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Drought tolerance in recombinant inbred lines (RIL_S) derived from the cross of BPT-5204/Sahbhagi dhan

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ABSTRACT

*Drought stress is a major constraint for rice (*Oryza sativa *L.) production and yield stability in rainfed ecosystems. Higher probability and greater spatial covariance of drought and less diversified farming systems with rice accounting for a larger share of household income are likely to be the main reasons for this higher cost of drought in eastern India. Farmers deploy various coping mechanisms but such mechanisms are largely unable to prevent a reduction in income and consumption, especially in eastern India. Therefore, identifying drought tolerant genotypes will help to develop rice cultivars suitable for water-limiting environments through marker-assisted breeding. F_4 progenies derived from the cross of BPT 5204 and Sahbhagi dhan were used to generate the RILs (Recombinant Inbred Lines) by advancing the F_4 generation to F_6. Eight hundred ten RILs were screened for drought tolerance in the rainout shelter. Scoring for leaf rolling, leaf drying and recovery was based on the 0-9 scale of SES (Standard Evaluation System) of IRRI. The screening results showed one hundred forty recombinant inbred lines were found highly resistant to drought (scale 0-3). Further confirmation of drought tolerance was based on the use of polymorphic microsatellite markers RM 3825, RM 1349, RM 24, RM 232, RM 212 and RM 3 linked to different drought related characteristics such as plant height, panicle length, test weight and grain yield.*

Key words: *Rice, Drought,Oryza, microsatellites*

Drought is one of the most important constraints in rice production resulting in large yield losses and limiting the average yield increase of the country. In India, 57 per cent of the area of rice cultivation is under rainfed condition (Pandey *et al*. 2007). The varying degree and duration of drought during the crop cycle can severely reduce rice grain yieldproduction in rainfed lowland condition. In a water-limited environment, a higher level of drought resistance and reduced yield loss by drought stress are required. It is a challenge posed by the environment to the survival and productivity of a crop that occupies a large area. This directly translates to economic loss to the farmer (s) who depend on the harvest. Therefore, there is an urgent need to enhance water-saving capacity or drought resistance (DR) of rice.Water stress has been identified as one of the major constraints to rice cultivation in rainfed lowland ecosystems, which occupy 28 percent of rice cultivation area in Jharkhand. A wide range of traits is found to be associated with drought resistance. As a breeding objective, the plant traits that could be selected among segregants are robust and efficient root systems, well-endowed shoot system and finally grain yield. Marker-assisted selection is expected to boost the pace of crop improvement especially for complex trait like drought. Therefore, in this study drought tolerant genotypes were identified by using SSR markers linked to various morphological and yield characteristics of the mapping population.

MATERIALS AND METHODS

The investigation was carried out during Kharif season 2013 at Birsa Agricultural University, Ranchi, Jharkhand. The experimental materials in research were F_6 generation population derived from the cross of BPT-5204(Samba Mahsuri) and Sahbhagi Dhan. Eight hundred ten recombinant inbred lines were screened for the drought tolerance in the rain out shelter at Birsa Agricultural University, Ranchi, Jharkhand and scoring for leaf rolling and leaf drying was done based on the SES (Standard Evaluation System) of IRRI, Philippines.The same set of RILs (Recombinant Inbred Lines) were also planted in the normal rainfed lowland condition. Observations for various morphological and yield contributing characteristics were recorded.

Field condition

The field experiment was conducted under a rainfed lowland environment at Birsa Agricultural University, Kanke, (latitude 23.18^{0}N, 85.19 ^{0}E longitude), Ranchi, Jharkhand during the 2013 wet season. The 810 recombinant inbred lines, and the parents BPT-5204 and Sahbhagidhan, were evaluated under rainout shelter and rainfed field conditions in the 2013 wet season. Water stress condition was imposed for 35 days during the vegetative stage. Screening of RILs was based on leaf rolling, leaf drying and recovery. The observations on recovery were recorded after 7 days of irrigation of imposing water stress duration. In the rainfed field condition, morphological and yield attributing characteristics were observed like plant height, days to 50% flowering, number of effective tillers, panicle length, biomass, harvest index, grain length, grain width, LB ratio, test weight and grain yield.

