RESEARCH PAPER

Evaluation of advanced back cross lines for drought tolerance in rice (*Oryza sativa* L.)

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Abstract : Drought is the largest abiotic constraint to rice production which cause significant yield loss depending upon the severity. Development of rice varieties with tolerance to drought and high use water use efficiency is the need of the hour. Despite the importance of drought as major abiotic constraint, the efforts to develop drought tolerant rice varieties are very low. Breeding efforts until recent past were focused on understanding and improvement of secondary traits that are putatively associated with drought tolerance. However, the genetic gain in yield by improvement of secondary traits is very low. Hence, improvement of yield per se under drought conditions will be better solution. Introgression of yield QTLs under drought in the genetic background of high yielding varieties will be helpful to overcome the problem to a certain extent. In the present study, 31 advanced back cross lines (BILs) derived from drought susceptible mega variety Samba Mahsuri (BT 5204) and a drought tolerant tolerant land race Azucena were evaluated under drought. Thirty one advanced back cross inbred lines (BC_2F_4) lines having yield QTLs viz., *qDTY3.1* on chromosome 3 and *qDTY2.1* on chromosome 2 were phenotyped under drought conditions. The results suggested that wide range of variation was observed for yield and its component traits in the BILs generated in the background of BPT 5204 and direct selection for yield under water stress coupled with marker assisted screening would help in development of drought tolerant version of mega varieties with improved yield under stress. Thermo tolerance studies indicated that high variability was observed for the BILs in terms of % seedling survival, % reduction in root and shoot growth under stress.

Key Words : Drought, Thermo tolerance, Rice, BILs, Yield

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important food crops grown worldwide and is the staple food for more than half of the world's population. India is the second largest rice-producing country in the world, but the productivity is low compared to other countries like

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China, Thailand etc. The major reason for dismal productivity of rice in the country is due to abiotic and biotic stresses. One of the major abiotic constraints responsible for declining rice production is drought stress. Drought is the most devastating factor and it depresses yield by 15 - 50% depending on the vigor and period of stress in rice. A total of 34 million ha of rainfed lowland and 8 million ha of upland rice in Asia suffers from drought stress of varying intensities every year (Wopereis *et al*., 1996). Drought is the most imperative and major limitation for production and productivity particularly in rainfed ecosystems (Nelson *et al.,* 2014, Pandey and Shukla, 2015). In India, 13.6 million hectare rice area is affected by drought with varying intensity.

Drought tolerance is considered as complex trait. Detailed studies of drought tolerance have enabled us to understand the physiological responses of plants, although our understanding of its genetic basis remains poor. Like yield and its components, traits that confer tolerance to drought are directly or indirectly controlled by many genes, with each contributing a relatively small effect on the overall phenotype. To achieve high production in rainfed areas, there is an urgent need to breed varieties with drought tolerance, and genetic improvement for drought tolerance should be a high priority theme of research in the current scenario (Panda *et al.,* 2021). Most of the past efforts to combine drought tolerance in high yield backgrounds have been a futile exercise, largely because of its genetically complex nature and lack of precise screening or selection protocols. Secondary traits like water stress, canopy temperature, leaf rolling, leaf drying, drought recovery contributes for drought tolerance (Kamoshita *et al*., 2008). Indirect selection for drought tolerant genotypes using secondary traits resulted in limited success compared to irrigated ecosystem. Availability of high density marker linkage maps, annotated genome sequence, and powerful biometric methods has enabled identification and use of trait-specific genes/QTL for directed improvement of rice. Potential markers and gene clusters have been identified for the various morpho-physiological indices of tolerance to moisture stress in rice (Lilley and Ludlow 1996; Lafitte *et al*., 2006, Bernier *et al.,* 2007 and Zhao *et al*., 2008).

