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Screening of rice genotypes for abiotic and biotic stresses using molecular markers

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Abstract
In the present study, a set of 12 rice genotypes was screened for submergence, drought and bacterial leaf blight disease using available tightly linked/gene based molecular markers. The PCR based molecular marker Sub1BC2, RM431 and four bacterial leaf blight resistance (Xa4, xa5S/xa5SR/R-Multiplex, xa13 prom, pTA248) were used to select the tolerant or resistant genotypes for submergence (*Sub1*), drought grain yield QTL (q*DTY1.1*) and bacterial leaf blight resistance gene (*Xa4*, *xa5*, *xa13* and *Xa21*), respectively. The genotypes RYC743, Purnendu and FR13A were identified as submergence tolerant. Based on the RM431 genotypic data, the three genotypes Vaidehi, Dudhi and Birar have drought grain yield QTLq*DTY1.1*. The bacterial leaf blight resistance gene linked markers revealed that the four genotypes Satyam, RYC743, Pansoradhan and Kajargod had *Xa4* gene, two genotypes Sudha and Desaria had *xa5* gene and one genotype Sudha indicated the presence of *Xa21* gene in heterozygous condition, while *xa13* gene was absent in all the genotypes screened. The new genotypes identified in the study can be used as potential donors for abiotic and biotic stress. Thus, the initial information generated here will lead to development of multiple stress tolerant rice varieties through a combination of molecular and classical breeding and can contribute significantly to yield stability and hence the livelihood security of Indian farmers.

Keywords: BLB, drought, molecular, rice, screening, submergence

Introduction
Rice (*Oryza sativa* L.) is the most important staple food crop for more than 60 percent of the global population and forms the cheapest source of food and energy. Globally, abiotic and biotic stresses are the major constraints in rice production by preventing a crop from reaching the genetically determined theoretical maximum yield. The major challenge to plant breeders is to overcome these constraints and produce high yielding rice varieties with multiple resistances to abiotic and biotic stresses. Nevertheless, with the availability of high quality reference genome sequence of rice, knowledge of exact position of genes/QTLs governing to stresses and availability of markers linked to desirable traits has opened up opportunities for breeders to identify plants with favorable alleles and transfer it in desired rice variety with required combination. Molecular markers are effectively complimenting the conventional breeding towards development of rice varieties with enhanced quality (Singh *et al.*, 2016) [1]. The immense potential of molecular markers as a tool for crop improvement has been extensively explored in rice by many researchers.
Impact of climate change has accelerated the emergence of new stresses that address the requirement for sustainable crop development and resistance to abiotic and biotic stresses. It is therefore necessary to relook for agronomically favourable alleles in the existing rice gemplasm. This will help the breeders to develop strategic breeding programmes in order to produce new stress tolerant varieties that can prepare agriculture for a stress free future. Thus, the present study aims at screening of rice genotypes for abiotic and biotic stress tolerance through tightly linked/gene based molecular markers.

Materials and Methods
In this study, twelve rice genotypes (Satyam, Sudha, Vaidehi, RYC743, Purnendu, FR13A, Desaria, Pansoradhan, Dudhi, Birar, Kalaladora and Kajargod) mostly originating from Bihar was used for molecular screening. DNA was extracted by using modified Dellaporta protocol (Dellaporta *et al.*, 1983) [2]. Hundred milligrams of fresh leaf tissue of 50 days old rice plants was grinded in 500 µl of DNA extraction buffer (100 mM Tris-HCl, 50 mM EDTA, 500 mM NaCl, 1% SDS and 4% PVP) using mortar and pestle without liquid nitrogen and incubated at 65° C for 45 minutes with frequent inverting. 200 µl of 5M potassium acetate (chilled)

was added to the sample, mixed vigorously and kept at -20^{0}C for 20 min. The samples were centrifuged for 8 minutes at 11,000 rpm and then 100 µl supernatant was transferred into another 1.5 ml Eppendorf tube. 100 µl of pre-chilled isopropanol was added and incubated at room temperature for 30 minutes. The samples were centrifuged for 8 minutes at 11,000 rpm at 28^{0}C and the DNA pellet was collected, washed twice with 70% ethanol and then final wash with 100% ethanol. After each washing, tubes were centrifuged at 3000 rpm for 5 min at 28^{0}C and then air dried the pellet. The DNA pellet was suspended in 25 µl of 100mM Tris buffer.

