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Analysis of Trait Introgressed Back Cross Progenies to Identify Superior Lines for Aerobic Cultivation in Rice (*Oryza sativa* L.)

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Abstract

Markers assisted back cross ($DCBC_3F_2$, Double Cross Back Cross) progenies introgressing root traits and WUE,were screened for yield performance under semi-irrigated aerobic cultivation.Based on the initial vigour 100 plants were selected for phenotyping for yield related traits. Effective surrogates such as leaf temperature for root traits, SCMR for WUEwere used for the identification of promising trait introgressed lines. The trait introgressed plants showed better yield than the recurrent parent, IR-64. Based on the variability in trait introgression, a few highly promising lines were selected using 16 traitsassociated polymorphic SSR markers. The molecular analysis suggests that the selected transgressiveseggregantswere having allelesintrogressed from the donor parents of the respective traits.

Keywords : Rice, introgression, SSR marker, superior, root, WUE.

Rice (*Oryza sativa* L.) is the major staple food for more than half of the world population. The consumption is projected to increase to 490 million tons in 2020 and to about 650 million tons by 2050 globally (Annon., 2016). These environmental crises have been the factors that decrease rice production around the world (IPCC, 2013). Drought is one of the major factors that target the rice yield (Fischer *et al*., 2012). Therefore, the challenge before the plant scientists is to increase the yield by minimizing the impacts of climate change through the adoption of modern molecular breeding approaches.

Plants have adopted several drought tolerance mechanisms to overcome drought effects ranging from cellular level to whole plant level. It maintains growth by maintaining tissue water relations and positive carbon gain by mining and providing water from deeper soil profiles (Farooq *et al.,* 2009). Water content of the leaf (turgor) and carbon gain under water limited conditions strongly dictates crop productivity. Turgidity of the leaf is strongly associated with water mining properties (Deep root, cooler canopy) and water use efficiency of the plant. It has been proven that, if these traits are pyramided together, the crop productivity increases (Raju *et al.,* 2014). This can be achieved by bringing together various drought adaptive traits with reasonably high acquired tolerance traits also referred to as cellular level tolerance (CLT) on to an elite genetic background.Although a conventional breeding approach to combine drought adaptive traits such as root and WUE led to the development of a significantly higher yielding cultivar namely KMP-175 (Sheshshayee *et al.,* 2011), introgressing such physiological traits are extremely difficult through the conventional breeding programmes. Therefore, a more focused marker assisted breeding strategy needs to be adopted.

Material and Methods

The major goal of the present study was to analyse backcross progenies and identify the superior lines with improved WUE and root traits for aerobic cultivation. Plant vigour is one of the main characters which are selected under semi-irrigated condition. Based on the initial vigour, 100 $DCBC_3F_2$ plants (the scheme for the development of $DCBC_3F_2$ is given in Fig. 1.) were selected for phenotyping. The parameters measured were chlorophyll content (SCMR value), leaf temperature (surrogate for root traits), tiller number, yield per plant (YPP) and total dry matter (TDM).

Chlorophyll content was measured using the SPAD meter (Soil Plant Analysis Development). The instrument measures the light attenuation at 430 nm (the peak wavelength for chlorophyll a and b absorption) and that at 750 nm (near infrared) with no

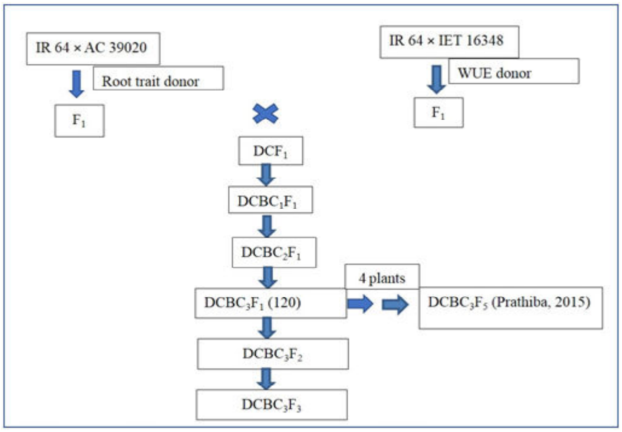

Fig. 1: The scheme for development of Marker Assisted Back Cross progenies

transmittance. The unit less value measured by the chlorophyll meter is termed as SCMR (SPAD Chlorophyll Meter Reading) which is a good estimate of chlorophyll content and therefore N content. Three readings were recorded on each leaves using SPAD chlorophyll meter. Necessary care was taken to avoid the interference of the midrib and sensor fully cover the leaf surface. Leaf temperature of the plants was measured using infra-red gun and expressed in degree celsius. Observations recorded just before irrigation twice during the crop cycle (mean values ofobservations were used for analysis). Infra-red gun was held pointing towards the leaf surface and the measurements were made at the midday when there was bright sunlight. Tiller number was computed by counting the number of productive tillers per plant. Grain yield per plant (YPP) was measured at physiological maturity by taking weight of all the grains from the 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.

