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Introgression of rice blast and bacterial leaf blight resistance genes into HPR2143 rice variety through marker assisted selection and Anther culture

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Abstract
The use of resistant rice cultivars is the most proficient way to overcome rice blast and bacterial blight diseases in rice. Cultivars, however, experience a rapid breakdown in their resistance, predominantly due to the advent of new pathotypes. Therefore, for durable resistance, it is important to combine multiple genes that impart resistance to more than one pathotypes into one genetic background. Foreground selection of BC_2F_2 plants of two crosses (HPR2143/DHMAS164 and HPR2143/PB1) was done to detect the presence of blast resistance genes i.e. *Pita* and *Pi9* in these plants. Plants homozygous for gene *Pita* were screened by a pair of gene specific markers YL87/155 (coupling dominant) and YL87/183 (repulsion dominant). Likewise, homozygous positive plants for gene *Pi9* were identified using the co-dominant marker *Pi9*STS-1. For bacterial blight (BLB) resistance genes, BC_2F_2 plants of two crosses (HPR2143/IRBB54 and HPR2143/PR114) were screened for genes *Xa21* and *Xa38* using gene-derived markers BLB248 and Oso4g53050-1, respectively. Anther culture of developed F_1s was attempted for fixation of genes.

Keywords: Rice, *Pi9*, *Pita*, *Xa21*, *Xa38*

Introduction

Rice (*Oryza Sativa* L.) is the substantial food crop of Southeast Asia where almost half the world's population reside (Mohiuddin *et al*., 2014) [17]. Approximately 92% of rice is grown and consumed by Asian population, which constitute 55% of the world population (Wilson and Talbot, 2009) [32]. It has been estimated that rice production must increase to approximately 136 million tonnes by 2050 in order to keep pace with the rising population (Anonymous, 2015) [2]. Although rice production has accelerated almost threefold over the past three decades (Christopher, 2002) [4], continued increase in yield productivity and number of varieties is necessary to meet the growing demand of the global population (De-Filippis, 2014) [9]. However, one of the primary restraints in achieving this target is the increased manifestation of certain pests and diseases (Yugander *et al*., 2018) [35].

In spite of continuous progression in rice breeding programmes, significant yield losses from diseases still prevail in rice. Of the most destructive diseases that abstain rice production, blast caused by the fungus *Magnaporthe grisea* (anamorph: *Pyricularia grisea*) ranks first because of its severity under favourable conditions. Another important disease of rice is bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* which is an endemic constraint to rice production worldwide (Narayanan *et al*., 2004) [19]. The utilization of resistant (R) genes and germplasm are efficient ways to combat these devastating diseases (Ni *et al*., 2015) [20].

The improvement of rice varieties for resistance to the diseases that are predominant and eradicative is necessary for the sustainability of rice grain yields (Pinta *et al*., 2013) [22]. Breeding for host-plant resistance is regarded as the most frugal and eco-friendly strategy for pest and disease management of crop plants. Due to high levels of variability in the disease populations in growing areas, past attempts to achieve varietal resistance to blast and bacterial leaf blight disease have been disappointing (Sreewongchai *et al*., 2010) [30].

The utilization of DNA markers in plant breeding programme is known as marker assisted selection (MAS) (Akhtar *et al*., 2010) [1]. The application of molecular markers can speed up

resistance breeding efforts, since segregating plants can be identified by molecular marker alleles instead of their phenotypes and introgression of multiple resistance genes or gene pyramiding can be monitored easily in a population (Sundaram *et al*., 2014) [31]. Marker assisted selection provides a potential solution to some of the problems that cannot be resolved by traditional breeding (Miah *et al*., 2013) [16]. DNA marker technology has contributed immensely to genetic advancement through the selection of desirable characteristics, such as resistance to disease. Rice has become a model plant for research in the field of breeding and genetics. Quantum leap in genomics and molecular biology, proteomics and metabolics has opened new channels to expend innovative approaches to rice breeding. Foreground selection has become an integral element of germplasm improvement. Molecular markers have their implication in trait improvement by tagging large number of genes/QTLs for different traits with them (Khush, 2013) [14]. Owing to genetic linkage, DNA markers can be used to mark the existence of allelic variation in the genes underlying these traits (Collard and Mackill, 2008) [10].

