

Physiological Characterization of Trait Introgressed Lines of Rice (*Oryza sativa* L.) for Drought Stress Tolerance

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ABSTRACT

Twenty-five lines of rice which were introgressed for root traits and water use efficiency (WUE) were physiologically characterized for drought adaptive traits in the present study. These lines along with the parents were screened for yield and other trait performance under semi-irrigated aerobic cultivation. Leaf temperature was selected as surrogate for root traits and SCMR for WUE. Apart from these traits, yield and yield associated traits, relative water content (RWC), membrane stability index and chlorophyll content were measured. The lower leaf temperature of trait introgressed lines than IR-64 suggests lines have better root biomass which enabled them to maintain its temperature. The yield performance of trait introgressed lines were better than recurrent parent, IR-64 under semi-irrigated aerobic cultivation. It indicates that the introgression of the respective traits onto an elite background, IR-64 improved the efficacy of the introgressed lines under water limited condition. Comparative analysis of introgressed lines for drought adaptive traits suggested G 25, G 4, G 12 and G 18 performed better than other lines. Therefore, these lines introgressed for drought tolerance traits could be utilized for other breeding programs for further improvement in drought tolerance in rice for the development of elite genotypes.

Keywords: Rice, Drought, Root, WUE, RWC.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the major staple food crops of Asia. It belongs to Poaceae family with chromosome number $2n=24$. Out of the 25 species of genus *Oryza* known so far, only 2 species are being cultivated i.e., *Oryza sativa* and *Oryza glaberrima*. On view of its importance as one of the staple foods, its enhanced production is needed to feed the world's growing

population. Rice is water intensive crop (Bouman, 2007). Due to shortage of fresh water, various technologies have been developed to grow rice in considerable less amount of water. Among the various technologies semi-irrigated aerobic approach had shown great potential to save water with a significant compromise in yield (Bouman et al., 2000).

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Therefore, to avoid such compromise, one needs to develop or breed for varieties which grow well under aerobic condition without penalty in yield.

Several attempts have been made so far, but plant breeders selected yield as a trait under water limited conditions. Due to high G X E interaction and narrow variability for yield among the improved lines, further selection for yield was not effective (Araus et al., 2008, & Reynolds & Tuberosa, 2008). Targeting its component traits led to a great deal of success (Araus et al., 2008). Genetic enhancement of rice by component trait based breeding is one of the most appropriate strategies to tackle drought. Among the various component traits, water acquisition by roots, WUE, water conservation through epicuticle wax load and cellular level tolerance (CLT) proved to have great impact during water limited conditions. Therefore, introgression of root and WUE traits onto an elite background appears to improve the yield under semi irrigated aerobic conditions.

Developing aerobic worthy rice varieties with yield potential will help in maximizing rice production in the challenging environment of water scarcity. Mean yield and relative yield performance under well-watered and water limited conditions are the most widely used criteria for selecting genotypes for water limited environments. The ability of crop cultivars to perform reasonably well in water limited condition is paramount for stability of production. The combination of high yield stability and high relative yield under water limited condition has been proposed as useful selection criteria for characterizing genotypic performance under varying degrees of water stress.

MATERIALS AND METHODS

The experimental material comprised of 25 trait introgressed lines (the scheme for the development of trait introgressed lines is given in Fig. A) and the parental lines (AC-39020 (Root donor), IET-16348 (WUE donor) and IR-64 (recurrent parent). These materials were

raised in blocks of 1m*1m size with three replications of blocks in Randomized Completely Block Design (RCBD) at Department of Crop Physiology, University of Agricultural Sciences, GKVK, Bangalore during kharif 2017. Irrigation was provided alternatively by means of surface irrigation. Cultural operations such as protection measurements, accurate nutrient supply by fertilizer application were followed as per the recommendation. All necessary measures were taken to ensure the development of healthy plants.

