DETERMINATION OF GENETICS IN F₁ POPULATION OF RICE (ADT45 x IR81869-B-B-195) FOR DROUGHT TOLERANCE AND GRAIN YIELD

S.P. Shalini and Srinivasan Bharathkumar*

PG & Research Department of Botany, Kandaswami Kandar's College, P.Velur,
Namakkal-638 182, Tamilnadu, INDIA.
Email: bharathkumar76kkc@gmail.com

ABSTRACT:

Here, we determined the genetics of 70 F_1 rice lines derived from a cross between ADT 45 and IR81869-B-B-195 (DTY2.1 and DTY3.1 QTLs) for drought tolerance and grain yield under drought condition. In the evaluation for drought tolerance, most of the rice lines were registered as highly tolerant and tolerant than both parental lines. Based on drought tolerance degree (DTD) analysis, a number of 63 and 30 rice lines were found to be more DTD value than recipient and donor parent, respectively. In the grain yield evaluation, a number of 28 and 49 lines were noted for having high percentage than the recipient and donor parent, respectively. In the heterosis analysis, many F1 rice lines are noted as positive heterosis for drought tolerance and seed setting. The percentage of heritability was found to be more for seed setting (21.97%) when compare to drought tolerance (19.60%). In statistical analysis, there was significant difference in mean value, variance, standard deviation and CV% between parental and F_1 rice lines for seed setting rather than drought tolerance. This study based on phenotype and genetic analysis will help the rice breeders to select the rice lines effectively and quickly to advance the improvement.

Keywords: Drought tolerance, grain yield, QTLs, Heterosis and Heritability, F1 rice lines.

INTRODUCTION:

Drought is an unavoidable one of the abiotic factors in rice cultivating rain-fed low and up land areas due to unexpected rainfall and lowering of underground water level (Nelson et al. 2014). It hampers the normal physiological and metabolic activities particularly related to the process of photosynthesis, respiration, etc in rice plants (Gupta et al. 2020; Barik et al. 2019). Drought stress occurs at any stage of life cycle of rice plant from seedling stage to reproductive stage. Seedling stage drought reduces the normal growth of the plant which leads to lowering the grain yield or causing the death of plants, whereas drought at reproductive stage leads to no grain yield due to inviability of pollens (Rollines, et al. 2013). During 2002 and 2009, India had faced severe drought which caused a drastic reduction in rice production (Directorate of Economics Statistics, 2009). Available genes/QTLs such as *OsPYL/RCAR5* (Kim et al. 2020), DTY 1.1 (Vikram, et al. 2011), DTY2.1 (Dixit et al. 2012), DTY 3.1 (Venuprasad et al. 2012), etc.for drought tolerance helps the rice plants at various stages of its growth. However, the selection of high-yielding rice varieties under drought stress is most important to increase the grain yield (Swamy et al. 2021). In the present study, we are improving local high-yielding rice variety (ADT 45) by introgression of DTY2.1 and DTY3.1 QTLs which are associated with drought tolerance during reproductive stage through conventional method. Here, we have derived a number of F1 seeds from cross between ADT 45 and IR81869-B-B-195 and these rice lines are evaluated for drought tolerance and grain yield

under stress condition to select effective rice lines to advance the improvement.

MATERIALS AND METHODS:

Derivation of F1 population

A small quantity of ADT 45 rice variety from Tamil Nadu Rice Research Institute (TRRI), Aduthurai, Tamil Nadu, and IR 81869-B-B-195 rice variety from National Rice Research Institute (NRRI), Cuttack, Odisha were obtained. To produce F1 population, ADT 45 and IR 81869-B-B-195 were used as recipient and donor, respectively, in cross pollination.

Evaluation for drought tolerance at seedling and reproductive stage

The F_1 seeds were germinated along with both parental lines and they were grown in a cup for 10-days. Then, they were transferred and grown in pot for another 15 days. After that, they were transplanted in field and the distance between two plants and two rows were maintained with 15 x 20 cm gap, respectively. Seedlings were grown with daily water irrigation for 30 days and then, the irrigation was withheld for 15 days for evaluation of drought impact on seedlings. During drought stress, the drought impact on seedlings was registered according to IRRI'scale (1976) (0-Highly tolerance; 1-Toerance; 3-Moderately tolerance; 5-Moderately susceptible; 7-Susceptibe; 9-Highly susceptible) and drought tolerant degree (DTD) (Zu, et al. 2018).

