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Parental Polymorphism Survey for Drought Tolerance In Rice By Using SSR Marker

Jyotima Tripathi[1], Shalini Nirmal Thagele[2], Ashish Kumar Sharma[3], Nilima Tripathi[4], Pratima Tripathi[5], Ajay Kumar Singh[6]

[1] *P.G. Student, department of Genetics and Plant Breeding, Banaras Hindu University, Varanasi, India*
[2]*P.G. Student, department Biochemistry, G.R. Medical College & J.A. group of hospitals, Gwalior, India*
[3, 6] *Tutor, Assistant professor, department of Biochemistry, Government Medical College, Ambedkar Nagar, India*
[4]*Lecturer, department of Zoology, Sanjay Memorial Women's College, Varanasi (U.P.) India*
[5] *Tutor, department of Microbiology, B.R.D. Medical College, Gorakhpur, India*
Corresponding Author: Shalini Nirmal Thagele

Abstract: *Rice (Oryza sativa L.) is one of the most-produced and consumed grains worldwide. Drought is a major environmental stress seriously limiting plant growth and crop productivity. In this study 60 SSR markers were used in which 41 were polymorphic. Among 41 makers 4 were highly polymorphic, these 4 marker shows polymorphism in all tolerant-susceptible combination.111 alleles were detected among the 41 SSR primer pairs, which were polymorphic between some of the tolerant-susceptible combinations among the 5 rice cultivars with an average allele frequency of 2.7 per marker. The Polymorphic information Content (PIC) values of 41 polymorphic primers ranged from 0.26 to 0.67.*

Keywords: *Rice, PIC, SSR marker, Polymorphism.*

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I. Introduction

Rice is the seed of the grass species *oryza sativa* (Asian rice) or *oryza glaberrima (*African rice). As a cereal grain, it is the most widely consumed staple food for a large part of the world's human population, especially in Asia. It is the agricultural commodity with the third-highest worldwide production [1]. In the present era of global climatic change where crops are more likely to undergo abiotic stresses, drought becomes quite important, particularly for high water consuming plant like rice [2]. Predictions of climatic change indicate an increased variability of rainfall in the next 40 years and an increased risk of high temperature [3]. Drought is a more complex phenomenon than most other stresses, such as salinity, submergence, pests, and diseases. The genetic mechanisms that order the expression of drought tolerance in rice plants are poorly understood. Since drought tolerance is a complex trait controlled by polygenes, it is one of the most difficult traits to study and characterize.

Genes associated with drought resistance are numerous and have been shown to interact with the environment, and thus the networks involved in drought tolerance are quite complex in nature. Therefore, progress in improving the drought tolerance of rice is slow [4]. The enormity of additive gene effect is particularly useful in the development of pure line varieties. Drought is predominantly controlled by additive gene. Mapping studies are performed to detect linkage of a molecular marker to a gene affecting a trait of interest. It then becomes possible to select for the desirable allele of those genes based on marker genotype rather than, or in addition to, field phenotype [5]. This technique, known as marker assisted selection (MAS), is theoretically more reliable than selection based solely on phenotype, as a marker tightly linked to the desirable gene would represent selection with a heritability of near unity for that specific gene [6]. The specific markers identified in the present study may also be used for identification of drought tolerant rice genotypes. The result would help in fine tuning of the breeding strategies and application of SSR markers for the development of the drought resistant rice varieties through marker assisted pyramiding multiple QTLs.