In the current situation, a sustainable approach to increase food production and decrease losses due to abiotic stresses particularly drought is required. One such approach is use of molecular markers to increase the efficiency of breeding to develop varieties which can endure drought. With the availability of large number of molecular markers linked to QTLs controlling drought tolerance Marker-assisted-backcross breeding (MABB) can be utilized as a potential approach Recently, QTLs with large effects on grain yield under drought stress, viz. *DTY1.1*, *DTY2.1*, *DTY2.2*, *DTY3.1*, *DTY3.2*, *DTY9.1* and *DTY12.1* have been identified from different landraces of rice *viz.,* Nagina 22, Apo, Adey Sel, Vandana, explaining 31-77% of the phenotypic variance.

Hence, present study was aimed to evaluate advanced back cross lines (BC_2F_4) derived from drought susceptible mega variety of ANGRAU Samba Mahsuri (BPT 5204) and tolerant Azucena for direct selection of yield under drought. Further these were evaluated under laboratory conditions for thermo tolerance.

MATERIAL AND METHODS

Screening of BILs for drought tolerance:

An experiment was initiated at RARS, Maruteru with an objective to introgress drought tolerant QTLs into mega variety BPT 5204. The mega variety BPT 504 which is drought susceptible was used as recurrent parent and Azucena, a traditional landrace of which is drought tolerant was used as donor. The F_1s generated were phenotyped and genotyped and were backcrossed twice to the recurrent parent BPT 5204 and were selfed thrice to generate BC_2F_3. Fifty advanced back cross lines were evaluated under drought conditions at Regional Agricultural Research Station, Maruteru. Based on the morphological data and foreground selection thirty one BILs (BC_2F_4) were selected and these were evaluated for confirmation. The experiment was laid out with two replications. Each line has 2 rows of 5m length with spacing of 20 cm between rows and 15 cm between

Fig. A : Phenotyping of BILs (BC_2F_4) of BPT 5204/Azucena for drought

Table A : Mean soil moisture % of stress period during the evaluation of the lines							
		Soil moisture (%) at different depths					
Situation	Stress	10 cm		20 cm		30 cm	
		I	A	I	A	I	A
Drought stress	50 days after transplanting to till harvest	76	9	79	12	80	22

I: Initial soil moisture % before stress   A: After stress Soil moisture %

plants. Field bunds were covered with thick polythene sheet to 1m depth to avoid seepage of water. Drought stress was imposed at 50 days after transplanting (booting stage) till the harvest of the crop. Data on yield and its components *viz.,* days to 50 % flowering, plant height in cm, ear bearing tillers per plant, Panicle length and spikelet fertility, 1000 grain weight and grain yield per plot converted to kilogram per hectare were recorded. (Fig. A).

The moisture condition of the soil was estimated using soil moisture tension meter (Delta T devices) at different depths before imposing the stress and after imposing the stress (50 days after transplanting) and data was furnished in Table A.

Screening of BILs for thermo tolerance using thermo induction response technique:

TIR approach involves the induction of high temperature followed by identification of promising genotypes. Phenotyping of rice genotypes for thermo tolerance was done as per Sudhakar *et al*., 2012 with certain modifications.

Surface sterilization of paddy seeds was done by treating with 0.1 per cent carbendizim for 30 minutes followed by washing with distilled water for 4-5 times followed by soaking in 1 per cent sodium hypochlorite for 1-2 minutes. After that the seeds were washed with distilled water for 4-5 times and kept for germination at 30 ^{0}C and 60 % relative humidity in the incubator. After 42 hours, in each genotype the uniform seedlings were selected and were transferred in aluminium trays (50 mm) filled with fertilized soil. The uniform seedlings in the trays were segregated into three sets. First set of seedlings in the aluminum trays were subjected to sub lethal temperatures (gradual temperature increasing from 36 ^{0}C – 52 ^{0}C for 5 hours in the environmental chamber ("WGC-450" Programmable Plant Growth Chamber). Treatment with sub lethal temperatures was followed by treatment with lethal temperatures (55 ^{o}C) (induced) for 2 hours. Second set of seedlings were directly exposed to lethal temperatures (non induced). Third set was the control with no exposure to temperature treatments.