DTD is defined as the mean of the ratios of green leaf length to total leaf length of the top three leaves in every plant after severe drought treatment. DTD values thus vary from zero to one. The green leaf length and the total leaf length of the first leaf are designated as F1 and F2, respectively. Similarly, the green leaf length and the total leaf length of the second leaf are separately designated as S1 and S2, and those of the third leaf as T1 and T2. The untreated control cultivars were handled in the same way to obtain their DTD values. The DTD value of

each material was calculated by the following formula:

$$Xj = \frac{1}{N} \sum_{i=1}^{n} [(F1/F2 + S1/S2 + T1/T2)/3]$$

DTD value = $(X_I + X_{II} + X_{III})/3$

After 15 days, the seedlings were re-irrigated until the initiation of flag leaf in plants. Again, the water irrigation to rice plants was withheld at the time of flag leaf initiation and percentage of seed setting was calculated and IRRI's score for grain yield was recorded as scale 1-100-90% seed recovered; 3-70-89%; 5-40-69%; 7-20-39%; 9-0-19%.

Statistical analysis

The mean value, variance (ᵟ), standard deviation (SD) and coefficient variance percentage (CV %) were calculated to study the difference between parental lines and F1 population. Study of Heterosis was done according to Turner (1953) for drought tolerance and grain yield characters as follows:

Heterosis over mid parent (H1) $HM\overline{P}(\%)$

$$= \frac{\overline{F1} - M\overline{P}}{M\overline{P}} \times 100$$

Heterosis over better parent (H2) $HB\overline{P}(\%)$

$$= \frac{\overline{F1} - B\overline{P}}{B\overline{P}} \times 100$$

Where, $F1$ = mean of $F1$, $M\overline{P}$ = mean of the two parents and $B\overline{P}$ = mean of the better parent.

The GCV (Genotypic coefficient of variance) and PCV (Phenotypic coefficient of variance) values were computed as per Burton and De vane (1953).

Genotypic and phenotypic variances were calculated as follows:

$\sigma 2\ g$ = $\dfrac{MS1 - MS2}{r}$

$\sigma 2$ ph = $\dfrac{MS1}{r}$

where $\sigma^2 g$ is genotypic variance; $\sigma^2 ph$ is phenotypic variance; MS1 is mean square for the entries; MS2 is mean square for the residuals; and r is replication.

Genotypic coefficient of variance (GCV) and phenotypic coefficient of variance (PCV) were determined as follows:

GCV% = $\dfrac{\sqrt{\sigma 2 g}}{X}$ x100,

PCV% = $\dfrac{\sqrt{\sigma 2\ ph}}{X}$ x 100,

where GCV is genotypic coefficient of variance; PCV is phenotypic coefficient of variance; $\sigma 2$ g is genotypic variance; $\sigma 2$ ph is phenotypic variance; and X is sample mean.

Heritability as per cent of mean was estimated following the method of Johnson et al. (1955).

RESULTS:

Drought tolerance screening

In the cross pollination between ADT 45 and IR81869-B-B-195, a number of one hundred and fourteen F1 seeds were derived. In the Drought tolerance screening of 70 F1 seedlings at seedling stage, we found different degree of leaf death in field experiment such as highly tolerant to highly susceptible (score 0 to 9) after 15 days stress (Table-1). In this screening, 1st and 2nd leaf of most of the seedlings were found to be highly tolerant (score 0) compared to 3rd leaf (Fig.1&2). The range of DTD value for drought tolerance varied from 0.24 to 1.0 in F1 population (Table-1). Among these, three and sixty seven seedlings accounted for less and more value compared to P1 parent (0.50), respectively,

whereas to P2 parent (0.76) there were thirty seedlings had more values (Fig.3). In the statistic analysis for DTD value, the mean value of the parental line accounted for lower value (0.33) compared to F_1 population (0.77). For variance, there was difference between the parental line (0.16) and F_1 population (0.07). SD value was noted as high in parental line (0.20) compared to F_1 population (0.03). In percentage of coefficient variance there was a huge difference between parental line (60.6) and F_1 population (4.21) (Table-4).