Genotyping

DNA extraction and amplification of PCR products

Genomic DNA was isolated from 1.0 g of leaf of the inbred lines using CTAB method described by Dellaporta *et al*. (1983). DNA extracted by 1% Cetyl trimethyl ammonium bromide (CTAB) solution in 100 mMTris, 500 mMNaCl and 50 mM EDTA. The crude DNA obtained after purification using chloroform: isoamyl alcohol was dissolved in 500 µl of TE buffer. After RNase treatment (3µl of 10 mg/ml solution) for 1 hour at 37^{o}C), the DNA was then purified by Phenol: Chloroform: Isoamyl alcohol (25:24:1). DNA samples were quantified on 1% agarose gel and concentration adjusted to approximately 25 ng µl^{-1}. PCR amplification was done with a 10µl reaction mixture having 50 ng DNA, 10xPCR buffer, 10 Mm dNTPs, 50 p mol primers, and 1.0 U/ µl Taq polymerase enzyme. To resolve the PCR products,2% agarose gels wereused. Parental polymorphism survey was done between BPT-5204 and Sahbhagidhan with 19 rice simple sequence repeat (SSR) markers taken from alreadyavailable rice genetic and sequence maps.

Genotyping of BPT5204/Sahbhagidhan population

Parental polymorphism was observed in 2% agarose gel using 19 SSR markers reported to be linked to drought. Genotyping of BPT5204/Sahbhagidhan population was carried out with 6 polymorphic SSR markers.

RESULT AND DISCUSSION

Phenotypic performance

Under rainout condition, the recombinant inbred lines along with the parents (BPT-5204 and Sahbhagi dhan) and susceptible check IR20 manifested significant differences in respect of leaf rolling, leaf drying and leaf drying recovery (Table 2, 3 and 4).

Eight hundred ten RILs were grouped into highly drought tolerant lines (score 0-3.0), moderately drought tolerant lines (score 3.1-5.0), moderately drought susceptible lines (score 5.1-7.0) and highly drought susceptible lines (score 7.1-9.0). Kanagaraj *et al*. (2010) also selected 11 lines which performed well (low scores) and 12 lines which performed very poorly (high scores) out of 330 RI lines and grouped into drought resistant and drought susceptible lines respectively, based on leaf rolling and leaf drying scores under water stress condition. Swain *et al*. (2014) studied 134 landraces that represented different geographic regions of India and a few from Indonesia and Philippines, with an SES (Standard Evaluation System) score of 0-3 in the 0-9 scale, seventy eight accessions were scored as tolerant with twelve having ‘0’ score, eighteen with ‘1’ and forty eight with the score ‘3’. Of the seventy eight genotypes, thirteen had recorded yield over 1.0 t/ ha while the tolerant (CR 143-2-2) and susceptible controls (IR 20) recorded 2.70 t/ ha and zero yield respectively.

Screening of the recombinant inbred lines was carried out under rainout shelter and the morphological observations were recorded in the normal rainfed lowland condition. The mean data of individual lines of

