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Validation of Markers Linked to Yield under Drought, Blast Resistance and Assessment of Polymorphism among the Donors for Use in Marker Assisted Selection in Rice

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Authors' contributions

This work was carried out in collaboration among all authors. Authors BV and BU carried out the major work and prepared manuscript and contributed to the refinement of the manuscript. Authors GU, PS, MSP and YH helped in foreground and background analysis of the study. Authors PS and AKS managed the literature searches. Author MSM designed the study, developed the structure and arguments for the paper and made critical revisions of the study. All authors read and approved the final manuscript.

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ABSTRACT

Rice is one of the most widely cultivated crop species in the world. Drought, a major constraint in upland rice (*Oryza sativa* L.) led to the unstable yields. The inconsistent yields often aggravated by the severe incidence of blast disease in many areas of rainfed ecosystem. To improve upland rice, introgression of two traits *viz.,* yield under drought stress and blast resistance is highly essential. We validated the markers linked to the $qDTY_{12.1}$ (exhibits grain yield under drought stress) and two

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important blast resistance genes (*Pi1* and *Pi54*) among the 12 important rice genotypes to use these markers in the foreground selection. Parental polymorphic survey was also conducted among the donor for the yield under drought (Vandana NIL possessing $qDTY_{12.1}$), upland rice variety Varalu and donor for blast resistance (line possessing *Pi1* and *Pi54* genes) using 500 SSR markers distributed across the genome. The polymorphism between Varalu, Vandana NIL is 30% whereas it is 24% between Varalu, blast donor and around 16% between three parents. The identified polymorphic markers which are linked to the genes as well as the parental polymorphic markers are useful to carry out foreground and background selection in marker assisted selection programme. In addition, the markers lies proximal and distal ends of the target genes from the respective chromosomes were also identified which can be used in recombinant selection. Thus the results emanated from this investigation are useful for the combining yield under drought and blast resistance traits through molecular breeding programme for development of rice variety suitable to the rainfed ecosystem.

Keywords: Drought; blast; SSR markers; parental polymorphism.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is the second staple food crop in the world and is a primary source of nutrition for more than half of the world's population. In changing global scenario, rice productivity has almost come to its plateau due to erratic rainfalls, unpredictable weather conditions coupled with an increased incidence of biotic and abiotic stresses. Worldwide, drought affects rice yield in approximately 23 million hectares of the rainfed ecosystem [1]. Vulnerability of rice to drought is likely to worsen in future due to the unpredicted global climate change [2]. Though many QTLs were identified for the yield, the major and stable QTL for yield under drought stress will be the right candidate for deployment. Bernier et al. [3] identified $qDTY_{12.1}$ for grain yield under reproductive stage drought stress on chromosome 12 and developed a donor line (IR 84984-83-15-18-B) having $qDTY_{12.1}$ in the genetic background of Vandana which suits the upland environments. During water stress conditions, rice blast disease caused by the filamentous ascomycete fungus, *Magnaporthe grisea* is also becomes a serious threat for rice production and leads to significant yield loss, as high as 70-80% during an epidemic [4]. Host plant resistance is very well documented and exploitation of host plant resistance is considered to be the best option for management of this disease [5]. Till date, more than 100 rice blast resistance genes and 340 QTLs were identified [6]. Among them, two major blast resistance genes *viz.,Pi1* and *Pi54* located on chromosome 11 are known to be very effective which were being used in introgression into several genetic backgrounds [7,8,9]. Therefore, we have chosen to validate the markers linked to $qDTY_{12.1}$ for improvising yield under drought stress and *Pi1* and *Pi54* for blast resistance.

To develop resistant varieties through marker-assisted selection (MAS), parental polymorphism has become a pre-requisite. Unless the parents are polymorphic for the traits of interest, the selection of plants with the traits of interest is not possible in further generations. Moreover, to assess the recurrent parent genome recovery, identification of polymorphic markers between the parents across their genome is also essential. Among the available molecular markers, simple sequence repeats (SSRs) are widely used due to the advantages like species specificity, genomic abundance, high reproducibility and co-dominant nature [10]. Hence in the present study, we have chosen SSR markers for the parental polymorphic study amongst the three parent's *viz.,* Varalu, Vandana NIL and BPT-LT used for the backcross breeding program.