Anther culture, an innovative method for accelerating the generation of homozygous doubled haploids (DH), can be used to hasten the varietal improvement programmes in rice (Herawati *et al*., 2010) [12]. Many desirable traits such as disease resistance, high grain weight, dwarf plant type and abiotic stress tolerance were introgressed into rice breeding population by means of anther culture (Sengsai *et al*., 2007) [27]. Double haploidy (DH) technology thus offered a technique to shorten breeding cycles and and increase genetic gain (Ren *et al*., 2017) [26]. In the light of the above, the present study was undertaken to exploit molecular marker technologies and anther culture for the efficient management of rice blast and bacterial leaf blight through the introgression of important rice blast (*Pita* and *Pi9*) and bacterial leaf blight (*Xa21* and *Xa38*) resistance genes into a popular variety, HPR2143 rice.

Materials and Methods

As resistance gene donors to rice blast, the rice genotypes DHMAS164 and PB1 consisting of the *Pita* and *Pi9* genes were used respectively, while genotypes IRBB54 and PR114 constituting genes *Xa21* and *Xa38* respectively were used as resistance gene donors against bacterial blight. However, rice cv. HPR2143 was used as a recipient parent. Previously established crosses between recurrent parent HPR2143 and resistance donors and their further backcrosses (BC_2F_2) developed through an ongoing programme in the Department of Agricultural Biotechnology, CSKHPKV Palampur at that time were used as an initial material for the research (Table 1).

The seeds of selected elite BC_2F_2 progenies of four crosses, HPR2143/DHMAS164 (*Pita*), HPR2143/PB1 (*Pi9*), HPR2143/IRBB54 (*Xa21*) and HPR2143/PR114 (*Xa38*) were sown in small separate plots (in nursery beds) in the cage house of the Department of Agricultural Biotechnology, CSK

HPKV Palampur.

Table 1: Details of the material used in the present study

Cross	Gene	Designated Plant Number	Generation
HPR2143 × DHMAS164	*Pita*	1-41	BC_2F_2
HPR2143 × PB-I	*Pi9*	42-83	BC_2F_2
HPR2143 × IRBB54	*Xa21*	84-125	BC_2F_2
HPR2143 × PR114	*Xa38*	126-167	BC_2F_2

Research Methodology

Foreground selection was carried out to select homozygous plants for genes *Pita*, *Pi9*, *Xa21* and *Xa38* using specific gene-derived markers. The selected gene-positive homozygous plants for the *Pita* and *Pi9* genes were crossed to obtain sufficient F_1 seeds (≈100) in order to combine two blast resistance genes (*Pita*+*Pi9*). In the similar manner, to combine two bacterial leaf blight resistance genes (*Xa21*+*Xa38*), the selected gene positive homozygous plants for genes *Xa21* and *Xa38* were crossed to obtain adequate seeds (≈100). Sufficient number of F_1 plants of crosses HPR2143/PB1 (*Pi9*) × HPR2143/DHMAS164 (*Pita*) and HPR2143/IRBB54 (*Xa21*) × HPR 2143/PR114 (*Xa38*) was raised under controlled conditions. Anthers of above-mentioned crosses were used to induce androgenic doubled haploids following previously standardised protocols in our lab (Chopra, 2006) [7].