The major goal of the present study was to physiologically characterize these lines and identify the superior lines with improved WUE and root traits. The parameters selected to meet the objective were chlorophyll content (SCMR value) (Surrogate for WUE), Relative water content (RWC), cell membrane stability (CMS), leaf temperature (surrogate for root traits) (LT), spikelet fertility (SF), yield per plant (YPP) and total dry matter (TDM). Nitrogen/Chlorophyll content was measured using the SPAD (Soil Plant Analysis Development) meter. This instrument measures the light attenuation at 430 nm (peak wavelength for chlorophyll a and b absorption) and at 750 nm (near infrared) with no transmittance. The value measured by chlorophyll meter is termed as SCMR (SPAD Chlorophyll Meter Reading) which is a unit less parameter. Three readings were recorded on each leaves using SPAD meter. Care was taken to avoid the interference of midrib and sensor should fully cover the leaf surface. Relative water content (RWC) is an important indicator of leaf water status. The leaf discs from the lines were collected separately and the fresh weight was measured and recorded. Leaf discs were floated on distilled water for 5 hours to attain full turgidity. Turgid weight was taken when the leaf discs were fully turgid. Later, leaf discs were dried in hot air oven at 70 °C to attain a constant weight and dry weight was recorded. RWC was calculated by equation as given by Barrs et al. 1962.

$$\text{RWC (\%)} = \{(\text{FW}-\text{DW}) / (\text{TW}-\text{DW})\} \times 100$$

where, FW- Fresh weight, DW- Dry weight, TW- Turgid weight

Cell membrane stability index/ membrane stability index (MSI) was calculated as

protocol given by Sairam et al. (2002) as follows:

$$\text{MSI} = [1 - (\text{EC1} / \text{EC2})] \times 100$$

Leaf temperature (surrogate for root traits) was measured using infra-red gun and expressed in degree celsius. Spikelet fertility was calculated by considering ratio of number of filled spikelets per panicle over total number of spikelets per panicle and expressed in percentage. Grain yield per plant (YPP) was measured at physiological maturity stage by taking weight of all the grains from one plant. Total dry matter (TDM) the biomass accumulated during the experimental period was computed by summing up leaf dry weight, stem dry weights and yield. The data were statistically analyzed by XLSTAT software. The means of trait introgressed lines were compared by Least significance difference (LSD) at 5 % level of significance.

RESULTS AND DISCUSSION

The main goal of the study was to phenotype 25 DCBC₃F₆ lines for drought adaptive traits along with the parents under semi-irrigated aerobic condition. Phenotypic data on various drought adaptive traits like SCMR (a surrogate for water use efficiency and chlorophyll content), leaf temperature (LT), yield attributes, spikelet fertility, relative water content and membrane stability index were recorded for each line along with the parents under semi-irrigated aerobic condition. The SCMR value of lines varied from 41.40 to 48.5 with a mean of 44.64. Yield per plant of IR-64, AC-39020 and IET-16348 was 15.43, 12.54 and 13.28 g pl⁻¹, respectively. Yield of introgressed lines varied from 18.26 to 24.66 g pl⁻¹. Total dry matter of IR-64, AC-39020 and IET-16348 was 65.10, 81.73 and 59.62 g pl⁻¹, respectively. However, total dry matter of introgressed lines ranged from 56.72 to 72.6 g pl⁻¹ with a mean of 65.10 g pl⁻¹. The yield attributes which determine the output of grain viz., spikelet fertility also differed considerably among the lines and parents. The

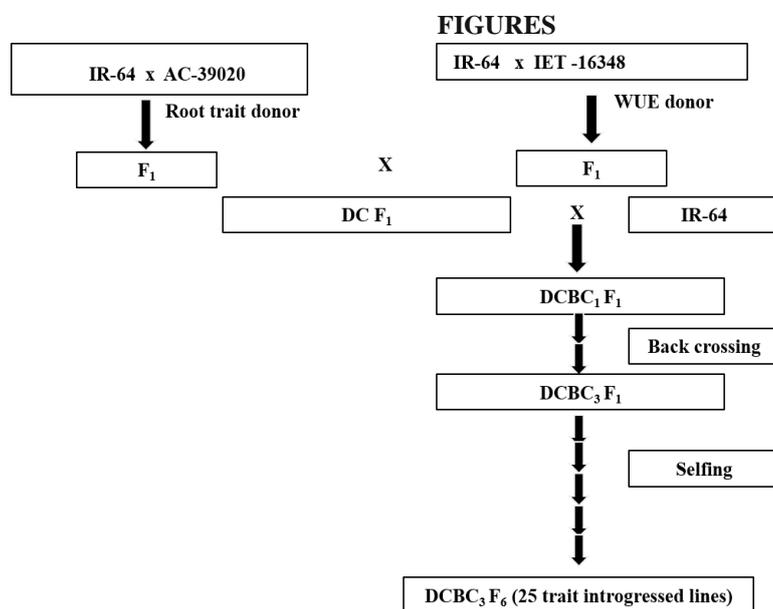
spikelet fertility percentage of lines varied from 80.71 to 94.54 *per cent* with a mean of 86.37 *per cent*. However, IR-64 had 75.65 % spikelet fertility. Relative water content (RWC) is the suitable measure of plant water status in terms of the physiological consequence of cellular water deficit. Relative water content of the tissue is primary factor affected under moisture stress. Relative water content of IR-64 and TILs differ significantly. RWC of lines varied from 85.24 to 89.92 *per cent* with a mean of 88.18 *per cent*. RWC value for IR-64 was 81.04 *per cent*. Leaf temperature (LT) of lines varied from 28.5 to 29.5 degree celsius with a mean of 28.98 degree celsius. LT value for IR-64 was 31.2 degree celsius. MSI also differed significantly between lines and IR-64.