2. MATERIALS AND METHODS

2.1 Plant Material

The rice varieties, Varalu, Vandana, Satya, BPT 5204, ISM, MTU1010, Tellahamsa, JGL3588, IR-50, Tulasi, Swarna Sub1 and BPT-LT (possessing *Pi1* and *Pi54* genes) collected from Indian Institute of Rice Research (IIRR), Hyderabad and Vandana NIL (possessing $qDTY_{12.1}$) from International Rice Research Institute, Philippines were kept for germination in Petri dishes to collect fresh leaf from 15-20 days old seedlings for DNA isolation.

2.2 Genomic DNA Extraction

Genomic DNA was extracted by a modified cetyl trimethyl ammonium bromide (CTAB) method [11]. About 200 mg of fresh leaf collected from 15-20 days old rice seedlings were used for DNA extraction with CTAB buffer (2% CTAB, 100 mMTris, 20 Mm ethylenediaminetetraacetate (EDTA), 1.4 M NaCl) preheated at 60°C). The quality and quantity of extracted DNA were estimated by comparing it with λ-DNA in agarose gel electrophoresis and purity was checked by using Nanodrop. The DNA was diluted to a final concentration of ~ 30 ng/µl using 1XTE buffer.

2.3 Polymerase Chain Reaction (PCR)

The PCR was performed as per the conditions described by Devi et al. [12] with minor modifications. Each PCR mixture was prepared using 20 ng template DNA, 5 picomoles of each primer (forward and reverse), 0.05 mMdNTPs, 1X PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$ and 0.01 mg/ml gelatin) and 1U of Taq DNA polymerase (Kappa, Germany) in a reaction volume of 10 µl. The PCR reaction was carried out in a thermal cycler by initial denaturation of template DNA at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 1 min. A final extension was done at 72°C for 7 min. The amplified products were electrophoretically resolved on 3% agarose gel for 2-3 hours at 120V and visualized under UV light in a gel documentation system. The size of the amplified fragments was calculated using a 100 bp DNA ladder (Fermentas, USA) as a reference.

2.4 Validation of *qDTY*$_{12.1}$ and Blast Resistance Genes (*Pi1* and *Pi54*)

To validate the presence of *qDTY*$_{12.1}$ and two major blast resistance genes (*Pi1* and *Pi54*) alleles among the 12 genotypes (Vandana, Varalu, Satya, BPT 5204, BPT-LT, ISM, MTU1010, Tellahamsa, JGL3855, IR-50, Tulasi, Swarna Sub1), a total of 5 linked SSR (RM511, RM28099, RM28130, RM28163, and RM28166) markers were selected [3]. For blast resistance genes, RM206 and Pi54 MAS for *Pi54* and RM224 markers for *Pi1* genes were used by following above mentioned PCR analysis [7].

2.5 Background and Recombinant Selection

A total of 500 SSR markers, each distributed randomly within every 5Mb distance across the twelve rice linkage groups (www.gramene.org) were used for the parental polymorphic survey among Varalu (upland rice variety), BPT-LT (possessing *Pi1*and *Pi54* genes) and Vandana NIL (possessing *qDTY*$_{12.1}$). Recombinant selection was carried among the three genotypes (Varalu, Vandana NIL and BPT-LT) using markers that are flanking to the target QTL/gene (e.g. less than 2 Mb on either side). For *qDTY*$_{12.1}$, a total of 20 SSR markers flanking on both sides of the QTL were selected whereas for blast genes *Pi1* and *Pi54*, 30 SSR markers were used.

2.6 Data Analysis

The genotypes whose marker allele coincides with that of the positive check (Vandana NIL or BPT-LT) were considered as positive for the QTL/genes. Similarly, the genotypes which were not correlating with the positive check marker allele were considered as negative for the QTL/genes. The parental polymorphic survey data was scored as “A” and “B” based the homozygous recipient allele, homozygous donor alleles and the data was imported into an Excel and further analyzed by GGT 2.0-software program. The percentage of markers homozygous for recipient parent (%A) and the percent donor alleles (%B) was calculated by using below mentioned formula [13].

$$G = [(X + 1/2Y) /100]/N$$

N = Total number of parental polymorphic markers screened
X = Number of markers showing homozygosity for recipient allele
Y = Number of markers showing homozygosity for donor alleles

3. RESULTS AND DISCUSSION

Rice productivity is being affected by several biotic and abiotic stresses among which the drought and blast respectively are the major ones. Molecular breeding has been proved to be the most economical and environmentally friendly approach to overcome these stresses. Molecular markers associated with the trait of interest increased the efficiency of gene introgression for developing resistant varieties.