Table 1. List of Primers used in the study

Primer Name	Primer Sequences	Annealing Temperature
RM212	F- CCACTTTCAGCTACTACCAGR- CACCCATTTGTCTCTCATTATG	46 ^{0}C
RM302	F- TCATGTCATCTACCATCACACR- ATGGAGAAGATGGAATACTTGC	47 ^{0}C
RM 3825	F- AAAGCCCCCAAAAGCAGTACR- GTGAAACTCTGGGGTGTTCG	51 ^{0}C
RM 1349	F- ATCCACCTGCTGATCAGCTCR- CGAGAAGCTCAAGGTGAACC	51 ^{0}C
RM34	F- GAAATGGCAATGTGTGCGR- GCCGGAGAACCCTAGCTC	48 ^{0}C
RM24	F- GAAGTGTGATCACTGTAACCR- TACAGTGGACGGCGAAGTCG	45 ^{0}C
RM112	F- GGGAGGAGAGGCAAGCGGAGAGR- AGCCGGTGCAGTGGACGGTGAC	61 ^{0}C
RM166	F- GGTCCTGGGTCAATAATTGGGTTACCR- TTGCTGCATGATCCTAAACCGG	53 ^{0}C
RM527	F- GGCTCGATCTAGAAAATCCGR- TTGCACAGGTTGCGATAGAG	48 ^{0}C
RM341	F- CAAGAAACCTCAATCCGAGCR- CTCCTCCCGATCCCAATC	49 ^{0}C
RM327	F- CTACTCCTCTGTCCCTCCTCTCR- CCAGCTAGACACAATCGAGC	51 ^{0}C
RM232	F- CCGGTATCCTTCGATATTGCR- CCGACTTTTCCTCCTGACG	48 ^{0}C
RM171	F- AACGCGAGGACACGTACTTACR- ACGAGATACGTACGCCTTTG	50 ^{0}C
RM17	F- TGCCCTGTTATTTTCTTCTCTCR- GGTGATCCTTTCCCATTTCA	47 ^{0}C
RM 5443	F- TACGGCTTACCCATAGCAGCR- AAACGGAGGGAGTATTTCCC	52 ^{0}C
RM537	F- CCGTCCCTCTCTCTCCTTTCR- ACAGGGAAACCATCCTCCTC	52 ^{0}C
RM136	F- GAGAGCTCAGCTGCTGCCTCTAGCR- GAGGAGCGCCACGGTGTACGCC	60 ^{0}C
RM3	F- ACACTGTAGCGGCCACTGR- CCTCCACTGCTCCACATCTT	52 ^{0}C
RM 3231	F- AACACGAAGACCGGCCTCR- CAGGTAGGAGCATGAGAGCC	53 ^{0}C

Table 2. Result of leaf rolling of the 810 recombinant inbred lines (RILs)

Scoring	No. of lines	Scale
8.0	BPT- 5204	Drought susceptible parent
1.0	SahbhagiDhan	Drought tolerant parent
7.5	IR 20	Drought susceptible Check
0-3.0	588	Highly drought tolerant lines
3.1-5.0	153	Moderately drought tolerant lines
5.1-7.0	52	Moderately drought susceptible lines
7.1-9.0	17	Highly drought susceptible lines

Table 3. Result of leaf drying of the 810 recombinant inbred lines (RILs)

Scoring	No. of lines	Scale
8.0	BPT- 5204	Drought susceptible parent
0.75	Sahbhagidhan	Drought tolerant parent
7.0	IR 20	Drought susceptible Check
0-3.0	441	Highly drought tolerant lines
3.1-5.0	224	Moderately drought tolerant lines
5.1-7.0	105	Moderately drought susceptible lines
7.1-9.0	40	Highly drought susceptible lines

the population was statistically analyzed to generate overall mean and other parameters. The variability in the mean values of different drought related traits (plant height, days to 50% flowering, number of effective tillers, panicle length, grain length, grain width, L/B ratio, test weight, grain yield, biomass, harvest index) among the RILs were distinguishable. These results were in agreement with the results of Singh *et al.*, (2004) and Manickaveli, *et al.*, (2006), showing significant variability for these traits.

Table 4. Result of leaf drying recovery of the 810 recombinant inbred lines (RILs)

Scoring	No. of lines	Scale
7.0	BPT- 5204	Drought susceptible parent
1.5	SahbhagiDhan	Drought tolerant parent
6.75	IR 20	Drought susceptible Check
1.0-3.0	221	Highly drought tolerant lines
3.1-5.0	268	Moderately drought tolerant lines
5.1-7.0	196	Moderately drought susceptible lines
7.1-9.0	125	Highly drought susceptible lines

Genotypic Performance

The parental DNA (BPT-5204 and Sahbhagidhan) was assessed for amplification using different primer sets. The amplified products were electrophoresed on 2% agarose gel. Out of the 19 SSR markers assessed, 6 were found to be polymorphic. These were RM 1349, RM 3825, RM 212, RM 232, RM 24 and RM 3.The polymorphic SSRs were further used with the 20 selected RILs found phenotypically drought tolerant under rainout shelter condition (Fig.1).