i. Marker aided foreground selection

Genomic DNA of 167 plants of aforementioned four crosses was extracted from fresh leaves at an appropriate stage, using CTAB method (Murray and Thompson, 1980) [18]. The PCR amplification was performed using gene-derived STS primers. Foreground selection for the blast resistance gene *Pita* was conducted using the gene derived markers, YL87/155 (coupling dominant) and YL87/183 (repulsion dominant) (Table 2). Likewise, homozygous positive plants for *Pi9* gene were screened using *Pi9*STS-1 marker (Table 3). The plants having bacterial blight resistant genes *Xa21* and *Xa38* were identified using the gene-derived markers Blb248 and Os04g53050-1, respectively (Table 4. For DNA amplification, a reaction mixture of 12.5 µl volume was prepared using 7.05 µl of PCR water, 2.0 µl template DNA (20 ng), 1.25 µl 10X buffer (10 mM), 1.0 µl dNTP mix (0.5 mM each of dATP, dGTP, dCTP, dTTP), 0.5 µl forward primer (5 pM), 0.5 µl reverse primer (5 pM) and 0.2 µl Taq polymerase (3 U/µl). The amplification was carried out in Veriti 96 wells Thermal Cycler (Applied Biosystem, CA, USA) following PCR amplification profiles given in Tables 2-4.

Hybridization among backcross derivatives containing single genes was attempted in order to pyramid 2 genes for each disease. Anthers of aforementioned crosses were used to induce androgenic doubled haploids following already standardised protocols in our lab (Chopra, 2006) [7].

Table 2 PCR profile used for amplification of YL87/155 and YL87/183 markers linked to gene, *Pita*

Steps	Temperature profile		Cycles
	YL87/155	YL87/183	
Initial Denaturation	94 °C for 3 minutes	94 °C for 3 minutes	
Denaturation	94° C for 45 seconds	94° C for 45 seconds	
Annealing	55°C for 45 seconds	55°C for 45 seconds	35 cycles
Extension	72°C for 1 minute	72°C for 1 minute	
Final extension	72 °C for 7 minutes	72 °C for 7 minutes	

Table 3 PCR profile used for amplification of *Pi9*STS-1 marker linked to the gene, *Pi9*

Steps	Temperature profile	Cycles
	*Pi9*STS-1	
Initial Denaturation	94 °C for 5 minutes	
Denaturation	94 °C for 1 minute	
Annealing	59 °C for 1 minute	35 cycles
Extension	72 °C for 1.30 minutes	
Final extension	72 °C for 5 minutes	

Table 4: PCR profile used for amplification of Os04g53050-1 and Blb248 markers linked to genes, *Xa38* and *Xa21*, respectively

Steps	Temperature profile		Cycles
	Os04g53050-1	Blb248	
Initial Denaturation	94 °C for 5 minutes	94 °C for 5 minutes	
Denaturation	94 °C for 1 minute	94 °C for 30 seconds	
Annealing	56 °C for 1 minute	55 °C for 1 minute	35 cycles
Extension	72 °C for 1 minute	72 °C for 1 minute	
Final extension	72 °C for 5 minutes	72 °C for 5 minutes	

ii. Anther culture of F₁ progenies to achieve fixing of two genes each in doubled haploids: The F₁s of two crosses i.e. (*Pita+Pi9*) and cross 2 (*Xa21+Xa38*) developed at the cage house of the Department of Agricultural Biotechnology, CSKHPKV were used as the source of anthers for developing blast and bacterial leaf blight resistant doubled haploids, respectively. Optimal fertilizers and plant protection measures were adopted to raise healthy plants.

Results and Discussion
i. Foreground selection for *Pita* gene homozygotes: For cross 1 (HPR2143 X DHMAS164), the gene specific markers

YL87/155 (coupling dominant) and YL87/183 (repulsion dominant) were used for foreground selection. In case of YL87/183 marker, out of 41 plants screened, bands were present in 25 plants including progeny number 3, 7 to 13, 15 to 17, 19 to 21, 23, 24, 26 to 29, 34, 35, 38, 40 and 41 (Fig. 1, lanes represented by blue arrows) which indicated the absence of *Pita* gene in these plants. The remaining 16 plants (1, 2, 4, 5, 6, 14, 18, 22, 25, 30, 31, 32, 33, 36, 37, and 39; lanes indicated by red arrows in Fig. 1) in which bands were absent therefore had the probability of having *Pita* gene. In order to confirm the existence of the *Pita* gene in these 16 plants, the coupling phase marker YL87/155 was amplified on the same set of plants. Further foreground selection using this marker indicated presence of desired amplified bands in 5 progenies having progeny nos. 6, 30, 36, 37 and 39 confirming the presence of *Pita* gene in these progenies (Fig. 2; lanes represented by red arrows.

