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Phenotyping of qDTY QTL introgressed Backcrossed Inbred Lines (BILs) of Rice for Drought Tolerance

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ABSTRACT: Drought is the major abiotic stress that affects rice cultivation under rainfed areas. In India, drought and water stress affects rice production in 17 Mha accounts for about 15.0 percent yield reduction. Improvement of varieties for drought tolerance is the crucial and challenging task in rice. In the present study, 120 BILs along with 3 parents *viz.,* ADT 45, Apo and Way Rarem were screened for vegetative stage drought tolerance under managed water stress condition at Tamil Nadu Rice Research Institute (TRRI) to identify genotypes with drought tolerance ability. The main challenge of this study The results revealed that among 120 BILs, 39 were found to be highly tolerant for vegetative stage drought stress while 29 BILs were susceptible. The recipient parent ADT 45 showed moderate drought tolerance under field conditions. Three BILs *viz.,* W 18, A 52, I 45 were promising with best scores for leaf rolling, leaf drying, leaf senescence and drought recovery indicating that these BILs are highly tolerant to vegetative stage drought stress. The BIL W171 has favourable drought scores and it also recovers rapidly after re-watering. Eight BILs *viz.*, W 97, W 195, I 39, I 40, I 74, I 76, I 92 have better drought scores but recovery rate is slower.

Keywords: Field screening, drought stress, leaf rolling score, leaf drying score, drought recovery score.

INTRODUCTION

Rice (*Oryza sativa* L.) is the major staple food crop that provides daily calorie intake for nearly half of the world's human population particularly in Asia (Sahebi *et al.,* 2018). According to UN report, the world human population is estimated to reach 8 billion and the demand for rice is estimated to be 2000 million metric tonnes by 2030 (FAO, 2016-17). To meet such increase in overall demand, there is a need to promote yield and productivity of rice in wide range of environments. Rainfed rice accounts for around 45% of world's rice area but accounts for only one-quarter of total rice production (Maclean *et al.,* 2002). Drought is the major yield reduction factor on such rainfed rice cultivation (Kumar *et al.,* 2014; Garg and Bhattacharya 2017). It reduced the agricultural productivity by 20 to 40 per cent in rainfed areas (Pandit *et al.,* 2016; Barik *et al.,* 2018). It affects the crop at both vegetative as well as reproductive stage. Drought at both stages is detrimental and severely affects the yield and productivity (Bunnag and Pongthai 2013). Hence there is a need to identify and develop elite genotypes that perform well under severe drought stress.

Rice plants respond to drought by altering morphological, physiological, biochemical and metabolic responses. It includes reduction in plant water content, reduced cell size, stomatal closure, reduction in gaseous exchange and disruption of enzyme-catalyzed biochemical processes (Ozga *et al.,* 2017; Islam *et al.,* 2018). Therefore, selection using morpho-physiological and metabolic traits may be effective for drought tolerance breeding in rice (Zaharieva *et al.,* 2001; Fukai *et al.,* 1995).

Survival and yield potential of rice crop under drought stress mainly depends on its ability to maintain plant water status under such water deficit conditions (Blum, 2009). Leaf rolling is one of the visible physiological response indicators to plant water deficit. It is an adaptive response to water deficit which helps in maintaining favorable water balance within plant tissues. The genetics of rice leaf rolling under vegetative stage drought stress was studied by Singh and Mackil (1991) and they reported major gene for leaf rolling. During vegetative stage drought stress, leaf rolling and leaf drying are the good selection criteria for screening and identification of genotypes for drought tolerance (Farooq *et al.,* 2010; Singh *et al.,* 2012; Chang, 1974; De data *et al.,* 1988). The capacity of a plant to recover from drought was regarded as more important than its drought tolerance (Maji, 1994). Chang, (1974); De Datta (1975); Gana *et al.* (2011) viewed drought recovery as the determining factor of grain yield under stress. Malabuyoc *et al.* (1985) also stated that poor recovery from stress could be a major factor in decreased grain yield.

Hence, the present study was taken up to identify the genotypes that perform well under vegetative stage drought stress based on physio – morphological traits such as leaf rolling, leaf drying, leaf senescence and drought recovery.