Observations on drought screening (leaf rolling, leaf drying and recovery) as well as various morphological and yield attributing characteristics under rainfed lowland condition categorized the population based on their level of drought tolerance and thus 20

Fig. 1. Agarose (2%) gel electrophoresis of RILs with primer RM 232**.**M- pBR 322 DNA/Hae III Digest;B- BPT-5204 (Susceptible parent); S- Sahbhagi dhan (Tolerant parent); 1- Line no. 4;2- Line no. 122;3- Line no. 125; 4- Line no. 135; 5- Line no. 137; 6- Line no. 152; 7- Line no. 161; 8- Line no. 162; 9- Line no. 165; 10- Line no. 179; 11- Line no. 180; 12- Line no. 181; 13- Line no. 193; 14- Line no. 194; 15- Line no. 205; 16- Line no. 418; 17- Line no. 586; 18- Line no. 808; 19- Line no. 809; 20- Line no. 810

highly drought tolerant inbred lines were further selected for the molecular studies using SSR markers linked to drought. The evaluation of the genotypes phenotypically and genotypically showed the presence of QTLs for drought in 20 RILs. In the present study, the six polymorphic primers used with the 20 selected RILs were linked to different QTLs governing drought tolerance (Kanagaraj *et al*. 2010). RM 1349 is reported to be linked to QTLs governing plant height (Lin *et al*. 2007) (drought tolerant lines were line number BS122, BS125, BS135, BS137, BS152, BS161, BSBS162, BS165, BS179, BS180, BS181, BS194, BS205, BS418, BS586, BS808, BS809 and BS810). RM 3825 is reported to be linked to QTLs for plant height, panicle length and grain yield (Boopathi, 2004; Beena, 2005) (drought tolerant lines were line numbers BS4, BS165, BS193 and BS808). RM 212 has been reported to be linked to QTLs for days to 50% flowering, biomass, harvest index, test weight, plant height, panicle length, grain yield and number of effective tillers (Price *et al*., 2000; Babu *et al*., 2003; Kamoshita *et al*., 2002; Bernier *et al*., 2007; Hittalmani *et al*. 2003) (drought tolerant line was line number BS193). RM 232 is reported to be linked to QTLs for test weight (Lin *et al*., 2007) (drought tolerant lines were line numbers BS122, BS125, BS135, BS 137, BS152, BS161, BS162, BS179, BS180, BS181, BS193, BS194, BS205, BS418, BS586, BS809 and BS810). RM 24 is reported to be linked to QTLs for plant height (Lin *et al*. 2007) (drought tolerant lines were line numbers BS193 and BS808). RM 3 is reported to be linked to the QTLs for yield (Lin *et al*. 2007) (drought tolerant lines was line number BS193).

In this analysis, 6 primers were found to be polymorphic. PCR amplification of the 20 inbred lines with these 6 polymorphic primers (RM 1349, RM 3825, RM 212, RM 232, RM 24, RM 3) confirmed presence of drought tolerant QTLs. Line numbersBS 4, BS122, BS 125, BS135, BS137, BS152, BS161, BS162, BS165, BS179, BS 180, BS181, BS193, BS194, BS205, BS418, BS586, BS808, BS809 and BS810 were found to be drought tolerant based on drought screening and molecular study. Several QTLs reported on chromosome 1, 3, 4, 5, 6, 7 and linked to drought traits like plant height, panicle length, grain yield, days to 50% flowering, biomass, harvest index and test weight were detected in different RILs. Rice cultivation is highly water-intensive. Drought is a major constraint affecting rice production especially in rainfed areas. The present study revealed the identification of drought tolerant inbred lines and presence of QTLs linked to drought. The study has identified lines that can be intercrossed to enable pyramiding of different QTLs which can then generate a mega variety for drought tolerance. Also, Marker assisted backcrossing and other methods can be used to introgress these identified QTLs in the desirable background.

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