Seed setting (SS)

In the parameter of SS under drought condition in field, the IRRI's score of parental and F1 population were noted in the range of 3 (good yield) to 9 (poor or no yield) and among them, most of the F1 plants (46) had accounted for score 5 (moderate yield) followed by score 7 in 18 plants. Only 3 F1 plants possessed score 3 and no plants had score 1 (Table-1; Fig.3). The seed setting percent in F1 population ranged from 9.0 to 84.0. Among them, forty two and twenty eight seedlings had lower and higher value, respectively, compared to P1 parent (50.66%) and forty nine seedlings was found to be higher than P2 parent (39.33 %) in SS percent (Fig.4). In the statistic analysis for SS%, the mean value of the parental line was low (45.14) compared to F_1 population (80.75). For variance, the value of parental line was lower (0.86) than F_1 population (45.35). For SD value, the parental line accounted for less value (0.11) compared to F_1 population (0.80), whereas the percentage of CV in parental line was lower (0.24) than F_1 population (0.99) (Table-4).

Heterosis:

In this analysis for drought tolerance, mid parental value and better parental value ranged from -0.37 to 1.62 and -0.95 to 2.10,

respectively. Among them, a number of fifty nine and eleven rice lines were accounted for negative and positive heterosis for mid-parental value, whereas to better parental value, a number of thirty five and thirty five rice lines were registered for positive and negative heterosis, respectively. In seed setting, mid parental value and better parental value ranged from -0.36 to 4.48 and -0.46 to 3.89, respectively. Among them, a number of twenty nine and forty one rice lines were noted for positive and negative heterosis, respectively, for mid-parental value, whereas to better parental value forty three and twenty seven rice lines had positive and negative heterosis, respectively (Table-2).

Heritability (H² %):

Percentage of PCV was more for drought tolerance (49.25%) compared to that of seed setting (9.88%), whereas the GCV percent was calculated as high for seed setting (44.97%) and low for drought tolerance (34.81%). Heritability in F1 population for drought tolerance was 19.60 % and 21.97 % for grain yield (Table-3).

Statistical analysis:

In the statistical analysis, mean value was high in F1 population for drought tolerance (0.33 and 0.77) and seed setting (45.44 and 80.75) compared to parental line. The value of variance in parental line for drought tolerance was more (0.86 and 0.07) and less for seed setting (0.11 and 0.80). Similarly, the value of SD was higher in parental line than F1 population for drought tolerance (0.11 and 0.03) and seed setting (0.11 and 0.80). The percentage of coefficient of variation was higher in F1 population for both drought tolerance (0.24 and 4.21) and seed setting (0.24 and 0.99) compared to parental line (Table-4).

DISCUSSION:

Generally, it is most important to do a proper drought screening for effective selection of high-yielding rice lines from drought susceptible lines under drought stress (Swamy, et al. 2012). With this connection, plant drought tolerance determining based on drought score is an alternative approach (Fen et al., 2015) and the visual scoring reflects the level of relative water content (RWC) of a plant under drought stress (Cabuslay, et al., 2002). And, genetic variation evaluation studies provides more information on parameters like genotypic coefficient of variation, phenotypic coefficient of variation, heritability estimates, and genetic advance and it is absolutely necessary to start an efficient breeding program. In the screening of F1 population at seedling stage for drought tolerance under field condition, first three leaves from top of the plant were taken into account for evaluating the impact of drought stress on seedlings. In this screening, we found that the second and third leaf had different degree of leaf death (scale 0-9) when compared to first leaf (scale 0). This type of result was noted in the first leaf of both parents also and most of the seedlings have accounted for highly tolerant category (scale 0) for second leaf. The different degree of leaf death is associated with the tolerance of the rice seedlings and it is used to measure drought occurrence (Gana, 2011). In the calculation of DTD value which is the mean of the ratios of the green leaf to total length of the top three leaves, many seedlings have highest DTD value (above 0.90) than P2 parent (donor) (0.75). It indicates the more ratio of green part in leaves under drought condition and the higher DTD value is linked with stronger drought tolerance. Some seedlings accounted for lower DTD value is