The DNA extracted was used for PCR amplifications. The molecular marker Sub1BC2, RM431 and four bacterial blight resistance (Xa4, xa5S/xa5SR/R-Multiplex, xa13 prom, pTA248) were used to select the tolerant or resistant genotypes for submergence (*Sub1*), drought grain yield QTL (q*DTY1.1*) and bacterial leaf blight resistance gene (*Xa4, xa5, xa13* and *Xa21*), respectively (Table 1). PCR amplification was done on a Agilent Mastercycler (Agilent Technologies, USA) in a total volume of 10 µl containing 1 × PCR buffer, 0.25 µM dNTPs, 0.25 µM each forward and reverse primer, 0.5 U Taq DNA Polymerase (Xcelris, India) and 1 µl template DNA using the following profile: a 4 minutes denaturation at 94°C and 35 cycles of 30 seconds denaturation at 94°C, 60 seconds annealing at 55°C for SSR/ gene specific markers and a 60 seconds extension at 72°C, followed by a final extension at 72°C for 5 minutes. The PCR products were resolved by electrophoresis in 1.5% agarose gels for pTA248, xa5 and xa13 prom markers and 2% agarose gels for xa4, RM431 and Sub1BC2 markers in 1X TAE buffer. The amplicons were visualized by UV light and documented (Uvitec gel doc system, UK). PCR Amplifications were carried out for at least twice for each sample. A DNA ladder 100 bp (Xcelris, India) was used as molecular markers to estimate the size of the amplicons.

Table 1: Molecular markers used for amplification in the study

S. No.	Gene/Qtl	Chromosome	Marker Name	Sequence(5'-3')	Approx. Band Size	References
1.	Sub1	9	Sub1bc2-F	Aaaacaatggttccatacgagac	268, 230	Septiningsih *et al.*, 2009 [3]
			Sub1bc2-R	Gcctatcaatgcgtgctctt		
2.	Qdty1.1	1	Rm431-F	Tcctgcgaactgaagagttg	180, 120	Vikram e*t al.*, 2011 [4]
			Rm431-R	Agagcaaaaccctggttcac		
3.	Xa4	11	Xa4-F	Atcgatcgatcttcacgagg	150, 120	Ma e*t al.*, 1999 [5]
			Xa4-R	Tgctataaaaggcattcggg		
4.	Xa5	5	Xa5s- F (Multiplex)	Gtctggaatttgctcgcgttcg	400, 300, 150	Sundaram e*t al.*, 2011 [6]
			Xa5s-R(Multiplex)	Tggtaaagtagataccttatcaaactgga		
			Xa5sr/R-F(Multiplex)	Agctcgccattcaagttcttgag		
			Xa5sr/R-R(Multiplex)	Tgacttggttctccaaggctt		
5.	Xa13	8	Xa13 Prom-F	Ggccatggctcagtgtttat	500, 250	Chu e*t el.*, 2006 [7]
			Xa13 Prom-R	Gagctccagctctccaaatg		
6.	Xa21	11	Pta248-F	Agacgcggaagggtggttcccgga	1000,700	Ronald e*t el*, 1992 [8]
			Pta248-R	Agacgcggtaatcgaagatgaaa		