Two markers, YL155/YL87 and YL183/YL87 belong to the functional marker class. However, both are dominant and cannot discriminate homozygous genotypes from heterozygous ones. Thus, we detected the same individuals using one marker, and then followed with the other to identify homozygous *Pita* plants. In literature, earlier workers have also used these two markers to infer homozygosity of the plants for *Pita* gene (Xiao *et al*., 2016) [34]. In the present case, out of a total population of 41 plants, five were assessed to be homozygous for *Pita* gene using YL87/183 and YL87/155 markers. This frequency is comparatively lower than the expected 25 per cent. This may be due to small size of the sampled plants and differential development accounting for the distorted ratio.

Fig 1: Foreground selection for blast resistance gene *Pita* in 41 BC₂F₂ progenies of cross HPR2143×DHMAS164 using repulsion phase marker YL87/183 *Lanes in the gel from left represent M: Molecular weight marker (1kb ladder), R: recurrent parent (HPR2143), D: donor parent (DHMAS164) and 1-41 BC₂F₂ progenies of HPR2143/DHMAS164 cross. PCR products were resolved on 1.5 per cent agarose and stained with ethidium bromide*
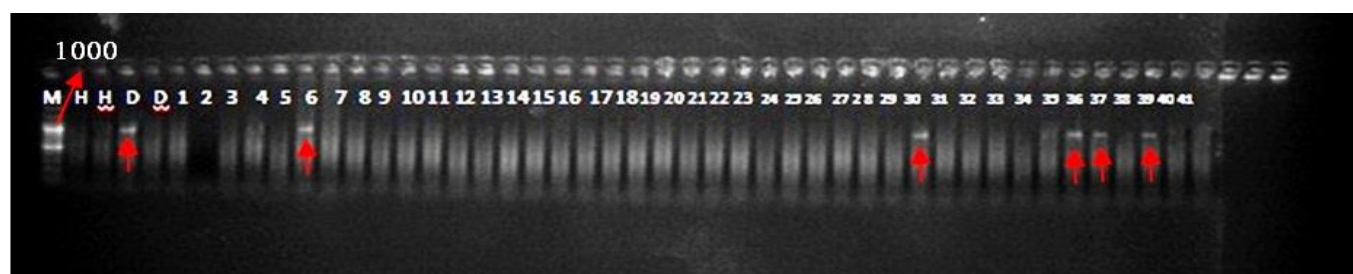
Fig 2: Foreground selection for blast resistance gene *Pita* in 41 BC₂F₂ progenies of cross HPR2143×DHMAS164 using dominant phase marker YL87/155 *Lanes in the gel from left represent M: Molecular weight marker (1kb ladder), H: recurrent parent (HPR2143), D: donor parent (DHMAS164) and 1-41 BC₂F₂ progenies of HPR2143/DHMAS164 cross. PCR products were resolved on 1.5 per cent Agarose and stained with ethidium bromide*

**ii. Foreground selection for *Pi9* gene homozygotes**
In cross 2 (HPR2143 X PB1), out of 42 plants screened with *Pi9*STS-1 marker linked to gene *Pi9*, 20 progenies (42 to 46, 51, 61, 63, 64, 66, 67, 69,72, 74 to 79 and 81) were found to

be homozygous positives for *Pi9 gene* (Fig. 3; Lanes indicated by red arrows). The *Pi9* broad-spectrum blast resistance gene derived from the wild tetraploid species, *Oryza minuta* (Sitch *et al*., 1989) [29] is most successful

against the blast races prevalent in various parts of India (Khanna *et al*., 2015; Rathour *et al*., 2011) [15, 24], which makes it an ideal choice for use in breeding programme. The gene-derived marker Nbs2Pi-9 and SSR markers linked to the allelic gene *Piz* (Fjellstrom *et al*., 2006) [10] have been widely used in MAS of the *Pi9* gene. However, the *Pi9*-linked SSR markers can sometimes result in misidentification of gene-positive plants due to the recombination between marker and gene. Considering the relevance of *Pi9* gene and