associated with more leaf death and weaker drought tolerance and it indicates the low water potential (Zu et al. 2017). In heterosis analysis, more number of F1 lines is noted as positive better parent for seed setting when compare to drought tolerance. The positive and negative relation with drought tolerance and seed setting character indicates strong and weak relationship with genetics, respectively (Wang, et al. 2015). In statistical analysis, the difference in the mean, variance and SD value between parental line and F1 lines reflects the variations in drought tolerance at phenotypic level. Higher percentage of CV in F1 lines (4.21%) is associated with significant drought tolerance when compare to parental line (0.24%) since the coefficient of variation only indicates the extent of total variability present for a character (Govindaraj, et al., 2011). In this study, the percentage of PCV and GCV was noted to be greater for drought tolerance and grain yield, respectively. This higher percentage of PCV is associated with the environment influence on the expression of character rather than genes, whereas the higher percent of GCV is a significant contribution of environment and genotypes for grain yield (Massaoudou, et al., 2018). When compare to drought tolerance (19.16%), we have registered high heritability for seed setting (21.97%). It indicates the important role of genotype than environment in determining the phenotype for seed setting. Similar result is presented for seed setting in a previous work (Adhikari, et al. 2018).

CONCLUSION:

It is more important to select high-yielding rice lines under severe drought condition to manage the ongoing climatic change process which is likely to further worsen the scenario in rice growing areas in future. In this study, many rice lines with more seed setting under drought stress have been identified from F1 population under upland ecological condition. Besides, these lines have showed significant variations from parental lines in the genetic evaluation studies such as mean, variance, SD, CV%, PCV, GCV, heterosis and heritability. This type of studies will support the rice breeders to fasten the selection process of high genetically different rice lines. Therefore, these rice lines will be more useful to advance the improvement process.

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Table-1. Parental lines and F1 population with different degree of IRRI's score for drought tolerance