Result and Discussion

Development of multiple stress tolerant varieties is one of the important objectives in rice breeding programs due to the occurrence of a number of stresses which adversely affecting rice growth and yield. However, this can be possible by accumulation of beneficial alleles from vast germplasm existing worldwide. Nowadays, molecular marker as a tool is used to determine germplasm containing beneficial alleles and assists to develop stress tolerant rice varieties with single and multiple resistance genes/QTLs. Many researchers have reported the use of molecular markers for searching for beneficial alleles in rice germplasm, such as the study of bacterial leaf blight resistance genes (Hittalmani *et al.*, 2013; Yadav *et al.*, 2013; Chungada *et al.*, 2016)[9, 10, 11], submergence tolerance (Samal *et al.*, 2014; Pradhan *et al.*, 2015; Singh *et al.*, 2015; Goswami *et al.*, 2017)[12, 13, 14, 15] and drought tolerance QTL (Chungada *et al.*, 2016; Singh *et al.*, 2016)[1,11]. This study was conducted to select the tolerant or resistant genotypes for submergence (*Sub1*), drought grain yield QTL (q*DTY1.1*) and bacterial leaf blight resistance gene (*Xa4, xa5, xa13* and *Xa21*) using tightly linked/gene based molecular markers.

Molecular screening for Submergence

Submergence is the serious problems in the flash flood prone rice cultivating areas. Submergence tolerance trait is governed by a major QTL on rice chromosome 9 (*Sub1*) derived from landrace FR13A that provides tolerance of two weeks of complete submergence (Septiningsih *et al*. 2013) [13]. The presence of Sub1 QTL was assured using Indel marker Sub1BC2 closely linked with the Sub1 gene (Septiningsih *et al.*, 2009) [3]. Result indicated the presence of an approximate 268 bp in submergence tolerance genotypes and approximately 230 bp for susceptible genotypes. This marker showed polymorphism of 38 bp size between the tolerant and susceptible genotypes. The three genotypes RYC743, Purnendu and FR13A were identified as submergence tolerant (Figure 1). Pradhan *et al.*, (2015) [13] also used Sub1BC2 markers to screen the various genotypes for submergence tolerance. In rice breeding, many researchers improved rice varieties for submergence tolerance using this *Sub1* locus specific marker through marker assisted breeding.

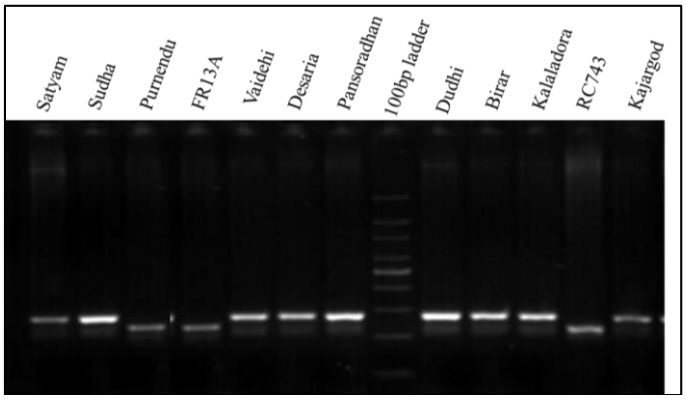

Fig 1: PCR amplification of Sub1BC2 marker linked to submergence tolerance

Molecular screening for Drought grain yield QTL

Rice is highly susceptible to drought throughout its life cycle and the reproductive stage is the most vulnerable. The loci controlling drought tolerance are widely distributed across the rice genome but only few major QTLs are mapped for a specific population and environment. A large number of drought-related QTLs have been reported in rice (Vikram *et al.*, 2011) [4]. The QTL q*DTY1.1* increase grain yield under drought conditions and closely linked markers located on chromosome 1 have been identified (RM431) at 38.89 Mb was used for grain yield under drought QTL selection by Singh *et al.* (2016). In the molecular analysis with RM431 marker, among the 12 genotypes, three genotypes Vaidehi, Dudhi and Birar indicated presence of the drought grain yield QTL (band size approximately 120 bp) (Figure 2). Recently, efforts have been made to introgress the QTL q*DTY1.1* into drought susceptible mega-varieties through the MABC programme. Therefore, the three new genotypes identified can be utilized as donor parent in breeding programmes for drought.