MATERIALS AND METHODS

The experimental material comprised of 120 qDTY QTL introgressed Backcrossed Inbred Lines (BILs)derived from the crosses ADT 45*3/Apo, ADT 45*3/Way Rarem and ADT 45*2/Apo//ADT 45*2/Way Rarem. ADT 45 is the recipient parent with high yield, whereas Apo and Way Rarem are drought tolerant donor parents for several qDTY QTLs. Field screening for drought tolerance of BILs along with the parents was carried out at Tamil Nadu Rice Research Institute, Aduthurai (Latitude 11^{o}N, Longitude 79.30^{o}E) during summer(March – April) 2022 under managed water stress condition.

The field experiment was conducted in well pulverized upland field in order to have a check on soil moisture status. The BILs along with the parents *viz.,* ADT 45, Apo, Way Rarem and the susceptible check IR 36 were evaluated through randomized complete block design in two replications. In each replication, the seeds were directly sown in dry soil in 2 rows of 1.5 m length each with the spacing of 20 cm between the rows. Recommended agronomic practices were carried out for proper crop maintenance. The crop was irrigated normally up to 45 days after sowing. On 45th day of sowing irrigation was withheld for a period of 15 days (45th day to 60th day) to impose water stress. During stress period, soil moisture content was monitored through periodical soil sampling at 30 cm depth. Leaf rolling was observed from 7to 10 days after the stress period in the susceptible check IR 36 and complete drying was observed on 14th day after stress. Scores for leaf rolling, leaf senescence and leaf drying were recorded on 15th day of stress period and IRRI Standard Evaluation System for rice, 2013 (IRRI-SES 2013) was followed to score the genotypes. Observations were recorded during mid - day between 12.00 to 2.00 PM. The crop was irrigated on 16th day after water stress and drought recovery score was taken at 7 days after re-watering. The observations were recorded on five randomly selected plants from each genotype and in each replication the mean data from five plants were used for statistical analysis. Statistical analysis was performed using softwares such as 'R' to analyze ANOVA and 'STAR' for cluster diagram.

RESULTS AND DISCUSSION

The results of ANOVA revealed that high significant difference between the genotypes for leaf rolling and drought recovery scores. Similar results were obtained by Anik *et al.* (2021); Verma *et al.* (2019); Yue *et al.* (2005). However, there is no significant difference between the genotypes for leaf drying and leaf senescence scores. However contrary to that, significant difference in leaf drying and leaf senescence and non-significant difference in leaf rolling was obtained by Pavithra *et al.* (2022).

Table 1: Analysis of Variance on mean sum of squares for drought scores.

SV	df	LRS	LDS	LS	DRS
Replication	1	0.65	0.05	15.29	23.48
Genotype	122	11.05**	4.88	6.10	5.65**
Error	122	5.60	4.14	5.78	3.12

(SV – Source of variation, df – degrees of freedom, LRS – Leaf rolling score, LDS – Leaf drying score, LS – Leaf senescence score, DRS – Drought recovery score, **Significant at 1% probability)

The rice genotypes can be categorized according to the Standard Evaluation System for Rice (IRRI – SES, 2013). Under vegetative stage stress, the genotypes showed variations in visual symptoms with score 1 to score 9 for leaf rolling, leaf drying, leaf senescence and drought recovery. The drought tolerant donor parents Apo and Way Rarem showed normal growth without any leaf rolling or drying whereas, the susceptible cultivar IR 36 extensive rolling and drying symptoms. All the 120 genotypes were compared with tolerant (Apo, Way Rarem) and susceptible (IR 36) genotypes to estimate the degree of drought tolerance, accordingly the 120 BILs were categorized into highly tolerant, tolerant, moderately tolerant/susceptible, susceptible and highly susceptible genotypes.

Table 2: Standard evaluation system (SES, 2013) of IRRI for leaf rolling and leaf drying.