Genotype	Drought tolerance				Seed setting		Geno type	Drought tolerance				Seed setting	
	IRRI score							IRRI score					
	1st leaf	2nd leaf	3rd leaf	DTD value	(%)	IRRI score		1st leaf	2nd leaf	3rd leaf	DTD value	(%)	IRRI score
ADT 45	0	5	9	0.50	50.66	5	F$_{1-35}$	0	0	7	0.88	56.91	5
IR-81869-B-B-195	0	0	7	0.76	39.63	7	F$_{1-36}$	0	0	1	0.91	44.44	5
F$_{1-1}$	0	1	9	0.60	27.86	7	F$_{1-37}$	0	0	0	1.0	50.0	5
F$_{1-2}$	0	5	9	0.54	34.78	7	F$_{1-38}$	0	0	5	0.81	61.01	5
F$_{1-3}$	0	1	7	0.67	53.77	5	F$_{1-39}$	0	7	9	0.44	42.64	5
F$_{1-4}$	0	3	9	0.48	47.11	5	F$_{1-40}$	0	0	0	1.0	40.0	5
F$_{1-5}$	0	5	9	0.59	51.51	5	F$_{1-41}$	0	0	3	0.87	63.85	5
F$_{1-6}$	0	0	5	0.85	23.0	7	F$_{1-42}$	0	0	3	0.82	59.09	5
F$_{1-7}$	0	0	5	0.83	48.03	5	F$_{1-43}$	0	0	0	1.0	65.34	5
F$_{1-8}$	0	0	0	1.0	28.0	7	F$_{1-44}$	0	0	0	0.98	48.18	5
F$_{1-9}$	0	0	5	0.85	9.23	9	F$_{1-45}$	0	0	0	0.98	49.18	5
F$_{1-10}$	0	5	9	0.52	61.85	5	F$_{1-46}$	0	0	5	0.84	57.0	5
F$_{1-11}$	0	0	7	0.74	46.53	5	F$_{1-47}$	0	0	0	1.0	38.98	7
F$_{1-12}$	0	1	5	0.81	40.44	5	F$_{1-48}$	0	0	3	0.88	50.0	5
F$_{1-13}$	0	1	7	0.65	44.11	5	F$_{1-49}$	0	0	9	0.51	43.07	5
F$_{1-14}$	0	0	1	0.24	54.28	5	F$_{1-50}$	0	0	9	0.68	42.1	5
F$_{1-15}$	0	0	5	0.86	17.85	9	F$_{1-51}$	0	0	0	0.94	33.8	7
F$_{1-16}$	0	0	0	0.97	47.43	5	F$_{1-52}$	0	0	0	0.96	49.52	5
F$_{1-17}$	0	1	3	0.71	79.22	3	F$_{1-53}$	0	0	3	0.88	50.68	5
F$_{1-18}$	0	1	3	0.68	52.38	5	F$_{1-54}$	0	0	5	0.81	21.42	7
F$_{1-19}$	0	1	9	0.60	31.42	7	F$_{1-55}$	0	0	9	0.66	54.12	5
F$_{1-20}$	0	0	3	0.77	45.45	5	F$_{1-56}$	0	0	9	0.66	60.67	5
F$_{1-21}$	0	1	9	0.60	34.28	7	F$_{1-57}$	0	0	9	0.66	30.88	7
F$_{1-22}$	0	0	5	0.84	42.18	5	F$_{1-58}$	0	0	9	0.66	51.11	5
F$_{1-23}$	0	1	9	0.63	50.7	5	F$_{1-59}$	0	0	9	0.66	50.84	5
F$_{1-24}$	0	0	3	0.80	37.03	7	F$_{1-60}$	0	0	9	0.66	34.61	7
F$_{1-25}$	0	7	9	0.45	53.01	5	F$_{1-61}$	0	0	9	0.66	41.02	5
F$_{1-26}$	0	0	9	0.66	38.46	7	F$_{1-62}$	0	0	9	0.66	84.84	3
F$_{1-27}$	0	0	1	0.91	40.62	5	F$_{1-63}$	0	0	9	0.66	25.37	7
F$_{1-28}$	0	0	0	0.97	46.92	5	F$_{1-64}$	0	0	9	0.66	53.33	5
F$_{1-29}$	0	0	0	0.94	72.34	3	F$_{1-65}$	0	0	9	0.66	32.43	7
F$_{1-30}$	0	0	5	0.79	55.71	5	F$_{1-66}$	0	0	9	0.66	13.58	9
F$_{1-31}$	0	0	0	1.0	56.52	5	F$_{1-67}$	0	0	9	0.66	33.33	7
F$_{1-32}$	0	0	3	0.87	41.79	5	F$_{1-68}$	0	0	9	0.66	25.6	7
F$_{1-33}$	0	0	3	0.87	54.76	5	F$_{1-69}$	0	0	9	0.66	50.56	5
F$_{1-34}$	0	0	0	1.0	60.91	5	F$_{1-70}$	0	0	9	0.66	35.55	7

Page 433

Table-2. Heterosis level for DT and SS characters.