10 $DCBC_3F_2$ plants were selected based on yield for molecular analysis. Genomic DNA was extracted from the leaves of 4 weeks old plants of the donor (AC-39020 for root and IET-16348 for WUE) and recipient parents (IR-64) and the selected progenies by using the CTAB (CetylTrimethyl Ammonium Bromide) method. Using Biospec-nano (Spectrophotometer for life science), advanced automated DNA quantifier, the ratios between 260 nm and 280 nm was estimated and used to estimate the DNA purity. A ratio of 1.8 to 2.0 for pure DNA samples is standard. PCR was performed in 15µl reactions containing 25ng of DNA template, 1.5µl Taq buffer (1X), 1.5µl of dNTPs (3.0mM), 0.3µl of $MgCl_2$ (2mM), 1.5 µL (5 pmole. µL^{-1}) each forward primer and reverse SSR primers, 1U of Taq polymerase and 7.40 µL of sterile water. Based on polymorphism among the three parents, 16 associated SSR markers were selected from previous study (Prathiba, 2015) as foreground markers for trait introgression. The 16 SSR markers (12 for roots and 4 for $\Delta^{13}C$) were used to screen the selected progenies along with the parents in the present study (Table I).

TABLE I

List of markers used for foreground selection

Trait	Trait component	Marker	Chr. No.	Position on chromosome (cM)
Root	RLD	RM80	8	103.7
	RWT	RM2584	8	45.8
	RLD	RM1388	4	77.9
	RLD	RM262	2	81.1
	R/S	RM239	10	25.2
	RV	RM3825	1	143.7
	RV	RM16	3	131.5
	RL	RM3276	4	102.4
	RV	RM247	12	32.3
	RLD	RM167	4	37.5
	RV	RM4455	10	21.8
	/S	RM71	2	49.8
Ä^{13}C	Ä^{13}C	RM493	1	79.9
	Ä^{13}C	RM586	6	7.4
	Ä^{13}C	RM149	8	122.1
	Ä^{13}C	RM131	4	148.8

A total of 800 $DCBC_3F_2$ plants were screened under semi irrigated aerobic conditions. Based on the early seedling vigour 100 $DCBC_3F_2$plants were selected for phenotyping.The parameters considered for phenotyping were SCMR value (chlorophyll content), canopy temperature (surrogate parameter for root traits), number of tillers, yield per plant and total dry matter. Further, advancing and characterisation of TILs depends on the identification of appropriate promising lines. Therefore, trait introgressed lines were selected based on marker data (foreground selection) and phenotyping data (grain yield and total dry matter).

RESULTS AND DISCUSSION

The SCMR value of 100 plants varied from 31.7 to 49.5 with a mean of 40.96. Tiller number of IR-64, AC-39020 and IET-16348 was 20, 11 and 25 per plant, respectively. The tiller number of 100 plants ranged from 9 to 37 per plant with a mean of 23 per plant. Yield per plant of IR-64, AC-39020 and IET-16348 was 20.45, 17.25 and 19.15 g pl^{-1}, respectively. Yield of 100 plants varied from 10 to 23 g pl^{-1} with a mean of 18.92. Total dry matter of IR-64, AC-39020 and IET-16348 was 51.05, 83.45 and 58.65 g pl^{-1}, respectively. The total dry matter of 100 plants ranged from 15 to 79 g pl^{-1} with a mean of 55.84 g pl^{-1}. Leaf temperature of IR-64, AC-39020 and IET-16348 was 35.7, 31.1 and 33.3 ^{0}C, respectively. Leaf temperature of 100 plants ranged from 30.90 to 35.90 ^{0}C with a mean of 33.91^{0}C.

Based on the yield per plant 10 lines were selected which were having lower leaf temperature, as leaf temperature is surrogate for root traits, and higher total dry matter. The selected lines were having better drought adaptive traits as these lines are having more SCMR value, cooler leaf and also enhanced yield associated parameters than the recurrent parent IR-64 and other lines of the same population (Fig. 2).

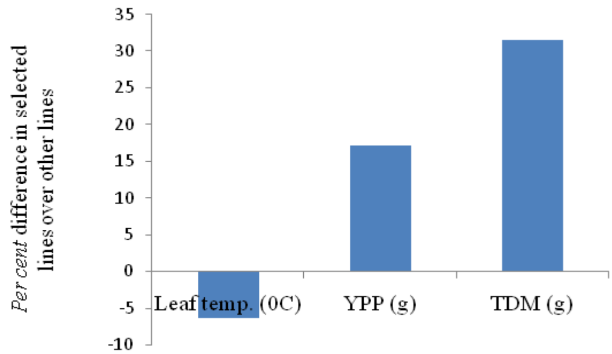

Fig. 2: Per cent difference in traits of the selected lines in comparison with non-selected lines

SCMR values varied from 34.7 to 46.7, leaf temperature 30.9 to 32.9^{0}C, TDM 64.90 to 78.65 g and YPP 20.95 to 23.05 g (Table II). It implies that the selected introgressed lines have better root system and cellular level tolerance mechanisms due to which these lines were performed better under semi-irrigated aerobic cultivation and shows the improved traits values. The