Scale	Leaf rolling	Scale	Leaf drying
0	Healthy leaves and no rolling symptoms	0	Leaves healthy and no drying symptoms
1	Shallow V-shaped folding of leaves begins	1	Slight tip drying
3	Deep V-shaped folding of leaves	3	Tip drying extended up to 1/4th length of leaves
5	Fully cupped U-shaped folding of leaves	5	1/4th to 1/2nd of all leaves fully dried
7	Rolling extends till leaf margins touching (O-shape)	7	More than two-third of all the leaves fully dried
9	Tightly and completely rolled leaves	9	All plants apparently dead. Length in most leaves fully dried.

Table 3: Standard evaluation scoring system (SES, 2013) of IRRI for leaf senescence and drought recovery.

Scale	Leaf senescence	Scale	Drought recovery score
1	Late and slow (Leaves have natural green colour)	1	90 to 100 % plants recovered
5	Intermediate (yellowing of upper leaves)	3	70 to 89 % plants recovered
9	Early and fast (all leaves become yellow and dead)	5	40 to 69 % plants recovered
		7	20 to 39 % plants recovered
		9	0 to 19 % plants recovered

The results showed that the leaf rolling score ranges from 0.33 to 9.0, leaf drying score from 0.50 to 7.67. Leaf senescence ranges from 1.0 to 9.0 and drought recovery score ranges from 1.0to 7.0.

Blum (1988) found that delayed leaf rolling under drought stress is an essential selection criterion for drought avoidance. Leaf rolling was thought to be a response to leaf water potential and correlated with leaf water potential of the plants. Delayed leaf rolling was thought to be favorable trait in rice (Maji*,* 1994). In the present study, 5 genotypes recorded the mean leaf rolling score of 0-1, 12 genotypes with the score 1-3, 26 genotypes with the score 3-5, 35 genotypes with the score between 5-7 and 45 genotypes were with the score between 7-9 (Table 4). The BILs W 195, I 39 and I 74 and also the donor parents Apo and Way Rarem showed no leaf rolling symptoms (Score 0-1) and hence they are highly tolerant to vegetative stage drought stress. Twelve BILs were tolerant to leaf rolling with shallow V-shaped folding. Most of the inter-mated BILs with more than one qDTY QTLs such as I 45, I 40, I 33, I 12, I 32, I 76 and I 92 falls under this category. Hence, it is evident that combination of qDTY QTLs contributes to better tolerance to drought than the single QTL. Twenty-six BILs were moderately tolerant to drought, 34 were susceptible and 45 genotypes were highly susceptible to drought stress. The recurrent parent ADT 45 was moderately susceptible with the leaf rolling score of 5-7. Most of the Way Rarem BILs such as W171, W216 exhibited high leaf rolling and hence they are highly susceptible to drought. This could be due to the non-effectiveness of single QTL (qDTY 12.1) for vegetative stage drought stress under this environment.

Table 4: Categorization of rice genotypes for vegetative stage drought tolerance using leaf rolling score based on Standard Evaluation System of IRRI.

Score	Number of genotypes	Name of the genotypes
Score 0-1	5	Apo, Way Rarem, W195, I39, I74
Score 1-3	12	W18, W97, A2, A95, A99, I12, I32, I33, I40, I45, I76, I92
Score 3-5	26	W11, W16, W24, W26, W27, W34, W39, W43, W99, W100, W106, W115, W117, W177, W191, W192, W194, A53, A70, A81, I5, I71, I85, I127, I140, I172
Score 5-7	35	ADT45, W33, W47, W61, W89, W90, W94, W95, W96, W013, W129, W179, W180, W187, W197, W199, W231, W237, W248, W249, W7-4-4, W18-8-7, A4, A14, A26, A58, A62, A67, A75, A78, A80, A82, A83, A88, A89
Score 7-9	45	W121, W125, W132, W135, W138, W145, W149, W162, W164, W168, W170, W171, W172, W214, W216, W220, W225, W234, W235, W242, W7-4-1, W7-4-2, A11, A16, A22, A24, A29, A35, A36, A41, A43, A59, A63, A91