Genotype	Drought tolerance		Seed setting %		Genotype	Drought tolerance		Seed setting %	
F1 lines	MPV	BPV	MPV	BPV		MPV	BPV	MPV	BPV
F_{1-1}	0.05	1	0.02	0.14	F_{1-36}	-0.28	-0.16	0.01	0.13
F_{1-2}	0.16	0.4	0.29	0.45	F_{1-37}	-0.37	-0.24	-0.09	0.01
F_{1-3}	-0.05	0.13	-0.16	-0.05	F_{1-38}	-0.18	-0.05	-0.26	-0.16
F_{1-4}	0.31	0.58	-0.04	0.07	F_{1-39}	0.43	0.72	0.05	0.18
F_{1-5}	0.06	0.28	-0.12	-0.01	F_{1-40}	-0.37	-0.24	0.12	0.26
F_{1-6}	-0.25	-0.1	0.96	1.2	F_{1-41}	-0.27	-0.12	-0.29	-0.2
F_{1-7}	-0.24	-0.08	-0.06	0.05	F_{1-42}	-0.23	-0.07	-0.23	-0.14
F_{1-8}	-0.37	-0.24	0.61	0.8	F_{1-43}	-0.37	-0.24	-0.3	-0.23
F_{1-9}	-0.25	0.1	3.89	4.48	F_{1-44}	-0.35	-0.22	-0.06	0.05
F_{1-10}	0.21	0.46	-0.27	-0.18	F_{1-45}	-0.35	-0.22	-0.08	0.03
F_{1-11}	-0.14	0.02	-0.02	0.08	F_{1-46}	-0.25	-0.95	-0.2	-0.11
F_{1-12}	-0.22	-0.06	0.11	0.25	F_{1-47}	-0.37	-0.24	0.15	0.29
F_{1-13}	-0.03	0.16	0.02	0.14	F_{1-48}	-0.28	-0.13	-0.09	0.01
F_{1-14}	1.62	2.1	-0.16	-0.06	F_{1-49}	0.67	0.49	0.04	0.17
F_{1-15}	-0.26	-0.11	1.52	1.83	F_{1-50}	-0.07	0.11	0.07	0.2
F_{1-16}	-0.35	-0.21	-0.04	0.06	F_{1-51}	-0.32	-0.19	0.33	0.49
F_{1-17}	-0.11	0.07	-0.43	-0.36	F_{1-52}	-0.34	-0.2	-0.08	0.02
F_{1-18}	-0.05	0.11	-0.13	-0.03	F_{1-53}	-0.28	-0.13	-0.1	0
F_{1-19}	0.05	0.26	0.43	0.61	F_{1-54}	-0.18	-0.05	1.1	1.36
F_{1-20}	-0.18	-0.01	0	0.11	F_{1-55}	-0.04	0.15	-0.16	-0.06
F_{1-21}	0.05	0.26	0.31	0.47	F_{1-56}	-0.04	0.15	-0.25	-0.16
F_{1-22}	-0.25	-0.09	-0.07	0.2	F_{1-57}	-0.04	0.15	0.46	0.64
F_{1-23}	0	0.2	-0.1	0	F_{1-58}	-0.04	0.15	-0.11	0
F_{1-24}	-0.21	-0.05	0.21	0.36	F_{1-59}	-0.04	0.15	-0.11	0
F_{1-25}	0.4	0.68	-0.14	-0.04	F_{1-60}	-0.04	0.15	0.3	0.46
F_{1-26}	-0.04	0.15	0.17	0.31	F_{1-61}	-0.04	0.15	0.1	0.23
F_{1-27}	-0.3	-0.16	0.11	0.24	F_{1-62}	-0.04	0.15	-0.46	-0.4
F_{1-28}	-0.35	-0.21	-0.03	0.07	F_{1-63}	-0.04	0.15	0.77	0.99
F_{1-29}	-0.32	-0.19	-0.37	-0.29	F_{1-64}	-0.04	0.15	-0.15	-0.8
F_{1-30}	-0.2	-0.03	-0.18	-0.09	F_{1-65}	-0.04	0.15	0.39	0.56
F_{1-31}	-0.37	-0.24	-0.2	-0.1	F_{1-66}	-0.04	0.15	2.32	2.73
F_{1-32}	-0.27	-0.12	0.08	0.21	F_{1-67}	-0.04	0.15	0.35	0.51
F_{1-33}	-0.27	-0.12	-0.17	-0.07	F_{1-68}	-0.04	0.15	0.76	0.97
F_{1-34}	-0.37	-0.24	-0.25	-0.16	F_{1-69}	-0.04	0.15	-0.1	0
F_{1-35}	-0.28	-0.13	-0.2	-0.1	F_{1-70}	-0.04	0.15	0.26	0.42

MPV- mid parental value; BPV- better parental value

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Table-3 Percentage of Heritability of DT and SS characters from parents.

6Cross combination (ADT 45 x IR 81869-B-B-195)	PCV (%)	GCV (%)	Heritability (%)
Drought tolerance (DT)	49.25	34.81	19.60
Seed setting (SS)	9.88	44.97	21.97

PCV- Phenotypic coefficient of variation; GCV- Genotypic coefficient of variation.

Table-4. Statistical analysis of parental line and F1s for drought tolerance, seed setting and panicle weight.