TABLE II

Comparison of selected trait introgressed lines with the parents for biomass and yield related parameters in $DCBC_3F_2$ generation

Plant No.	SCMR	Leaf temp. (°C)	Panicle per plant (#)	TDM (g)	YPP (g)
IR-64	39.9	35.7	20	51.05	20.45
AC-39020	37.6	31.1	11	83.45	17.25
IET-16348	38.5	33.3	25	58.65	19.15
37	46.7	31.6	26	64.90	21.05
38	43.9	31.8	24	75.35	20.95
40	41.9	30.9	37	69.05	22.30
45	41.7	32.2	33	72.40	22.05
49	43.7	31.3	32	68.85	21.70
61	42.5	32.7	32	74.65	20.95
65	44.9	31.8	29	73.55	23.05
69	34.7	31.8	28	69.55	22.55
72	41.5	32.5	25	78.65	21.60
75	34.9	32.9	21	65.35	21.75

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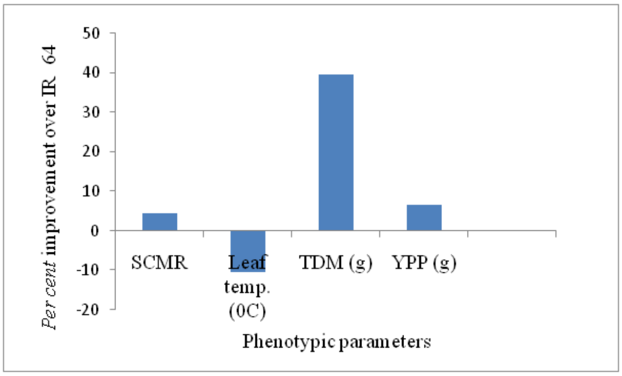

Fig. 3: Per cent improvement of traits for selected lines over the recurrent parent

parental lines differed significantly for all the traits specially leaf temperature and total dry matter.

The selected lines (mean of the 10 lines) show 10 per cent lower leaf temperature than the recurrent parent IR-64 (Fig. 3). This signifies that the selected lines were having improved root traits, due to which these lines were able to explore more water from the deeper layer of soil and hence maintain lower leaf temperatures by transpiring more. Ability to extract water from deeper soil profiles is an extremely important determinant of crop growth under water limited conditions. Identification of TILs with good root system under semi-irrigated aerobic condition holds the key for success in crop improvement programmes.

Further, these lines show 40 per cent improvement over recurrent parent for total dry matter. It implies that these lines were able to gain more carbon than IR-64 either by means of more water use efficiency or by keeping stomata opens for longer time or combination of both. There was significant improvement for SCMR value of the selected TILs over recurrent parent. This suggests that the TILs were able to maintain better chlorophyll content under aerobic conditions, which is also needed for more vigorous growth. Hence, the phenotypic characterization proved that the selected lines were having drought adaptive traits.

TABLE III

Foreground marker distribution of the selected DCBC$_3$F$_2$ lines

Trait	Marker	45	61	49	37	40	65	75	69	38	72
Root	RM3825	-	✓	✓	-	-	-	-	✓	-	-
Root	RM262	✓	-	-	-	✓	-	-	✓	✓	✓
Root	RM71	✓	✓	-	✓	✓	✓	-	-	-	-
Root	RM16	-	-	✓	✓	-	✓	✓	-	-	-
Root	RM1388	-	✓	-	✓	✓	-	-	-	-	-
Root	RM3276	✓	✓	-	-	✓	✓	✓	-	-	-
Root	RM167	✓	-	-	✓	✓	-	-	✓	✓	-
Root	RM80	✓	-	✓	-	-	✓	✓	-	-	✓
Root	RM2584	-	-	✓	✓	✓	✓	✓	-	-	-
Root	RM4455	-	✓	-	-	-	-	-	-	✓	-
Root	RM239	✓	-	-	✓	-	✓	✓	✓	-	-
Root	RM247	✓	✓	✓	-	✓	-	-	-	-	-
$\Delta^{13}C$	RM493	✓	-	✓	✓	-	-	-	-	✓	-
$\Delta^{13}C$	RM131	-	✓	✓	-	-	-	-	-	-	✓
$\Delta^{13}C$	RM586	✓	✓	-	-	-	✓	✓	✓	-	-
$\Delta^{13}C$	RM149	-	✓	✓	-	-	-	✓	-	-	✓
	Total (#)	**9**	**9**	**8**	**7**	**7**	**7**	**7**	**5**	**4**	**4**

The marker distribution indicated that the number of foreground markers varied from four alleles to nine alleles per plant. The foreground marker distribution of the selected 10 lines is given in Table III. The plants with higher foreground as well as higher yield and cooler leaf temperature were selected. It suggests that the lines with more proportion of introgressed traits / markers performed better in terms of yield performance under semi-irrigated aerobic condition.

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Molecular characterization leads to the identification of plants with higher foreground markers. Physiological parameters support the results of molecular characterization. Based on molecular and phenotyping data, 10 TILs with improved drought tolerance were identified.

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