Field screening results for leaf drying showed that two BILs were with the score of 0-1, 21 with the score of 1-3, 55 with the score of 3-5, 37 with the score of 5-7 and 3 BILs with the score of 7-9. The BILs W 99 and I 45 shows no drying symptoms indicating that these genotypes are highly tolerant to drought stress. The donor parent Apo also falls under the same category. 21 BILs *viz.,* W11, W16, W18, W43, W84, W89, W94, W96, W97, W106, W117, W195, W199, A16, A22, A35, A52, I39, I40, I74, I76 and I92 are tolerant to drought stress showing slight tip drying. The donor parent Way Rarem also falls under the same category. Majority of the BILs exhibited moderate resistance for leaf drying. 31 Way Rarem derived BILs, 17 Apo derived BILs and 7 inter-mated BILs falls under this category. Thirty-seven BILs along with the recipient parent ADT 45 shows more than two-third of the leaves fully dried and hence susceptible to drought. The BILs A24, A83 and A89 were fully dried and apparently dead indicating that they are highly susceptible to leaf drying.

Table 5: Categorization of rice genotypes for vegetative stage drought tolerance using leaf drying score based on Standard Evaluation System of IRRI.

Score	Number of genotypes	Name of the genotypes
Score 0-1	3	Apo, W99, I45
Score 1-3	22	Way Rarem, W11, W16, W18, W43, W84, W89, W94, W96, W97, W106, W117, W195, W199, A16, A22, A35, A52, I39, I40, I74, I76, I92
Score 3-5	55	W24, W26, W27, W33, W34, W39, W47, W51, W60, W61, W63, W64, W90, W92, W95, W100, W115, W121, W125, W138, W149, W177, W179, W180, W187, W191, W197, W214, W231, W248, W249, A4, A11, A26, A32, A36, A41, A43, A63, A67, A70, A75, A81, A82, A88, A91, A95, A99, I5, I12, I33, I85, I127, I140, I172
Score 5-7	38	ADT45, W7-4-1, W7-4-2, W7-4-4, W18-8-7, W132, W135, W145, W162, W164, W168, W170, W171, W172, W192, W194, W216, W220, W225, W234, W235, W237, W242, A14, A29, A53, A58, A59, A62, A78, A80, I69, I71
Score 7-9	3	A24, A83, A89

Leaf senescence score showed that 60 BILs exhibited mean score values between 1-3, 51 BILs with the score of 4-6 and 9 BILs with the score of 7-9. Most of the BILs have leaf senescence score less than 3 indicating that these genotypes retain their natural green color and rate of senescence is slow. All the three parents exhibited late and slow leaf senescence. BILs such as W 18-8-7, W 7-4-1, W 7-4-2 and W 7-4-4 exhibited intermediate leaf senescence with yellowing of upper leaves alone. Nine BILs *viz*., W 164, W 168, W 171, W 172, W 235, W 242, W 248, A 24 and A 83 exhibited early and fast leaf senescence. No inter-mated BILs exhibited score more than 5 indicating that they are better adopted to drought when compared to single cross BILs of Apo and Way Rarem.

Table 6: Categorization of rice genotypes for vegetative stage drought tolerance using leaf senescence score based on Standard Evaluation System of IRRI.

Score	Number of genotypes	Name of genotypes
Score 1-3	63	ADT 45, Apo, Way Rarem, W11, W16, W18, W24, W26, W27, W34, W39, W43, W47, W60, W61, W63, W64, W84, W89, W90, W92, W94, W95, W96, W97, W99, W100, W106, W117, W149, W187, W191, W195, A26, A36, A11, A91, A43, A88, A16, A22, A35, A52, A67, A70, A75, A81, A82, A95, A99, A80, I32, I74, I76, I92, I5, I12, I33, I39, I40, I45, I69, I85
Score 4-6	51	W18-8-7, W7-4-1, W7-4-2, W7-4-4, W33, W51, W62, W69, W85, W86, W103, W115, W138, W121, W125, W129, W162, W170, W177, W179, W180, W192, W194, W197, W199, W214, W216, W220, W225, W231, W234, W237, W249, A4, A15, A29, A41, A53, A58, A59, A62, A63, A78, I71, I89, I127, I140, I172
Score 7-9	9	W164, W168, W171, W172, W235, W242, W248, A24, A83

