Evaluation of advanced backcross lines for drought tolerance in rice

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ABSTRACT

Drought is one of the serious abiotic stresses limiting rice productivity under prolonged dry spells. The present study was aimed to estimate heritability of yield and its components under lowland stress for direct selection of yield in fifty advanced back crosslines derived from drought susceptible Samba Mahsuri and tolerant Azucena besides screening with yield QTL linked molecular markers. High heritability for broad sense was observed in days to 50% flowering followed by spikelet fertility and grain yield plant⁻¹ indicating direct selection for yield under stress is practicable in evolving drought tolerant rice varieties with yield potential. Thirty one advanced back cross lines (BC₂F₃) lines co segregating for simple sequence repeats (SSRs) linked to yield QTLs under low land stress viz., RM 520 linked to DTY 3.1 on chromosome 3 and RM 236 linked to DTY 2.1 on chromosome 2 exceeding yield of susceptible parent were advanced to BC₂F₄. These results suggested that direct selection for yield under water stress coupled with marker assisted screening would help in precise selection of genotypes for drought prone areas.

Key words: drought tolerance, rice, SSRs, yield

Drought is one of the major constraints in rice production during crop growth period due to erratic rainfall caused by adverse climate changes. Besides rainfed areas, tail end areas of irrigated rice ecosystem are affected with drought during terminal growth period resulting in low yields. Drought tolerance is considered as a complex trait. 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. Direct selection of yield under drought is practicable for evolving drought tolerant varieties (Kumar et al., 2008, Gouda et al., 2012). QTLs with large effects on grain yield under drought stress, namely DTY_{1.1}, DTY_{2.1}, DTY_{2.2} DTY_{3.1}, DTY_{4.1}, DTY_{9.1} and DTY_{12.1} have been identified explaining 31-77% of the phenotypic variance (Bernier et al., 2007, Venuprasad et al., 2009, Vikram et al., 2011). Hence present study was aimed to evaluate advanced back cross lines (BC_2F_2) derived from drought susceptible Samba mahsuri (BPT 5204) and tolerant Azucena for direct selection of yield under drought by studying heritability of yield and its components and using yield QTL linked SSR markers *viz.*, RM 236 (DTY 2.1 on chromosome 2) and RM 520 (DTY 3.1 on chromosome 3).

Fifty advance backcross lines (BC_2F_3) developed from widely adopted mega variety samba mahsuri (susceptible parent) and drought tolerant Azucena were used to estimate heritability of yield and its components under drought. The study was conducted at Andhra Pradesh Rice Research Institute and Regional Agricultural Research Station, Maruteru in dry season, 2010. A field experiment was carried out with fifty BC_2F_3 lines in two replications with 2 rows of 5m length with spacing of 20cm between rows and 15cm between plants. Field bunds were covered with polythene sheet to 1m depth to avoid seepage of water. Stress was imposed at 30 days after transplanting till the harvest of the crop. Data on yield and its components viz., days to 50% flowering, plant height, ear bearing tillers plant⁻¹, Panicle length and spikelet fertility were recorded in 5 randomly selected plants

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replication⁻¹. Soil moisture condition before imposing and after stress was measured using soil moister tension meter (Delta T devices) at different depth at 30 days after transplanting till harvest (Table 1).

| Table 1. | Mean soil moisture % of stress period during dry |
|----------|--|
| | season of 2010 |

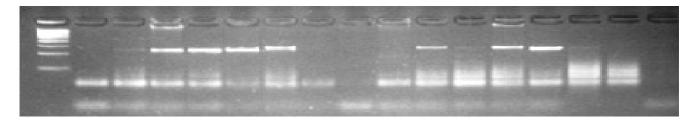
| Stress | Soi | Soil moisture (%) at different depths | | | | | | |
|-----------------------------|--------|---------------------------------------|--------|--------|--------|--------|--|--|
| | 10cm | | 20cm | | 30cm | | | |
| | Before | After | Before | After | Before | After | | |
| | stress | stress | stress | stress | stress | stress | | |
| 30 days after transplanting | | | | | | | | |
| till harvest | 79 | 11 | 81 | 16 | 82 | 26 | | |