Induced and non induced rice seedlings were allowed to recover at 30 ^{o}C and 60 per cent relative humidity for 48 hours.

The following parameters were recorded from the seedlings:

$$\text{Per cent survival of seedlings} = \frac{\text{Number of seedlings survived at the end of recovery}}{\text{Total number of seedlings sown in the tray}}$$

Per cent reduction in root growth = Actual root growth of control seedlings - (Actual root growth of treated seedlings / Actual root growth of control seedlings) x 100

Per cent reduction in shoot growth = Actual shoot growth of control seedlings – (Actual shoot growth of treated seedlings / Actual shoot growth of control seedlings) x 100

Foreground selection with linked markers:

The molecular evaluation of the BILs was taken up at Rice Biotechnology laboratory of RARS, Maruteru. Fresh leaf samples were collected from young leaves and DNA was extracted from the leaf samples Cetyl Trimethyl Ammonium Bromide (CTAB) method (Murray and Thompson, 1980) using Qiagen tissue lyser with certain modifications. The quality and quantity of DNA was estimated using Nanodrop spectrophotometer and was also checked on 0.8% agarose gels with standard DNA marker. Two SSR markers linked to yield QTLs under drought RM 520 and RM 236 were used for PCR amplification. PCR amplifications were performed in 10 µl of reaction mixture containing 1 µl of 10X buffer with MgCl$_2$ 0.5 µl of dNTPs (25 m M. L^{-1}), 1 µl (5 µ molar) each of forward and reverse primers, 1 µl Taq DNA polymerase (0.5 U/micro litre), 3 µl of template DNA (10 ng/ µl) and 2.5 µl of sterilized distilled water. The polymerase chain reaction was performed by using Eppendorf thermo cycler with the following temperature profiles. The initial denaturation was at 94°C for 5 min, followed by 35 cycles of denaturing at 94 ^{o}C for 0.5 min, annealing at 55 ^{o}C for 0.5 min, extension at 72 ^{o}C for 1.0 min and 7 min at 72 ^{o}C for the final extension. The PCR products were electrophoresed in 3% agarose gel at 100 volts for 2 hrs in 1X TAE buffer. A 100 bp lader (Genei) was used for appropriate sizing of the products. The gel

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was photographed under UV light using Ingenius gel doc system.

RESULTS AND DISCUSSION

Mega variety BPT 5204 was crossed to Azucena for introgression of yield QTLs under drought into BPT 5204. The backcrosses were taken up and the handling of the population was presented in Table 1.

Based on the phenotyping and genotyping results in BC_2F_3, thirty one lines were selected from 50 lines and were evaluated for yield and its contributing traits under drought. Significant variation was observed among the thirty one BILs for all the traits studied under stress. Twenty six BILs (BC_2F_4) lines co segregating for simple sequence repeats (SSRs) linked to yield QTLs under stress *viz.*, RM 520 linked to DTY 3.1 on chromosome 3 and RM 236 linked to DTY 2.1 on chromosome 2 out of 31 lines were selected and promoted to yield trials. (Fig 1).

Days to 50% flowering :

The DFF ranged from 91 days (2199-43-1, 2199-44-1 and 2199-65-1) to 112 days (2199-54-1) with a mean of 97 days in BILs whereas it was 102 days in the recurrent parent BPT 5204 and 86 days in Azucena. (Table 2).

Plant height :

A mean plant height of 113 cm was recorded in BILs with highest in 2199-73-1 (135 cm) and was lowest in 2199-70-2 (100 cm) while the parents BPT 5204 and Azucena recorded 106 and 126 cm, respectively.

Ear bearing tillers/m2:

A wide range of variation was observed in the number of ear bearing tillers per m2 from 178 (2199-178-1) to 363 (2199-46-1) with a mean of 264 tillers. The recurrent parent BPT 5204 recorded 162 tillers while it was 242 in the donor parent Azucena.

