{-1}, plant vigor and leaf rolling score were positively skewed (towards Moroberekan) indicates predominant contribution of genomic portion of Moroberekan to the population for above traits.

Biomass yield plant^{-1} (65.6%), panicle length (49.6%), test weight (49.6%), days to maturity (44.4%) and number of spikelets panicle^{-1} (35.6%) (Table 6) exhibited maximum per cent of superior transgressive segregants which provides ample opportunity in selection of drought tolerant and high yielding genotypes. That transgressive segregates that exceed the best parent of a cross for a character can be fixed through inbreeding and selection in further generations (Smith, 1952).

Susceptible check (IR50) and some F_2 genotypes start leaf rolling on the 11th day after inducing stress at 12.8% soil moisture content (Fig.1). Many of the drought tolerant genotypes in a segregating population did not roll and leaves remained to meet the transpiration demand of the plant. Results from the *Chi*-square test was found to fit a ratio of 3 rolled to 1 unrolled (Table 7) implying segregation is at one locus only (Allah, 2009).Based on leaf rolling plasticity F_2 genotypes were classified in to six drought resistance categories (Fig. 3).

Based on yield and drought related traits, F_2 genotype number 141, 107, 101, 116, 160, 60, 48, 151, 126 and 100 were selected as superior genotypes. These genotypes are high yielding and drought tolerant (low leaf rolling score) under low moisture stress aerobic condition. Based on LR plasticity, 64 and 45 F_2genotypes were classified as highly drought resistant and drought resistant respectively. The result of this study suggests harvest index, biomass yield plant^{-1}, productive tillers plant^{-1}, spikelet fertility, plant vigor, panicle exsertion and leaf rolling can be used as major traits in selection of high yielding drought tolerant

Table 5 Test of normality of the distribution curve of all the 18 traits recorded in F_2 population of Moroberekan x IR64, *japonica x indica* rice cross

Traits	PHT	LW	CA	TN	PTN	DF	DM	PL	PW
W test	0.97319	0.83706	0.70950	0.93159	0.92668	0.97139	0.96576	0.97997	0.96392
Probability	P<0.0266	P<0.000	P<0.000	P<0.0000	P<0.0000	P<0.0122	P<0.0007	P<0.2522	P<0.0002
Traits	TW	NOS	BY	YLD	HI	LR	PE	SF	PV
W test	0.98891	0.97319	0.88764	0.86314	0.24254	0.86818	0.96555	0.88029	0.93484
Probability	P<0.8991	P<0.0265	P<0.0000	P<0.000	P<0.000	P<0.000	P<0.0006	P<0.0000	P<0.0000

Abbreviations for the traits are the same as in Table 1

Table 6. Superior transgressive segregants for fourteen quantitative traits in F_2 population of Moroberekan/ IR64 rice cross under aerobic condition during dry season 2012

Character	Mean		No. of Superior trasgressive segregants
	Moroberekan	IR64	
TN	7.78	36	14 (5.6%)
PTN	4	21.3	32 (12.8%)
DF*	134	119.7	79 (31.6%)
DM*	164.08	150.07	111 (44.4%)
PL	24.48	21.11	124 (49.6%)
PW	4.41	2.42	51 (20.4)
TW	2.91	2.58	124 (49.6%)
NOS	135.7	90.2	89 (35.6%)
BY	48.27	57.15	164 (65.6%)
YLD	16.8	47.7	31 (12.4%)
HI	0.24	0.46	10 (4%)
LR*	8.69	7.89	8 (3.2%)
SF	71.29	89.36	65 (26%)
PV	8.69	11.77	183 (73.2%)

Abbreviations for the traits are the same as in Table 1 *superior segregants having lower value than the lower parent value.

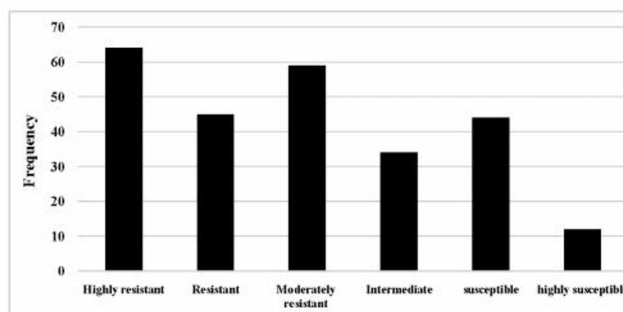

Fig.3 Frequency distribution of F_2 genotypes of Moroberekan/IR64 cross for drought resistance based on leaf rolling plasticity.

Table 7. Observed and expected frequencies of plants in F_2 generation of the rice cross Moroberekan x IR64 along with "Test of goodness of fit" for leaf rolling trait

Cross name	Observed frequency		Expected frequency		Expected ratio	*Chi* Calculated value	*Chi* Table Value at 1%
Moroberaken x IR64	192	58	187.5	62.5	3:1(Rolled: Unrolled)	0.432	6.635

** Significant at 0.01 level.

genotypes. These above mentioned characters can be best used in deriving the drought tolerant high yielding genotypes in rice.

ACKNOWLEDGEMENTS

The financial support of DBT New Delhi for Dr. Shailaja Hittalmani and Pioneer Hi-Bred Research International Fellowship for Mr. Manjappa is gratefully acknowledged.

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