II. Material And Methods

The experimental material for this investigation comprised of five rice genotypes in which three are tolerant (EKHA KEHA, SOLOI, NP125) and the remaining two are susceptible (HUR105 and SARJU52). Genomic DNA was extracted by modified cetyl-trimethyl ammonium bromide (CTAB) method [7]. 15 - 20 days rice leaves were extracted with DNA extraction buffer (2% CTAB, 100 mM Tris, 20 Mm ethylene-diaminetetra acetate (EDTA), 1.4 M NaCl). The genomic DNA was subjected to PCR amplification as per the

procedure described by Chen *et al.*, 1997 [8] with minor modifications. The PCR were carried using a programmable thermo cycler for DNA amplification. Agarose gels Ethidium bromide was added while pouring the gel so that the DNA fluoresces when gel was exposed to UV light. The DNA fragments was then visualized under UV Trans illuminator and the banding pattern was observed and recorded using gel documentation unit (Gene flash) which was stored for further scoring and permanent records. Quantitative Multistate traits depicting an array of characters were converted into binary characters [9] based on the variations present. Markers were scored for the presence and absence of the corresponding bands of the genotypes. The score 1 and 0 indicates the presence and absence of bands, respectively. To measure the in formativeness of the markers, the polymorphism information content (PIC) for each genomic SSR and EST-SSR markers was calculated according to the formula [10].

$PIC=1-(\Sigma P_i^2)$

Where, '*i*' is the total number of alleles detected for SSR marker and 'P*i*' is the frequency of the *i*th plus allele in the set of 5 genotypes investigated.PIC value estimate the discriminatory power of the SSR marker.

III. Results

Parental SSR marker polymorphism survey was carried out between five tolerant-susceptible rice lines possessing drought stress resistance genes, in which three are Drought resistant EKHA KEHA, SOLO I, NP125 and two are susceptible HUR 105 and SARJU 52. A total of 60 SSR primer pairs distributed across the rice genome were used for molecular characterization of the 5 rice varieties mentioned above, selected for the study. Out of these 17marker did not show any bands, 2 markers shows single band 41 markers were polymorphic. Interestingly, out of 60 SSR markers, a total of 4 SSR markers were observed to be highly polymorphic between all the genotypes analyzed. The allelic data for each SSR marker is listed below in table1 and given in figures 1, 2, 3 and 4.