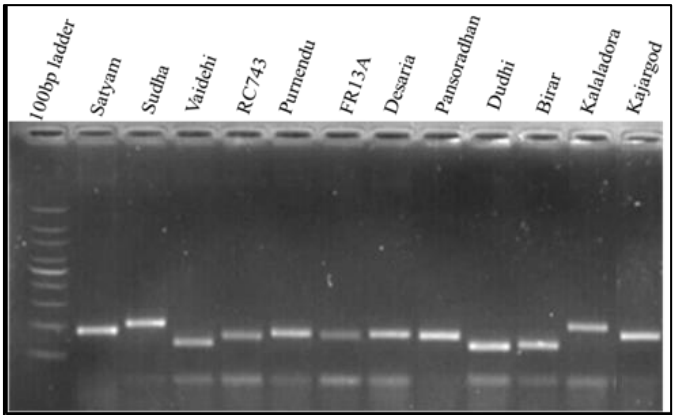

Fig 2: PCR amplification of RM431 marker linked to drought tolerance (Grain yield under drought)

Molecular screening for Bacterial leaf blight (BLB)

Bacterial blight is a serious disease in rice and it is especially prevalent in irrigated and rainfed lowland rice growing areas. The availability of markers tightly linked to BLB resistance genes has facilitated the screening of genotypes and marker assisted breeding without inoculation with the pathogen. In the present study, the genotypes were screened for the presence of four bacterial blight resistance genes i.e., *Xa4*, *xa5*, *xa13* and *Xa21* using molecular markers, Xa4, xa5 (Multiplex), xa13 prom and pTA248, respectively. The presence of resistance specific band of 120bp, 400bp and 150bp, 500bp and1000bp, will indicate the presence of BLB resistance genes, *Xa4*, *xa5*, *xa13* and *Xa21*, respectively. Our result is therefore indicates that among the studied rice genotypes, four genotypes were positive for *Xa4*, two genotypes for *xa5* and one genotype (heterozygous condition) for *Xa21* (Figure 3A, 3B, 3C). None of the genotypes showed presence of *xa13* gene in the rice genotypes used in this study (Figure 3D). Huang *et al.* (1997) [17], Sundaram *et al.* (2008) [18], Basavaraj *et al.* (2010) [19], Kumari and Rani (2015) [20] also used markers xa13 prom and pTA 248 linked to BLB resistance genes *xa13* and *Xa21*, respectively for marker assisted selection. We also found that genotype Sudha contained both *xa5* and *Xa21* gene and RYC743 have both *Sub1* and *Xa4* (Table 2). Genotype Sudha showed *Xa21* gene in heterozygous condition. Davierwala *et al.* (2001) [21] and Hittalmani *et al.* (2013) [9] also reported heterozygosity for *Xa21* gene. Many researchers used tightly linked molecular markers to introduce *X4*, *xa5*, *xa13* and *Xa21* into a BB susceptible lines. However, the *xa5* and *xa13* genes are recessive in nature. Pradhan *et al.* (2015) [22] reported that three gene combinations appeared to be the most effective among which *Xa21* is contributing the largest component of resistance. In the present study, rice genotypes with the resistance genes *Xa4*, *xa5* and *Xa21* will accelerate the breeding efforts for the development of bacterial leaf blight resistant rice cultivars through pyramiding approaches using marker assisted selection.