Character	Drought tolerance		Seed setting (%)	
Genotype	Pl	F1s	Pl	F1s
Mean	0.33	0.77	45.44	80.75
Variance	0.86	0.07	0.86	45.35
SD	0.11	0.03	0.11	0.80
CV%	0.24	4.21	0.24	0.99

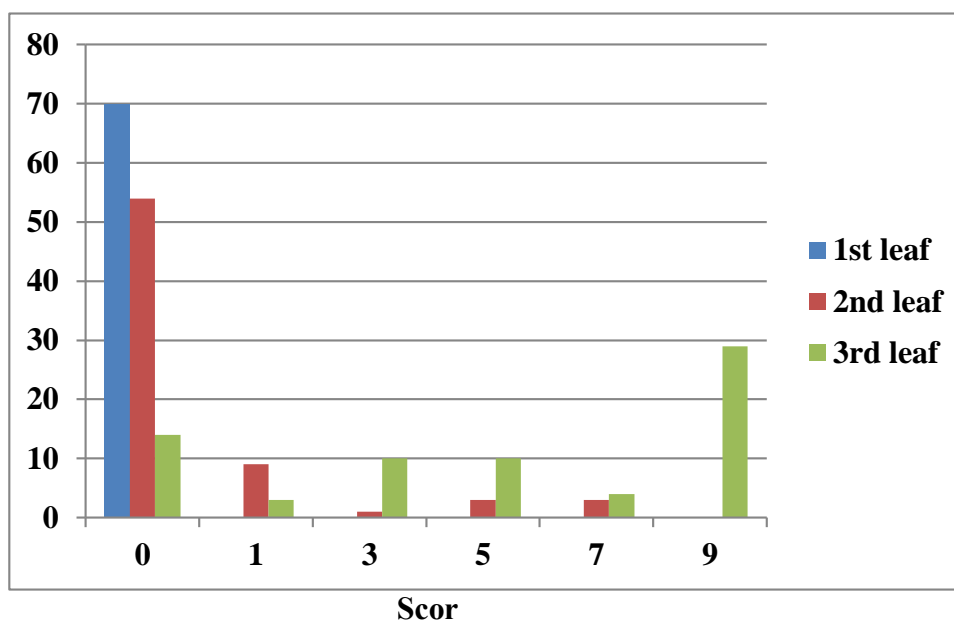Pl – parental line; F1s – F1 population; SD – standard deviation; CV % – coefficient of variantion percentage.