Table 1: No. of polymorphic markers with Donors and Susceptible parents

	SUSCEPTIBLE	
TOLERANT	**HUR 105**	**SARJU 52**
EKHA KEHA	33	26
SOLO 1	27	25
NP125	18	16

Fig-1: Amplified banding pattern of the marker RM 154

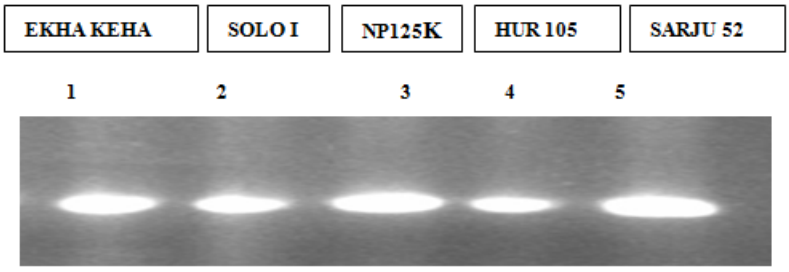


Fig-2 Amplified banding pattern of the marker RM 431

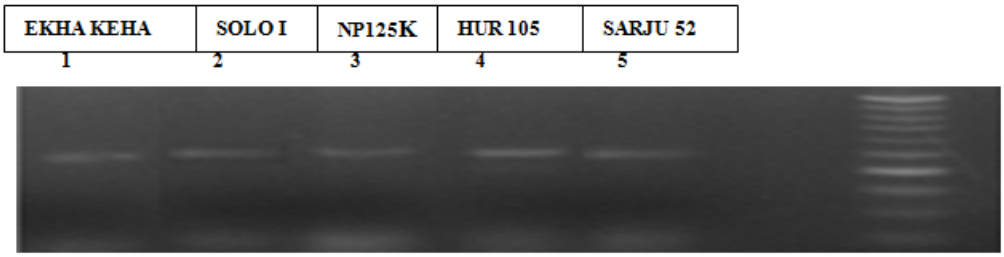


Fig-3 Amplified banding pattern of the marker RM118

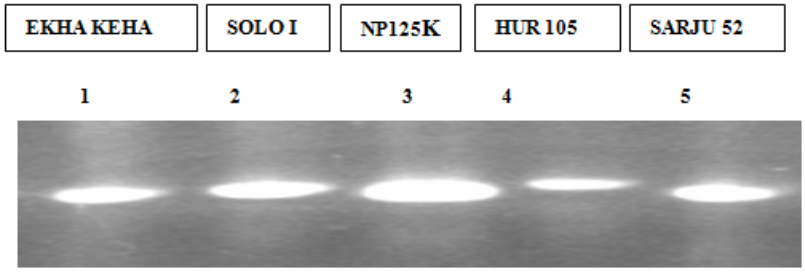
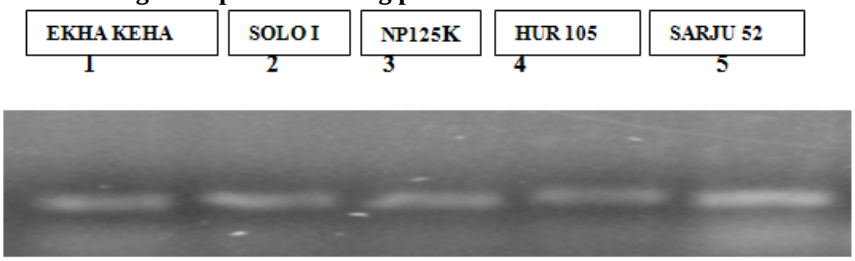


Fig-4 Amplified banding pattern of the marker RM284



Among the 60 polymorphic markers, 41 SSR primer pairs were polymorphic between some of the tolerant-susceptible combinations. Among these RM1, RM 144, RM 5891 amplified maximum of four alleles, while the markers RM495,RM 283, RM 259,RM 237, RM431, RM 489, RM 338,RM 55RM307, RM124, RM413, RM334, RM125, RM118, RM152, RM25, RM44, RM284, RM316, R105, RM215, RM536, RM19, RM11943, RM589 amplified 3 alleles. The SSR markers RM312, RM5, RM154, RM452, RM507, RM161, RM510, RM78, RM454, RM162, RM 455, RM 28166 amplified two alleles among genotypes analyzed in the present study.

IV. Discussion

In the current study "parental polymorphism survey for drought tolerance in rice" five rice varieties were use in which three were tolerant (EKHA KEHA, SOLO I, NP125) and two were susceptible (HUR105 and (SARJU52). Polymorphism Information Content (PIC) value is the reflection of allelic diversity and frequency among the verities or lines. To measure the in formativeness of each SSR marker, PIC values were calculated. The PIC values of 41 polymorphic primers ranges from 0.26 to 0.67. The primer pair RM 455 posses a minimum PIC value 0.26 and RM1, RM118, RM144, RM5891 posses maximum PIC value 0.67 and the average PIC value is 0.52. Lapitan *et al.*, 2007 [11] studied on the genetic diversity of Philipine rice cultivars using SSR markers and reported PIC value 0.68 in their experiment. Brondani *et al.*, 2008 [12], Rajendra kumar *et al.*, 2009 [13] and Matin *et al.*, 2012 [14] had reported average PIC Values of 0.61, 0.61 and 0.634, 0.685 respectively with different set of rice genotypes using SSR markers. The result of PIC value obtained was much higher than the observations made by Lapitan *et al.*, 2007 [11], Brondani *et al.*, 2008 [12], Rajendra kumar *et al.*, 2009 [13] and Matin *et al.*, 2012 [14]. In the present study, we have selected the hyper variable SSR markers which covered uniformly across the genome, hence results a fair amount of PIC values for these markers. Among 41 polymorphic SSR markers, 4 marker namely, RM 1, RM 589, RM 118 and RM 144 amplified a maximum of four alleles, and these four markers located on chromosome 1, 6, 7 and 11 as well as high PIC value 0.67. Markers with PIC values of 0.5 or above are highly informative for genetic studies since they are extremely useful in distinguishing the polymorphism rate of a specific locus [15]