Fig. B shows percent improvement of traits of trait introgressed lines over IR-64. IR-64 is recurrent parent, therefore, the traits has been compared with IR-64. Yield per plant for trait introgressed lines was 35.82, TDM (43.33), SF (14.17), RWC (8.81), SCMR (9.04), MSI (10.35) *per cent* improved than IR-64. Leaf temperature for introgressed lines was reduced to 7.10 *per cent* than IR-64. *Per cent* improvement over IR-64 for the traits like SCMR value, yield, leaf temperature and cell membrane stability signifies the importance of trait based breeding. Leaf temperature improvement over IR-64 suggests root traits has been improved for introgressed lines.

Drought stress/ Water limited condition suppresses leaf expansion and photosynthesis (Kramer & Boyer, 1995) and reduces photosynthetic rates and leaf area due to early senescence (Nooden, 1988). Chlorophyll pigment dictates photosynthetic capability of genotypes and generally used to quantify leaf senescence. The reduction in chlorophyll content reduces the carbon fixation which ultimately affects the

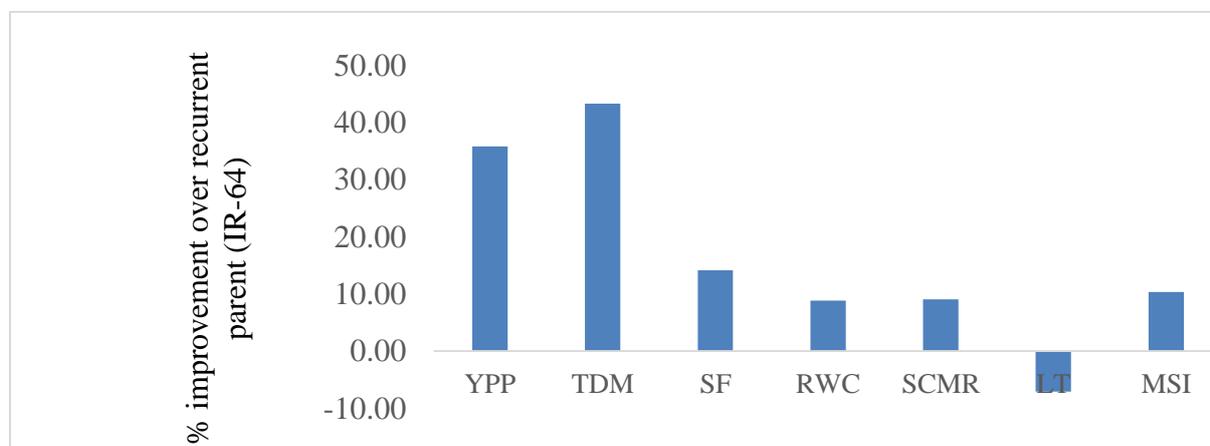
photosynthesis of genotype. Plasma membranes are one of the primary sites of water stress and MSI measure the electrolyte leakage due to cell membrane injury. Electrolyte leakage from plasma membranes is reported as one of the most important selection criterion for identification of drought tolerant plants. All of these factors are responsible for a reduction in dry matter accumulation and grain yield under drought. The drought tolerant variety expected to perform better under water limited condition by developing these traits. Higher RWC and a lower electrolyte leakage can be used as markers of membrane integrity under drought stress condition.

Comparative study of trait introgressed lines for physiological traits under aerobic condition had identified superior lines based on ranking of physiological traits. The trait introgressed lines G 25, G 4, G 12 and G 18 scored higher ranks followed by G 3, G6, G 13, G 114, G 15, G 21 and G 22 based on performance ranks (Table 1). Therefore, trait introgressed lines G 25, G 4, G 12 and G 18 identified for drought tolerance in the present study based on comparative study which could be used for further improvement in rice for drought tolerance in future breeding programme.