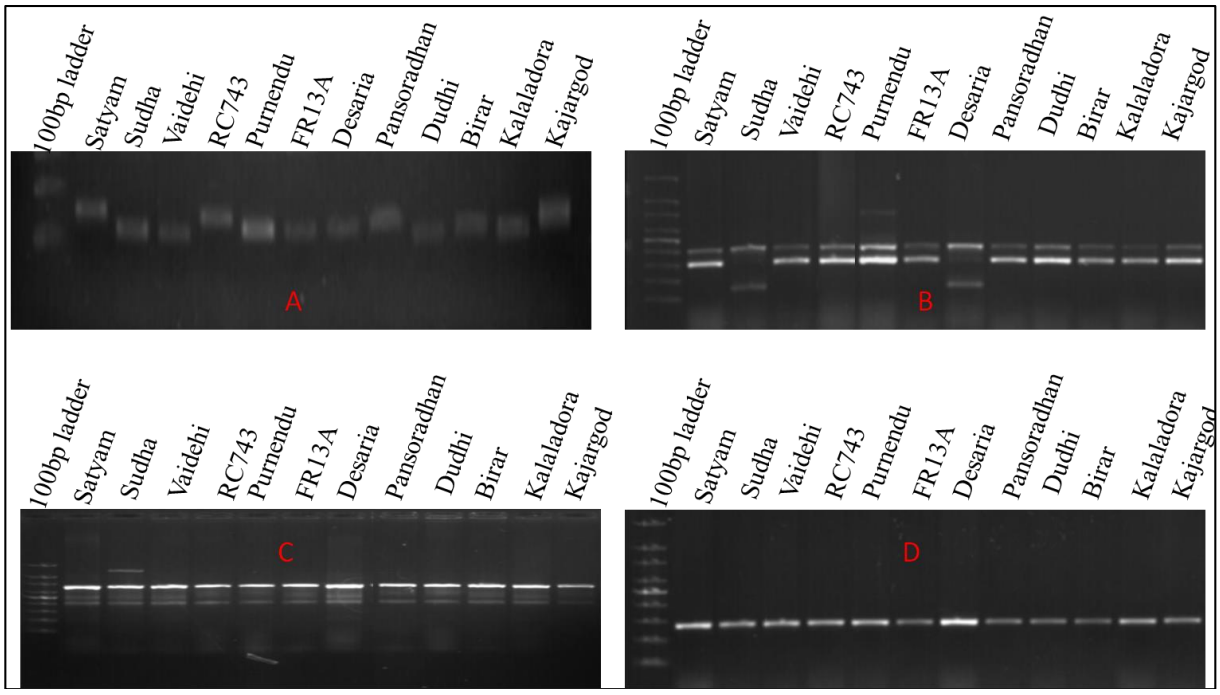

Fig 3: PCR amplification of markers linked to bacterial leaf blight resistance genes, *Xa4*, *xa5*, *Xa21* and *xa13* using primers A) Xa-4 B) xa5 (Multiplex) C) pAT248 and D) xa13 prom, respectively

Table 2: Molecular screening of Rice genotypes for submergence (*Sub1*), drought grain yield QTL (q*DTY1.1*) and Bacterial leaf blight resistance gene (*Xa4, xa5, xa13* and *Xa21*) using Sub1BC2, RM431 and four bacterial blight resistance (Xa4, xa5S/xa5SR/R-Multiplex, xa13 prom, pTA248) marker

Genotype	Origin	Gene/QTL status					
		Sub1	***qDTY1.1***	***Xa4***	***xa5***	***xa13***	***Xa21***
Satyam	Bihar	-	-	+	-	-	-
Sudha	Bihar	-	-	-	+	-	+
Vaidehi	Bihar	-	+	-	-	-	-
RYC743	Bihar	+	-	+	-	-	-
Purnendu	West Bengal	+	-	-	-	-	-
FR13A	Orissa	+	-	-	-	-	-
Desaria	Bihar	-	-	-	+	-	-
Pansoradhan	Bihar	-	-	+	-	-	-
Dudhi	Bihar	-	+	-	-	-	-
Birar	Bihar	-	+	-	-	-	-
Kalaladora	Bihar	-	-	-	-	-	-
Kajargod	Bihar	-	-	+	-	-	-

+ Presence of gene, - absence of gene

Conclusion

The availability of molecular markers which are linked to genes or QTLs allows us to genotype large number of samples and selects the genotypes of specific traits with minimum time and cost. The genotypes identified in the present study will be use as a donor parent in future breeding programmes for the development of new breeding population resistant or tolerant to abiotic and biotic stress. Hence, more resistant or tolerant varieties produced will improve yield of the rice and help to enhance food security.

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