Panicle length:

The panicle length ranged from 18.6 cm (2199-70-1) to 28.5 cm (2199-65-1) with a mean of 25 cm while BPT 5204 had a panicle length of 14.2 cm and Azucena had 18.6 cm.

Spikelet fertility:

Spikelet fertility is one of the key components for yield under stress. The spikelet fertility ranged from 62.71% (2199-379-1) to 93.15% (2199-22-1) in the BILs with a mean of 82.0% and BPT 5204 had 64.2% fertility and Azucena had 82.6% spikelet fertility.

1000 grain weight:

A mean test weight of 26 g was recorded in BILs with highest test weight in 2199-153-1 (28.6 g) and lowest in 2199-403-1 (16.8 g) and the donor parent Azucena had 18.24 g and BPT 5204 had 14.38 g test weight.
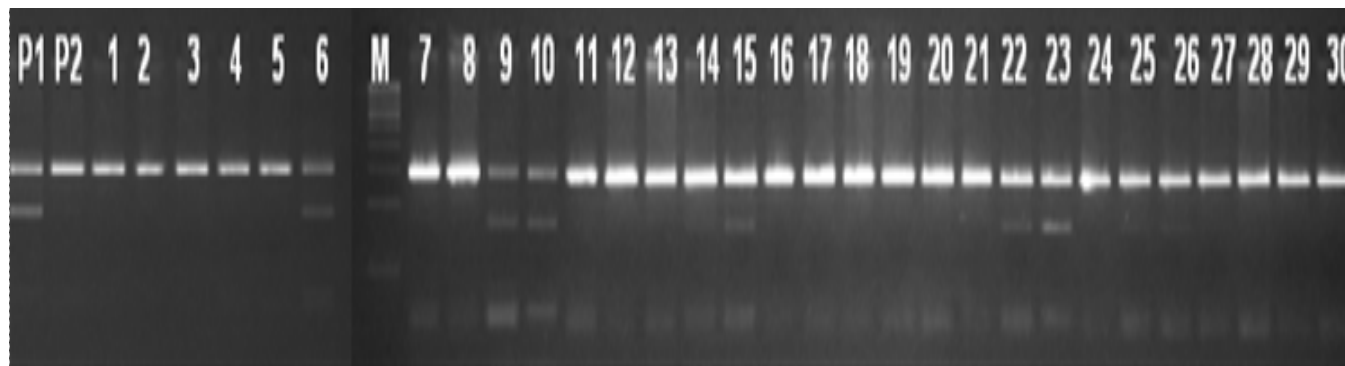
Fig. 1 : Phenotyping of BILs (BC_2F_4) of BPT 5204/Azucena for drought

Table 1 : Phenotyping and genotyping of mapping population

Markers selected for fore ground selection	Association with QTL/gene	No. of plants selected at BC_2F_1	No. of plants studied in BC_2F_2	No. of plants selected in BC_2F_2	No. of progenies selected in BC_2F_3	No. of progenies selected in BC_2F_4	Preliminary trial
RM 520	DTY 3.1	5 out of 24	2000	200	31 out of 50	26 out of 31	26 entries
RM 236	DTY 2.1	plants					studied

Grain yield:

A wide range of variation was observed in grain yield in the BILs ranging from 2530 kg/ha (2199-70-2) to 5948 kg/ha (2199-54-1) with a mean grain yield of 4811 kg/ha while the recurrent parent recorded 2890 kg/ha and the donor gave 4120 kg/ha (Table 2). Similar genetic variation for yield and its components under water stress have been reported earlier (Kumar *et al.*, 2008, Gouda *et al.,* 2012 and Girija Rani *et al.,* 2013).

Screening of BILs for thermo tolerance:

The thirty one BILs were screened for thermo tolerance using TIR technique and the genotypes showed significant genetic variability for per cent survival of seedlings, per cent reduction in root and shoot growth, respectively.