Fig.1 F1 population with various degrees of IRRI's score for drought tolerance

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Fig.2 The different degree of leaf death among F1 seedlings under drought stress condition
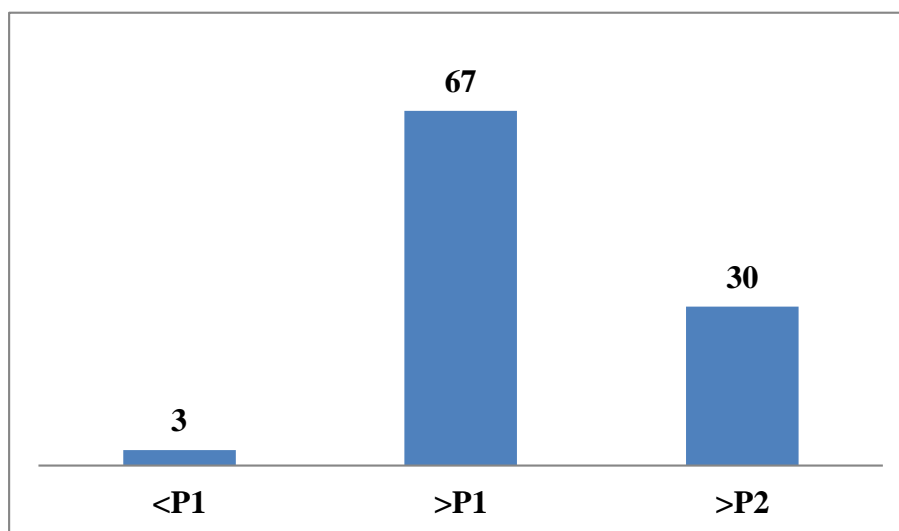
Fig. 3 Number of F1 plants with more and less value than P1 and P2 parent for DTD value.
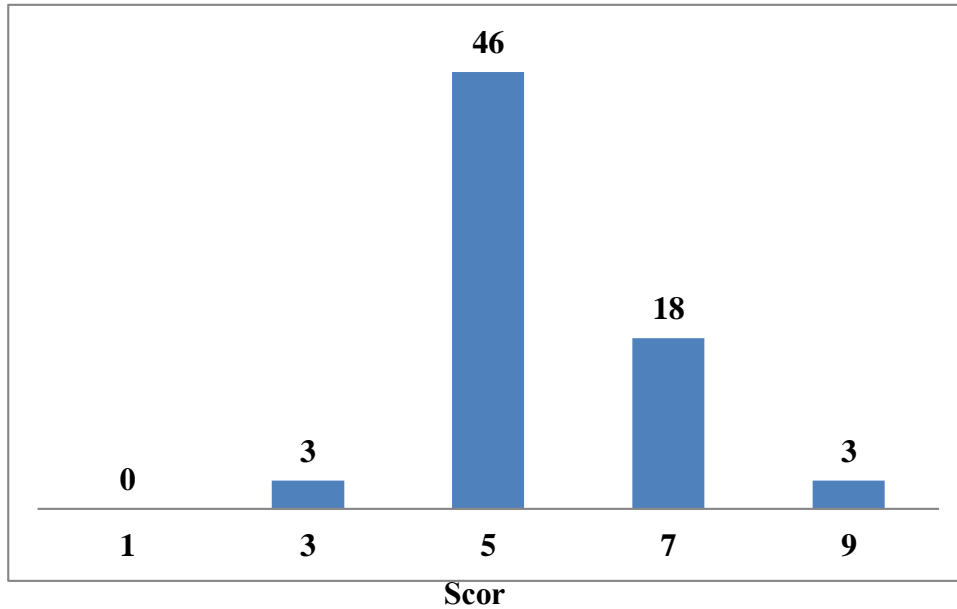
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Page436

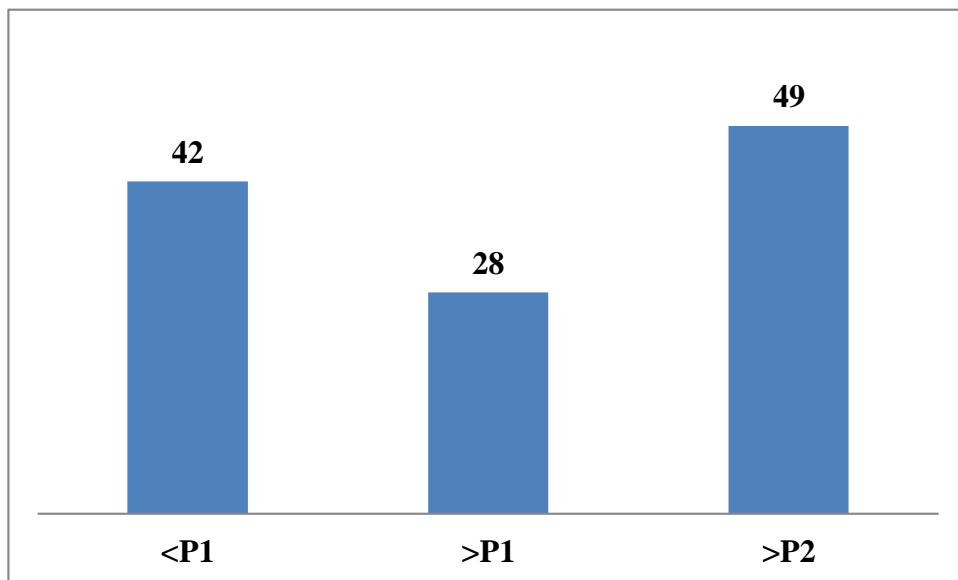**Fig.4** F1 population with various IRRI's score for Seed setting %

Fig. 5 Number of F1 plants with more and less value than P1 and P2 parent for seed setting SS% .

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