shortcomings of the currently available markers for this gene, Rathour *et al*. (2016) [25] developed co-dominant gene-derived markers for efficient marker-assisted selection of the *Pi9* gene by making use of the publicly available DNA sequence of the cloned *Pi9* gene. Of the three STS markers designed by Rathour *et al*. (2016) [25], the marker *Pi9*STS-1 showed a fragment size of 1073 bp in *Pi-9* containing line Pusa 1637 and 1185 bp in PB1, while *Pi9*STS-2 amplified a 753 bp fragment in Pusa 1637 and 844 bp in PB1.
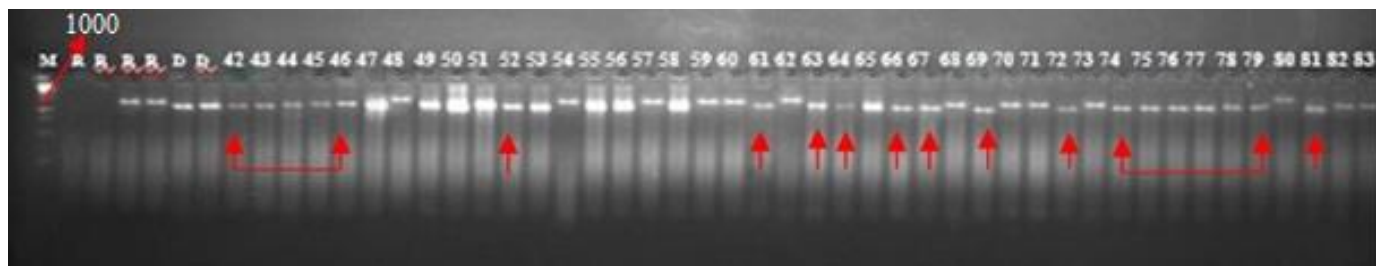
Fig 3: Foreground selection for blast resistance gene *Pi9* in 42 BC$_2$F$_2$ progenies of cross HPR2143×PB-1 using *Pi9*STS-1 *Lanes in the gel from left represent M: Molecular weight marker (1kb ladder), R: recurrent parent (HPR2143), D: donor parent (DHMAS164) and 42-83: BC$_2$F$_2$ progenies of the cross, HPR2143 X PB-1. PCR products were resolved on 2.5 per cent Agarose and stained with ethidium bromide*

iii. Foreground selection for *Xa21* gene homozygotes
In cross 3 (HPR2143 X IRBB54), out of 42 plants screened using marker *BLB 248*, 12 (86, 91, 96, 97, 98, 106, 110, 111, 112, 115, 123 and 125) were found to be homozygous positives (lanes represented by red arrows in Fig. 4), 23 were homozygous negatives (84, 85, 89, 92, 93 to 95, 100, 101, 103 to 105, 107 to 109, 113, 114, 116, 117, 120 to 122 and 124, represented by blue arrows) and 7 were heterozygous (87, 88, 90, 99, 102, 118, and 119, represented by yellow arrows) for gene *Xa21* (Fig. 4).

In the past, several former researchers had reported introgression of various BLB resistance gene(s) into elite