Per cent survival of seedlings:

The per cent seedling survival ranged from 40

Table 2 : Evaluation of BILs for yield and its component under drought

Sr. No.	Entry	Days to 50% flowering	Plant height (cm)	Ear bearing tillers/m2	Panicle length (cm)	Spikelet fertility (%)	1000 Seed Wt. (g)	Grain yield (kg/ha)
1.	2199-54-1	112	108.4	317	24.44	89.71	26.2	5948
2.	2199-178-1	97	127.0	178	26.68	89.65	27.4	5872
3.	2199-83-2	109	124.4	244	25.28	88.70	22.6	5816
4.	2199-73-1	95	135.0	264	26.52	88.95	27.0	5792
5.	2199-44-1	91	116.2	257	25.80	88.66	26.8	5780
6.	2199-46-1	96	112.0	363	24.88	87.24	26.8	5769
7.	2199-52-1	96	114.4	297	25.78	81.69	27.6	5758
8.	2199-35-1	93	112.2	264	26.22	88.05	26.2	5721
9.	2199-153-1	107	124.6	264	25.42	86.44	28.6	5706
10.	2199-53-1	95	112.4	238	27.04	84.19	25.6	5560
11.	2199-43-1	91	113.0	224	20.10	90.15	28.0	5528
12.	2199-47-1	95	114.2	337	26.30	74.19	25.6	5520
13.	2199-88-1	95	112.8	231	27.14	80.36	27.8	5370
14.	2199-65-1	91	112.0	257	28.46	82.31	27.6	5320
15.	2199-41-1	96	110.8	238	25.10	78.42	26.4	5230
16.	2199-16-1	95	109.6	277	26.96	79.74	26.0	4980
17.	2199-95-1	105	127.6	323	22.88	83.67	25.2	4960
18.	2199-50-1	95	113.8	297	25.04	80.90	26.0	4940
19.	2199-5-1	95	106.6	271	26.34	70.99	27.6	4930
20.	2199-153-2	93	110.6	271	23.60	87.31	25.4	4910
21.	2199-22-1	93	110.8	218	24.90	93.15	27.8	4640
22.	2199-7-1	96	108.8	244	26.82	81.32	25.6	4370
23.	2199-6-1	96	104.0	224	25.94	86.00	26.8	3990
24.	2199-4-1	95	104.0	297	25.86	76.42	27.4	3870
25.	2199-403-1	98	102.6	218	21.04	86.60	16.8	3810
26.	2199-379-1	101	104.0	224	24.04	62.71	26.4	3670
27.	2199-83-1	97	122.8	218	24.52	70.58	22.6	3460
28.	2199-62-1	96	115.2	337	25.70	72.10	26.2	3400
29.	2199-174-1	96	102.2	257	25.90	74.60	25.0	3310
30.	2199-70-1	96	108.6	231	18.62	83.33	21.6	2690
31.	2199-70-2	99	100.0	297	20.82	73.93	17.8	2530
	Mean	97	112.9	264	24.97	82.00	25.6	4811
	BPT 5204	102	105.6	162	14.16	64.20	14.3	2890
	Azucena	86	126.4	242	18.64	82.64	18.2	4120

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(2199-62-1) to 100 % (2199-6-1, 2199-54-1, 2199-70-2, 2199-95-1, 2199-153-1, 2199-174-1 and 2199-178-1). The parents BPT 5204 and Azucena recorded 50 and 100 % survival respectively (Table 3).

Per cent reduction in root growth :

The per cent reduction in root growth ranged from nil to nearly 50% in the BILs. Out of 31 BILs, twenty two BILs had no reduction in root growth in the treatment when compared to control. Highest reduction in root growth was observed in 2199-6-1 (48.2%) while BPT 5204 had 29.5% reduction in root growth and Azucena had nil reduction.