Prathibha, 2015

Fig. (A) The scheme for development of 25 Trait Introgressed Line



YPP-Yield per plant; TDM-Total Dry Matter; SF-Spikelet fertility; RWC-Relative Water Content; SCMR-SPAD value; LT-Leaf Temperature; MSI-Membrane Stability Index

Fig. (B) Per cent improvement of traits in trait introgressed lines over recurrent parent (IR-64)

Table 1: Comparative study of ranks of trait introgressed lines of rice based on physiological performance under aerobic conditions as compare to IR-64
% I- Per cent Improvement

Lines	RWC		SCMR		CMS		LT		SF		YPP		TDM		Repeated ranks					Total
	% I	Rank	% I	Rank	% I	Rank	% I	Rank	% I	Rank	% I	Rank	% I	Rank	1	2	3	4	5	
G 1	10.51	3	12.81	8	8.61	20	-6.41	17	89.67	6	42.06	7	25.74	23			1			1
G 2	9.48	11	7.64	17	10.6	6	-7.69	10	91.57	4	40.44	10	33.47	21				1		1
G 3	10.25	5	5.67	20	11.3	1	-6.73	16	88.62	8	20.29	24	25.69	24	1				1	2
G 4	8.00	18	19.46	1	10.3	7	-5.77	21	92.89	3	38.43	12	54.45	5	1		1		1	3
G 5	9.07	14	2.22	24	10.7	3	-6.41	18	83.56	19	26.31	21	40.31	16				1		1
G 6	9.87	8	11.08	10	9.46	13	-8.65	2	85.22	13	59.82	1	44.76	11	1	1				2
G 7	7.90	19	6.65	19	8.37	21	-8.01	7	83.97	18	45.69	4	41.30	15				1		1
G 8	7.60	20	8.87	15	8.23	24	-7.69	9	85.12	14	44.98	6	24.88	25						0
G 9	10.25	4	13.55	7	9.24	15	-8.01	8	84.76	15	35.71	13	36.06	20				1		1
G 10	9.37	12	4.68	23	8.31	22	-8.33	6	81.50	22	34.48	14	39.08	18						0
G 11	9.83	10	10.10	14	9.15	18	-7.37	11	84.23	17	23.14	22	31.66	22						0
G 12	9.16	13	5.42	22	10.6	5	-6.73	15	90.64	5	27.74	18	59.11	2		1			2	3
G 13	10.70	2	7.64	16	9.21	16	-7.37	12	88.17	9	51.98	2	53.35	6		2				2
G 14	9.86	9	5.67	21	7.17	25	-8.33	4	93.21	2	21.84	23	53.04	7		1		1		2
G 15	9.01	16	1.97	25	9.05	19	-8.65	1	94.52	1	18.34	25	42.51	13	2					2
G 16	5.18	25	15.02	4	9.39	14	-5.77	20	89.35	7	40.51	9	39.63	17				1		1
G 17	6.19	23	11.08	11	10	9	-5.45	25	86.34	11	29.94	17	42.12	14						0
G 18	10.96	1	10.34	12	8.28	23	-8.33	5	85.59	12	50.36	3	46.48	9	1		1		1	3
G 19	10.02	7	13.79	5	9.72	11	-5.45	24	84.33	16	26.51	20	43.57	12					1	1
G 20	8.64	17	7.14	18	9.2	17	-5.77	19	82.22	21	38.95	11	51.70	8						0
G 21	5.45	24	13.79	6	10.6	4	-7.37	14	87.45	10	41.35	8	59.84	1	1			1		2
G 22	7.10	21	11.82	9	10.9	2	-5.77	22	80.71	25	30.59	16	57.75	3		1	1			2
G 23	9.06	15	16.26	2	9.62	12	-5.45	23	83.41	20	27.41	19	44.85	10		1				1
G 24	10.13	6	10.34	13	9.94	10	-7.37	13	80.77	24	33.12	15	37.14	19						0
G 25	6.69	22	15.52	3	10.1	8	-8.65	3	81.34	23	45.50	5	54.82	4			2	1	1	4

CONCLUSION

In the present study G25, G 4, G 12 and G18 performed better than the other introgressed lines and IR-64 under aerobic condition. These lines could be used for further breeding programmes or released as variety after field trials in drought prone areas.

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