Markers can be applied in backcross breeding at three stages *viz.,* foreground, recombinant and background selections. SSR markers have been used widely for genotyping plants over the past twenty years as they are highly informative, co-dominant and multi-allelic genetic markers that are experimentally reproducible and transferable among related species and these can be effectively applied for developing unique DNA profiles of rice genotypes because of having a high level of polymorphism and greater information [14]. Several studies reported that SSR markers are highly used in DNA fingerprinting, genetic diversity assessment, introgression, molecular mapping QTLs/genes and marker-assisted selection [15].

In marker-assisted breeding, markers can be used for the selection of target gene or QTL which referred to as foreground selection [16]. It can also be used to select for reproductive-stage traits in the seedling stage, allowing the best plants to be identified for backcrossing. Furthermore, recessive alleles can be selected, which is difficult to do using conventional methods. In the present study, the SSR markers were used for validation of $qDTY_{12.1}$ and also to study the polymorphism between Varalu, BPT-LT and Vandana NIL. Validation of $qDTY_{12.1}$ alleles among the 12 genotypes revealed that none of the tested entries posses the QTL alleles. Among the five linked SSR markers for the *qtl12.1*, the peak marker RM28099 clearly distinguished the positive check (Vandana NIL) from all the tested popular varieties whereas other linked marker RM28130 showed variation with all the varieties except BPT-LT. Another linked marker RM511 is monomorphic with all the varieties except Varalu. The remaining two SSR markers RM28163 and RM28166 showed polymorphism with a set of four (Vandana, Varalu, MTU1010, Swarna Sub1) and six (Vandana, BPT 5204, ISM, Tellahamsa, JGL3588 and Swarna Sub1) varieties respectively (Fig. 1). Similarly, PCR based approach was also followed for the validation of $qDTY_{12.1}$ introgressed lines of FUNAABOR-2, a popular Nigeria upland rice variety Ofada by using the SSR markers RM511, RM28099, RM1261, RM28130 and RM28166 [17]. Awasthi et al. [18] also reported the validation of two SSR markers RM263 and RM3825 linked to drought QTLs, *qDTY2.3, MQTL1.1, MQTL1.2* and *qDTY3.2* among the crosses (HUR 3022 × Nagina 22) × HUR 3022, (HUR 3022 × Birsa Gora) × HUR 3022, (Sarjoo 52 × Nagina 22) × Sarjoo 52 and (Sarjoo 52× Birsa Gora) × Sarjoo 52) and found suitable for use in marker-assisted backcross breeding programs. Singh et al. [19] reported that the association of RM252 with the root thickness using the DH lines derived from the cross of CT9993 × IR68766 and recombinant inbred lines (RILs) derived from cross of IR66366 × IR66876 as well as on a set of twenty cultivars / landraces adapted to the rain fed lowland and upland ecosystems.

Genotyping of 12 genotypes for two major blast resistance genes (*Pi54* and *Pi1*) revealed that, all the genotypes showed the absence of these genes. RM206 linked to *Pi54* and RM224 linked to *Pi1* showed clear polymorphism with positive check, BPT-LT from all the tested genotypes whereas another marker for *Pi54*, Pi54 MAS is monomorphic for three genotypes (Varalu, Vandana and Vandana NIL). These markers can be used for the foreground selection in the introgression of $qDTY_{12.1}$, *Pi54* and *Pi1* genes in the tested genotypes. The genetic dissection of the offspring with closely linked molecular markers for the gene of interest is an effective way of speeding up the selection process at each generation for developing the superior varieties with maximum RPG recovery [3]. Patroti et al. [7,20] used same markers (RM206, PI54 MAS and RM224) for the foreground selection of *Pi54* and *Pi1* genes in to the genetic background of Swarna Sub1 and Improved Samba Mahsuri. Swathi et al. [8] carried out foreground selection of blast resistant gene, *Pi54* using functional marker Pi54 MAS in the genetic background JGL1798.