Based on drought recovery score the BILs were grouped as follows: 25 BILs with the score of 1-2, 46 BILs with the score of 3-4, 36 BILs with the score of 5-6 and 11 BILs with the score of 7. The results clearly showed that no BILs have a score of 9 indicating that at least 20 percent of plants get recovered from drought in every genotype. In 27 BILs, more than 80 percent of the plants got recovered and in these genotypes, apparently the rolling and drying symptoms were very minimum with 0 to 3 score. Drought tolerant parents Apo and Way Rarem also falls under this category since they did not exhibit any leaf rolling or drying symptoms during drought stress and therefore, these genotypes quickly regained without any impact of drought stress. In 46 BILs, recovery percentage was between 70 to 90 per cent indicating that these BILs are tolerant to drought stress. Thirty six BILs along with the parent ADT 45 showed 40 to 70 percent recovery and hence they are moderately tolerant / moderately susceptible to drought. 11 BILs showed susceptibility by having 20 to 40 percent recovery from drought.

Table 7: Categorization of rice genotypes for vegetative stage drought tolerance using drought recovery score based on Standard Evaluation System of IRRI.

Score	No. of genotypes	Name of the genotypes
Score 1-2	27	Apo, Way Rarem, W11, W18, W24, W26, W34, W86, W179, W197, W237, W242, W248, W249, A11, A16, A26, A35, A41, A52, A58, A62, A63, A70, A81, A82, A95, I12, I71
Score 3-4	46	W16, W27, W33, W39, W62, W63, W64, W69, W85, W95, W96, W99, W129, W135, W138, W162, W164, W170, W171, W177, W187, W191, W192, W194, W199, W214, W216, W220, W225, W231, W234, W235, A4, A24, A29, A43, A53, A59, A67, A78, A88, A91, A99, I33, I40, I45
Score 5-6	36	ADT45, W43, W47, W51, W61, W84, W90, W94, W97, W100, W106, W115, W117, W121, W125, W132, W145, W149, W168, W172, W180, W195, W7-4-4, A22, A36, A80, A83, A89, I5, I32, I39, I74, I76, I85, I92, I140, I172
Score 7-8	11	W60, W89, W92, W103, W7-4-1, W7-4-2, W18-8, A14, A75, I69, I127
Score 9	-	-

Cluster analysis was performed with the drought scores of 120 BILs and 3 parents. Based on the variation, the 120 BILs and the three parents are grouped into five clusters by using Ward's method. Number of genotypes in each cluster is given in the Table 8.

Table 8: Grouping of 120 BILs and 3 Parents in different clusters by Ward's method based on drought scores.

Cluster	Number of genotypes	Name of genotypes
I	26	ADT 45, W 47, W 60, W 61, W 84, W 90, W 92, W 94, W 96, W 100, W 103, W 115, W 177, W 180, A 22, A 75, A 14, A 80, A 99, I 69, I 85, W 7-4-1, W 18-8-7, I 127, I 140, I 172
II	25	Apo, Way Rarem, W 11, W 16, W 18, W 24, W 26, W 27, W 34, W 39, W 95, W 187, W 191, A 43, A 88, A 16, A 35, A 52, A 70, A 81, A 82, A 95, I 12, I 33, I 45
III	29	W 33, W 51, W 63, W 64, W 69, W 85, W 121, W 125, W 129, W 138, W 149, W 179, W 197, W 199, W 214, W 216, W 220, W 225, W 231, W 249, A 26, A 29, A 36, A 63, A 91, A 11,,A 41, A 67, A 4
IV	14	W 43, W 89, W 97, W 99, W 106, W 117, W 195, I 32, I 74, I 76, I 92, I 5, I 39,I 40
V	29	W 62, W 86, W 132, W 135, W 145, W 162, W 164, W 168, W 170, W 171, W 172, W 192, W 194, W 234, W 235, W 237, W 242, W 248, A 24, A 62, A 59, A 53, A 58, A 78, W 7-4-4, A 83, A 89, I 71, W 7-4-2

Distribution pattern of dendrogram (Fig. 1) showed that Cluster I contains 26 genotypes which include recipient parent ADT 45 and 25 BILs *viz.,* W 47, W 60, W 61, W 84, W 90, W 92, W 94, W 96, W 100, W 103, W 115, W 177, W 180, A 22, A 75, A 14, A 80, A 99, I 69, I 85, W 7-4-1, W 18-8-7, I 127, I 140 and I 172. Cluster II contains 25 genotypes which includes drought tolerant parents Apo and Way Rarem and the BILs W 11, W 16, W 18, W 24, W 26, W 27, W 34, W 39, W 95, W 187, W 191, A 43, A 88, A 16, A 35, A 52, A 70, A 81, A 82, A 95, I 12, I 33 and I 45. Genotypes in these cluster are better performing genotypes under drought stress. Cluster III and V contains large number of genotypees while cluster IV contain least number of genotypes.