backgrounds using marker-assisted selection. Most of these were combinations of 3-4 genes together, followed by two and a single gene. Single gene, *Xa21* is reported to have been successfully introgressed into Chinese hybrid rice Minghui 63 (Chen and Ronald, 1999) [6] and *Xa25* into rice transgenic cultivar, HPTER (Gao *et al*., 2006) [11]. Similarly Bustamam *et al*. (2002) reported successful pyramiding of 2 genes (*Xa4+xa5* and *Xa4+Xa7)* into two rice cultivars, Angke and Conde in Indonesia. Most of these introgressions have been reported to be associated with better and durable resistance against BLB.
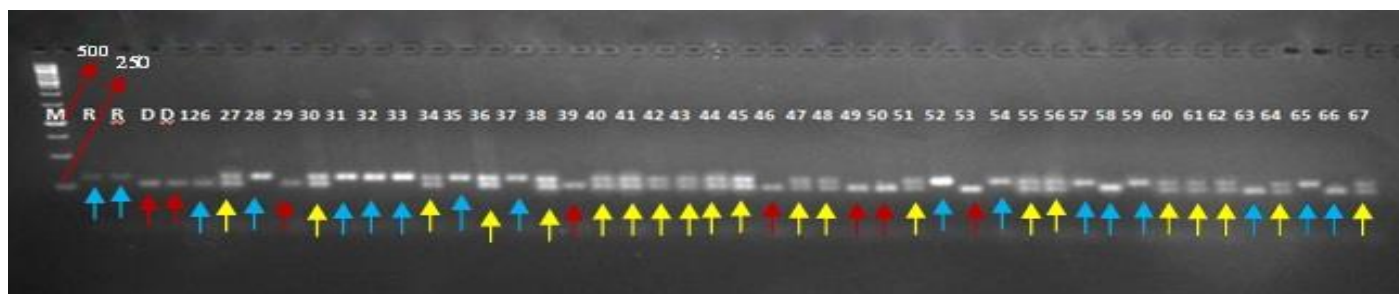

Fig 4: Foreground selection for blast resistance gene *Xa21* in 42 BC$_2$F$_2$ progenies of cross HPR2143×IRBB54 using Blb248 *Lanes in the gel from left represent M: Molecular weight marker (1kb ladder), D: recurrent parent (HPR2143), R: donor parent (DHMAS164) and 84-125: BC$_2$F$_2$ progenies of HPR2143 X IRBB54. PCR products were resolved on 1.5 per cent Agarose and stained with ethidium bromide*

iv. Foreground selection for *Xa38* gene homozygotes
In cross 4 (HPR2143 X PR114), a total of 42 plants were screened with marker *Oso4g 53050-1*, out of which 6 progenies (129, 139, 146, 149, 150, and 153, represented by red arrows) were found to be homozygous positives and 15 (126, 128, 131 to 133, 135, 137, 152, 154, 157 to 159, 163, 165 and 166, represented by blue arrows) were inferred to be homozygous negatives. Twenty one progenies (127, 130, 134, 136, 138, 140 to 145, 147, 148, 151, 155, 156, 160 to 162, 164 and 167, represented by yellow arrows) were found to be heterozygous for gene *Xa38* (Fig. 5).
Xa38, a novel BLB resistance gene was identified from *Oryza nivara* acc. IRGC 81825 (Cheema *et al*., 2008) [5] and was mapped on chromosome 4L in a 38.4 kb region that provided high level of resistance against multiple virulent isolates of

BLB pathogen (Bhasin *et al*., 2012) [3]. Precise foreground selection is the key to the success of MABB and the highly reliable marker Os04g53050-1 was used for detection of *Xa38* (Bhasin *et al*., 2012) [3]. Since the gene is a novel one, identified and mapped recently, not many reports on its introgression and pyramiding are documented. More recently Yugander *et al*. (2018) [35] have reported incorporation of the novel bacterial blight resistance gene *Xa38* into the genetic background of elite rice variety Improved Samba Mahsuri (ISM) that already possesses BLB pyramid of genes *xa5+xa13+Xa21*. The resultant 18 backcross-derived lines (BDLs) exhibited very high level of resistance against multiple *Xoo* strains and displayed agro-morphological traits, grain qualities and yield levels similar to or better than those of recurrent parent Improved Samba Mahsuri (ISM).