The parental polymorphism analysis by marker screening plays a crucial role in the process of selecting the individuals having higher recurrent parent genome. A total of 500 SSR markers over 12 chromosomes were used for testing polymorphism between three parents which includes two donors and one upland rice variety (Vandana NIL, BPT-LT and Varalu) which indicated a clear polymorphism between them. Out of 500, 150 SSR markers (30%) exhibited polymorphism between Varalu and Vandana NIL (donor for $qDTY_{12.1}$). Among the 12 chromosomes, the highest polymorphism (42.5%) was recorded on chromosome 12 and the least (20%) on chromosome 8 (Table 1). In between Varalu and BPT-LT (donor for blast resistance genes), a total of 120 polymorphic SSR markers were observed with a percentage of 24%. The highest (31.58%) polymorphism was observed on chromosome 9 while the least (18.18%) on chromosome 2 (Table 1). Interestingly, among the three parents, 80

polymorphic markers (16%) were found with highest (25%) polymorphism on chromosome 3 and least (10.87%) chromosome 10 (Table 1 & Fig. 2). The identified markers can be useful for the background selection of the tested genotypes in the rice improvement programme. Background selection refers to the markers that are unlinked to the target gene/QTL on all other chromosomes, in other words, markers that can be used to select against the donor genome. This is extremely useful because the recurrent parent genome recovery can be greatly accelerated. Challa et al. [21] observed 95 (13.17%) polymorphic SSR markers after conducting a parental polymorphism survey between Kasturi and Chaw Khao using 721 microsatellites (SSR) markers. Polymorphism survey between C14-8, CARI Dhan 5 and donor IRBB 60 using 200 highly variable SSR markers reported 36 and 48 polymorphic SSR markers for C14-8 and CARI Dhan 5 respectively when compared with the donor [22]. Yerva et al. [23] screened two parents namely PR122 and IR10M196 for parental polymorphism using 647 SSR markers, of which 108 markers exhibited polymorphism with 16.69%. Marathi et al. [24] reported maximum polymorphism was observed in chromosome 4 with overall polymorphic percentage of 32.93. For the identification of sheath blight tolerance QTLs in rice, 637 SSR markers were used between the parents HP2216 and Tetep and found only 74 markers were polymorphic [25]. For introgression of genes/QTLs, parents were selected based on genetic diversity with adequate polymorphism at molecular level [21].