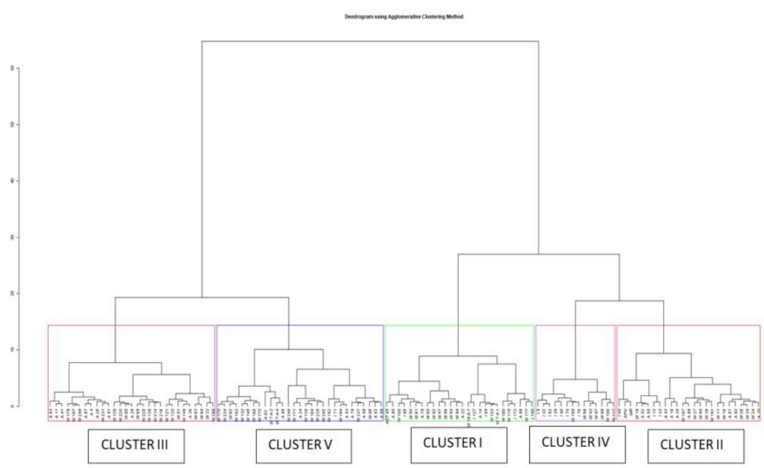

Fig. 1. Dendrogram of 120 BILs and 3 Parents using Ward's method.

Table 9: Mean drought score values of different clusters

Cluster	LRS	LDS	LS	DRS
Cluster 1	5.89	4.13	2.79	5.81
Cluster 2	4.16	3.24	1.93	2.36
Cluster 3	7.59	4.69	3.76	3.41
Cluster 4	2.40	2.19	1.52	5.29
Cluster 5	7.08	6.29	5.74	3.61

(LRS – Leaf rolling score, LDS – leaf drying score, LS – Leaf senescence score, DRS - Drought recovery score)

Mean performance values of five clusters for all the drought scores were computed to evaluate the superiority of the clusters, which is useful in improvement of drought tolerance. Based on which the genotypes with best drought tolerance ability are clustered in cluster II. Genotypes in cluster II are highly tolerant to leaf drying, leaf senescence and tolerant to leaf rolling and have highest drought recovery. The next best cluster was IV which comprised of genotypes with preferable scores for leaf rolling, leaf drying and leaf senescence and the recovery from stress was intermediate in these genotypes. Majority of the inter-mated BILs are grouped into this cluster IV. Cluster I contain genotypes with moderate leaf rolling and drying and moderate drought recovery percentage. Genotypes in cluster III showed high leaf rolling and drying with moderate drought recovery. Genotypes in cluster V are highly susceptible to drought with poor leaf rolling, leaf drying and leaf senescence scores.

SUMMARY AND CONCLUSION

The results derived from the present field study on screening of BILs for drought tolerance revealed that 39 genotypes present in cluster II were found tolerant to drought while 29 genotypes present in cluster V were susceptible. The recipient parent ADT 45 exhibited moderate drought tolerance under field stress conditions. Based on all the physio-morphological traits, three BILs *viz.,* W 18, A 52 and I 45 exhibited best scores for leaf rolling, leaf drying, leaf senescence and drought recovery indicating that these BILs are high tolerant to vegetative stage drought stress. Hence, these genotypes may be concentrated and promoted as drought tolerant cultures. The BIL W 171 has very high drought scores however it recovers fast after re-watering. This genotype may be useful to study the physiological mechanism and inter relationship among the drought tolerant traits.

FUTURE SCOPE

The drought tolerant BILs identified by the present study of field screening can be forwarded to next generation and can be utilized for variety release programme or it can be used as a potential donor parent for drought tolerance with desired QTLs.

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