Leaf was collected from field at 20 days after transplanting. DNA was extracted by the Cetyl Trimethyl Ammonium Bromide (CTAB) method (Murray and Thompson, 1980) using Qiagen tissue lyser. The quality and quantity of DNA was estimated using ND 8000 eight channel spectrophotometer and checked on 0.8% agarose gels. Two SSR markers RM 520 and RM 236 used for PCR amplification. PCR amplifications were performed in 10 μ l of reaction mixture containing 1 μ l of 10X buffer with MgCl₂ 0.5 μ l of dNTPs (25 m M. L⁻¹), 1 μ l (5 μ molar) each of forward and reverse primers, 1 µl Taq DNA polymerase(0.5 U/micro litre), 3 µl of template DNA $(10 \text{ ng } \mu l^{-1})$ 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°C for 0.5 min, annealing at 55°C for 0.5 min, extension at 72°C for 1 min and 7 min at 72°C for the final extension. The PCR products were electrophoresed with ethidium bromide (10mg ml⁻¹) at 100 volts for 2 hrs in 1X TBE buffer. A 100 bp lader (Genei) was used for appropriate sizing of the products. The gel was photographed under UV light using Ingenius gel doc system. Analysis of variance was carried out to check the genetic variance among the 50 BC₂F₂ lines of all the traits using Crop stat 7.2 version. Heritability at broad sense (H) was calculated for each trait from the covariance values using the formula, $H = \frac{\delta^2 g}{\delta^2 g} + \frac{\delta^2 e}{r}$, where $\delta^2 g$ and δ^{2e} are genetic and residual variance, respectively and r number of replications.

Significant variation was observed among the fifty BC_2F_3 lines for all the traits studied under stress (Table 2). Similar genetic variation for yield and its

| | Mean | Range | Standard deviation | Heritability in broad sense |
|---|-------|------------|--------------------|-----------------------------|
| Days to 50 %flowering | 98.2 | 90-104 | 2.80 | 0.96 |
| Plant height cm | 98.83 | 89-109 | 5.15 | 0.82 |
| Ear bearing tillers plant ⁻¹ | 8.7 | 4-11 | 1.74 | 0.58 |
| Panicle length cm | 23.7 | 20.1-27.34 | 2.09 | 0.32 |
| Spikelet fertility % | 75.81 | 42-90.7 | 11.03 | 0.95 |
| Grain yield plant-1 gms | 18.69 | 8.6-28.35 | 5.57 | 0.93 |

Table 2. Trait mean values for advanced backcross lines during dry season of 2010



Lanes 1-ladder, 2-Samba Mahsuri (susceptible parent), 3-Azucena, 4-onwards advanced back cross lines

Fig.1. Screening of advanced back cross lines with RM 520 SSR marker linked to DTY 3.1

□ 298 □

components under water stress have been reported earlier (Kumar et al., 2008, Gouda et al., 2012). High heritability for broad sense was observed in days to 50% flowering followed by spikelet fertility, grain yield plant⁻¹ and plant height indicating use of these traits for direct selection for yield under stress is effective. But low heritability was observed for panicle length, followed by ear bearing tillers plant⁻¹. Earlier workers Kumar et al., 2008 observed moderate to high heritability of grain yield under stress. Thirty one advanced backcross lines (BC_2F_3) lines co-segregating for simple sequence repeats (SSRs) linked to yield QTLs under lowland 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 50 lines were selected. It was observed that these lines performed better in yield than susceptible parent Samba mahsuri. Hence, by correlating marker data and yield data 31 advanced back cross lines (BC_2F_2) were selected for $BC_{2}F_{4}$ generation. 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.

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