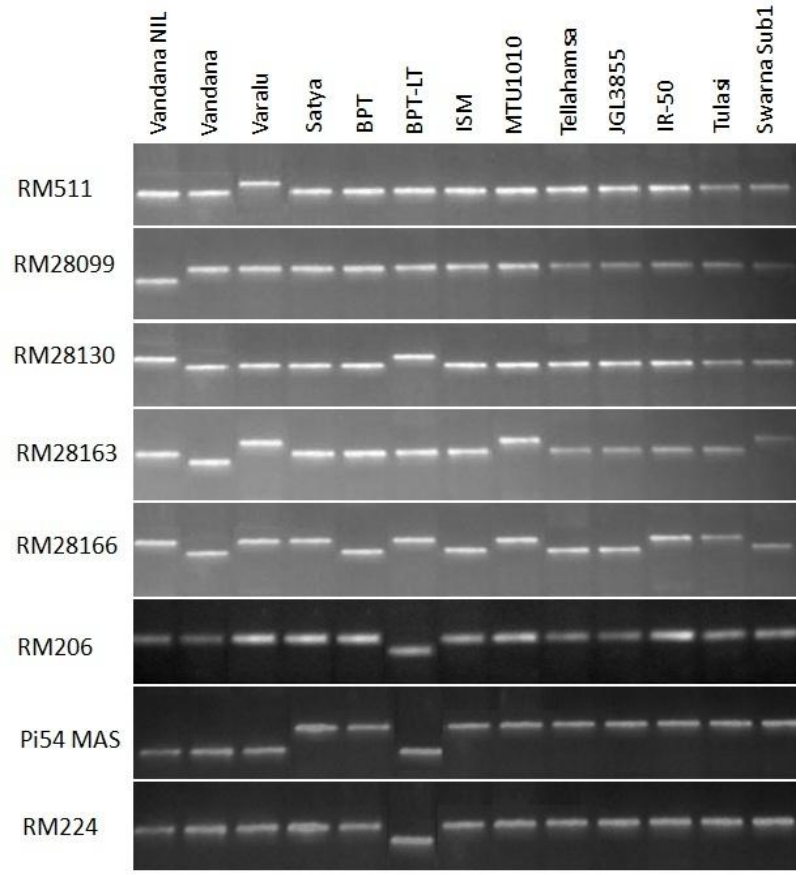

Fig. 1. Validation of *qDTY*$_{12.1}$ and blast resistance genes among the 12 rice genotypes. First five SSR markers (RM511, RM28099, RM28130, RM28163 and RM28166) are linked to *qDTY*$_{12.1}$ and next two markers are linked to *Pi54* (RM206 and Pi54 MAS) gene and last SSR marker (RM224) is linked to *Pi1* gene

Table 1. Chromosome wise details of parental polymorphism percentage among the three-parent lines (Varalu, Vandana NIL and BPT-LT) using SSR markers

Chrom number	No. of markers screened	No. of markers polymorphic for Varalu and Vandana NIL	% of polymorphism between Varalu and Vandana NIL	No. of markers polymorphic for Varalu and BPT-LT	% of polymorphic between Varalu/ Vandana NIL/BPT-LT	No. of markers polymorphic between Varalu/ Vandana NIL /BPT-LT	% of polymorphism
1	42	12	28.57	10	23.81	10	23.81
2	44	10	22.73	8	18.18	8	18.18
3	32	11	34.38	10	31.25	6	18.75
4	37	10	27.03	9	24.32	5	13.51
5	38	12	31.58	11	28.95	8	21.05
6	45	16	35.56	10	22.22	6	13.33
7	43	13	30.23	11	25.58	7	16.28
8	50	10	20.00	9	18.00	6	12.00
9	38	15	39.47	12	31.58	6	15.79
10	46	10	21.74	9	19.57	5	10.87
11	45	14	31.11	10	22.22	5	11.11
12	40	17	42.50	11	27.50	8	20.00
Total	**500**	**150**	**30**	**120**	**24**	**80**	**16**