Table 3: Evaluation of BILs for thermo tolerance using TIR technique

Sr. No.	Entries	% Survival of seedlings	Root length		Shoot length		% reduction in root growth	% reduction in Shoot growth
			Control	Treatment	Control	Treatment		
1.	2199-6-1	100	4.83	2.50	12.30	9.70	48.2	21.1
2.	2199-7-1	50	3.40	2.50	7.00	7.00	26.5	0.0
3.	2199-44-1	60	2.70	3.00	6.00	6.40	0.0	0.0
4.	2199-54-1	100	2.70	4.33	8.00	8.50	0.0	0.0
5.	2199-65-1	90	3.00	2.00	8.87	7.70	33.3	13.2
6.	2199-70-1	90	2.83	3.30	11.70	10.70	0.0	8.5
7.	2199-83-2	100	3.00	2.73	7.70	7.50	9.0	2.6
8.	2199-178-1	100	3.00	3.00	8.20	8.40	0.0	0.0
9.	2199-4-1	80	2.70	3.40	9.00	6.00	0.0	33.3
10.	2199-5-1	90	2.00	3.40	7.70	6.40	0.0	16.9
11.	2199-16-1	70	3.40	2.40	8.00	5.40	29.4	32.5
12.	2199-22-1	70	1.83	2.50	8.00	5.70	0.0	28.8
13.	2199-35-1	50	2.50	2.20	9.70	3.20	12.0	67.0
14.	2199-41-1	70	2.50	2.50	9.70	7.40	0.0	23.7
15.	2199-43-1	50	1.70	2.00	9.50	6.00	0.0	36.8
16.	2199-46-1	70	1.83	1.83	10.00	6.00	0.0	40.0
17.	2199-47-1	50	2.50	3.70	6.43	5.40	0.0	16.0
18.	2199-50-1	60	2.83	2.83	7.00	5.40	0.0	22.9
19.	2199-52-1	60	3.20	3.40	5.40	6.40	0.0	0.0
20.	2199-53-1	60	2.60	3.20	4.80	5.00	0.0	0.0
21.	2199-62-1	40	3.40	2.40	7.70	5.50	29.4	28.6
22.	2199-70-2	100	3.40	4.00	5.40	4.80	0.0	11.1
23.	2199-73-1	80	3.00	3.00	6.50	4.70	0.0	27.7
24.	2199-83-1	50	3.40	3.20	6.70	5.20	5.9	22.4
25.	2199-88-1	60	3.20	5.20	5.70	4.10	0.0	28.1
26.	2199-95-1	100	2.70	4.40	5.50	4.73	0.0	14.0
27.	2199-153-1	100	2.83	3.50	7.70	7.00	0.0	9.1
28.	2199-153-2	50	1.50	3.40	4.83	4.70	0.0	2.7
29.	2199-174-1	100	2.17	2.70	4.40	5.70	0.0	0.0
30.	2199-379-1	90	1.50	2.44	4.50	4.17	0.0	7.3
31.	2199-403-1	50	2.70	2.34	7.70	4.00	13.3	48.1
	BPT 5204	50	3.83	2.70	7.00	4.70	29.5	32.9
	Azucena	100	2.37	2.72	4.36	5.28	0.0	0.0

Per cent reduction in shoot growth:

The per cent reduction in shoot growth varied greatly among the BILs with no reduction to as high as 67% (2199-35-1). Out of 31 BILs, Seven BILs *viz.,* 2199-7-1, 2199-44-1, 2199-52-1, 2199-53-1, 2199-54-1, 2199-174-1 and 2199-178-1 had no reduction in shoot growth in the treatment when compared to control. The recurrent parent BPT 5204 had 32.9 % reduction in shoot growth and Azucena had nil reduction.

Conclusion:

It was observed that these lines performing better in yield than susceptible parent samba mahsuri under drought stress. Hence, by correlating marker data and yield data 26 advanced back cross lines (BC_2F_4) were selected from 31 lines. These results revealed that direct selection for yield under drought is effective coupled with marker assisted screening in enhancing yield potential in drought prone areas. Further, the lines 2199-54-1 and 2199-178-1 which had higher yield under drought coupled with 100 per cent seedling survival in TIR were promising and were advanced for further evaluation in yield trials.

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