In present study, a total of 111 alleles were detected across 5 rice varieties. The markers located on target chromosome 1, 3, 6 and 12. 111 alleles were detected among the 41 SSR primer pairs, which were polymorphic between some of the tolerant-susceptible combinations among the 5 rice cultivars with an average allele frequency of 2.7 per marker, and 16 alleles were detected among the 4 highly polymorphic SSR primer pairs. An average allele frequency of 4 alleles per marker was observed. The number of alleles generated per locus by each marker is 2.7. Similar results were reported by Pachauri *et al.*,2013 [16] and Shah *et al.*, 2013[17] with an average number of alleles per locus detected was 2.79 and 2.75 respectively in molecular and morphological characterization of Indian farmers rice varieties and genetic diversity in Basmati and non-Basmati rice varieties. However, the result observed in present study was higher than the result reported by Kibria *et al.*, 2009 [18] with an average number of alleles 1.78 per locus in to assess the genetic diversity among aromatic rice genotypes using simple sequence repeat (SSR) and randomly amplified polymorphic DNA

(RAPD) markers through marker aided selection (MAS) which were nearly comparable. The results obtained in present study were lower than the observations made by Rabey *et al*., 2013 [19], Shah *et al.*, 2013 [17]. Average numbers of alleles detected were 3.5, 3.83 and 2.75 in Tiwan rice germplasm, genetic diversity of eight rice cultivars and diversity within the aromatic and non-aromatic rice varieties respectively.

In the present study 60 SSR markers were used in which 41 were polymorphic. Among 41 markers 4 were highly polymorphic, it means markers show 68.33% result. The result of present study is much better, if we compare the result of Naguyen *et al*., 2012 [20]. In this study 765 SSR markers were used for parental survey and of which 226 markers showed clear polymorphism (29.54%). While, Kumar, 2011 [21] used much higher parental polymorphic SSR marker than those used by earlier workers, in his study, where he used 150 polymorphic SSR markers for biotic resistance of rice, in which 85 SSR markers were polymorphic. Out of 85 polymorphic markers he got 20 high polymorphic SSR markers. The result of present study is much lower than the result reported by King *et al*., 2005 [22]. They documented the isolation and characterization of 23 SSR markers for endangered Kirtland's warber (*dendroica kirtlandii*). These marker shows high level of allelic diversity that is 7.7 alleles per locus and genotypic frequency of these marker were 95%. It means that, out of 23 markers, 22 were polymorphic.

V. Conclusions

In this study 60 SSR markers were used in which 41 were polymorphic. Among 41 makers 4 were highly polymorphic, these 4 marker shows polymorphism in all tolerant-susceptible combination, these markers located on target chromosome 1, 3, 6, 12. 111 alleles were detected among the 41 SSR primer pairs, which were polymorphic between some of the tolerant- susceptible combinations among the 5 rice cultivars with an average allele frequency of 2.7 per marker, and 16 alleles were detected among the 4 highly polymorphic SSR primer pairs with an average allele frequency of 4 alleles per marker. The Polymorphic information Content (PIC) values of 41 polymorphic primers ranged from 0.26 to 0.67. The primer pair RM 455 posses a minimum PIC value 0.26 and RM 1, RM118, RM144, RM5891 posses maximum PIC value 0.67 average value of total PIC value is 0.52 . The specific markers identified in the present study may also be used for identification of drought tolerant rice genotypes. The result would help in fine tuning of the breeding strategies and application of SSR markers for the development of the drought resistant rice varieties through marker assisted pyramiding multiple QTLs.

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