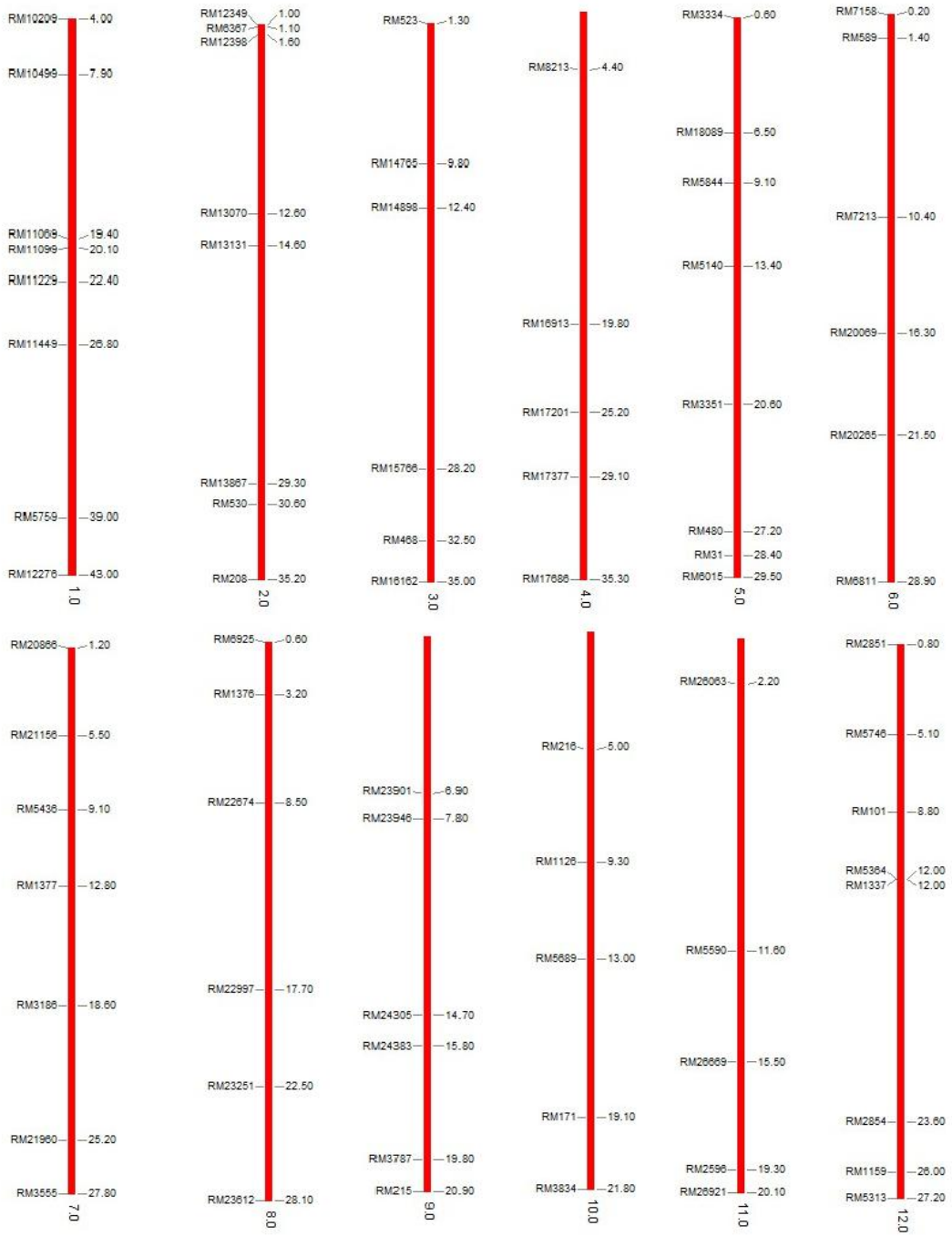

Fig. 2. Graphical representation of polymorphic markers among the three-parent lines (Varalu, Vandana NIL and BPT-LT)

In the present study, three genotypes were analyzed for the recombinant selection. Out of 20 SSR markers near the *qDTY*$_{12.1}$ locus, nine markers showed polymorphism between Varalu and Vandana NIL at *qDTY*$_{12.1}$ region on chromosome 12. Among them six markers (RM27973, RM1337, RM27970, RM7102, RM28076 and RM28089) at proximal end and three markers (RM28173, RM3448 and RM28404) at distal end were observed. Similarly at *Pi54* locus, identified two markers (RM26998 and RM26999) at proximal end and RM27132 at

distal end while at *Pi1* locus RM27172 and RM27282 at proximal end and three markers (RM27310, RM27322 and RM2136) at distal end were showed polymorphism between Varalu and BPT-LT on chromosome 11. The purpose of recombinant selection is to reduce the size of the donor chromosome segment containing the target locus (i.e. size of the introgression). This is important because the rate of decrease of this donor fragment is slower than for unlinked regions and many undesirable genes that negatively affect crop performance may be linked to the target gene from the donor parent which refers as linkage drag [26]. The identified markers can be used to minimize the linkage drag. Feng et al. [27] introgressed blast resistant gene *pi21* in to the genetic background of Kongyu-131 by following foreground, background and recombinant selection and obtained recurrent parent genome of 99.92%. Through MAS, basmati variety, PB1 was improved with *Xa13* and *Xa21* with minimum linkage drag above 1.3cM [28]. Jamaloddin et al. [9] observed 0.5-3.5 Mb of linkage drag on proximal and distal ends of improved lines of Tellahamsa for blight and blast resistance.

4. CONCLUSION

In the present study validated the markers linked to the $qDTY_{12.1}$, blast resistance genes (*Pi1* and *Pi54*) among the 12 popular genotypes, and confirmed none of the popular variety is having blast resistance and yield under drought stress. Parental polymorphism among upland rice variety Varalu, Vandana NIL and BPT-LT revealed 30% and 24% respectively while between three parents 16% polymorphism was observed. Further, identified nine polymorphic flanking markers for $qDTY_{12.1}$, eight closest markers for *Pi54* and *Pi1* genes on proximal and distal ends of the target loci to carry recombinant selection. The identified polymorphic markers from the present study will be utilized for foreground and background selection in marker assisted selection programme to improve yield in drought-prone environment and blast resistance.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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