Fig 5: Foreground selection for blast resistance gene *Xa38* in 42 BC$_2$F$_2$ progenies of cross HPR2143×IRBB54 using Oso4g53050-1 *Lanes in the gel from left represent M: Molecular weight marker (1kb ladder), R: recurrent parent (HPR2143), D: donor parent (DHMAS164) and 126-167: BC$_2$F$_2$ progenies of the cross,* HPR2143 X PR114. *PCR products were resolved on 1.5 per cent Agarose and stained with ethidium bromide*

v. Anther plating and callus induction

For cross 1 (*Pita × Pi9* and reciprocals), out of 22562 anthers plated on callus induction medium, 2080 calli were induced with an overall callus induction frequency of 9.21 per cent. Similarly, for cross 2 (*Xa21 × Xa38* and reciprocals) 1534 calli were induced from a total of 16894 plated anthers with a callus induction frequency of 9.08 per cent (Table 5). Different stages of culture and callus induction in cultured anthers of F$_1$s of crosses among selected backcross progenies carrying different gene combinations (*Pita × Pi9* and reciprocals) and (*Xa21 × Xa38* and reciprocals) are shown in Plate 1.1.

Table 5: Frequency of callus induction in anthers of F$_1$s of crosses among selected backcross progenies

Cross	Total number of anthers plated	Total number of calli induced	% Callus induction
Pi-ta × Pi-9 and reciprocals	22562	2080	9.21
Xa21 × Xa38 and reciprocals	16894	1534	9.08
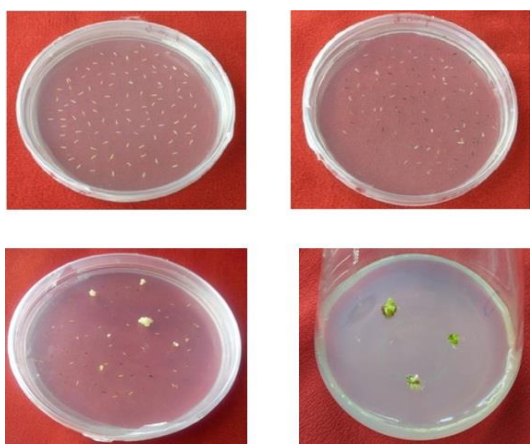
Plate 1: Different stages of anther culture and callus induction in cultured anthers of F$_1$s of crosses among selected backcross progenies carrying different gene combinations (*Pita × Pi9* and reciprocals) and (*Xa21 × Xa38* and reciprocals)

vi. Plant regeneration from induced calli and overall anther culture response

For cross combination 1 (*Pita + Pi9* and reciprocals), all the induced calli (2080) were transferred to the regeneration media. Out of 1264 calli transferred, 137 calli regenerated giving rise to green and albino plantlets with an overall callus regeneration frequency of 6.5 per cent. The frequency of green plantlet regeneration recorded was 29.9% as 41 green plantlets were regenerated from 137 calli transferred on regeneration medium. However, out of total planlets regenerated, a good proportion of regenerated plantlets (96) were albino with a frequency of 70.0%. The overall anther culture efficiency came out to be 0.18% as a total of 41 green plantlets regenerated from a total of 22562 anthers cultured initially (Table 6). The stages of plantlet regeneration from calli are shown in Plate 2.

Similarly, for cross 2 (*Xa21 + Xa38* and reciprocals), all the 1534 induced calli were transferred to the regeneration media, out of which a total of 88 plantlets were regenerated, reflecting a callus regeneration frequency of 5.7 per cent. Out of total number of 88 regenerating calli, 20 were green (22.7%) and 68 were albino (77.2%). Hence, the overall efficiency of anther culture in F$_1$ of cross (*Xa21 + Xa38* and reciprocals) was observed to be 0.11% (Table 6).

Overall anther culture efficiency in rice is known to be low. A significant increase in anther culture efficiency was reported in *indica* rice cultivars by substituting sucrose with 9.00 per cent maltose as carbon source in MO19 basal medium (Raina, 1997) [23]. Mohiuddin *et al*. (2014) [17] used two types of media for plantlet regeneration through callus culture and have reported 96% green plant regeneration in BR 802-78-2-1-1 from KC medium whereas, only 63% was regenerated from KA medium. The frequency of albino plants was reported to be 37% from KA media, whereas, 4% from KC in the same line. The occurrence of large proportion of albino in the anther culture derived plantlets is a common feature in rice. In the present study, the frequency of albino plant regeneration varied from 70 to 77.2 per cent for cross 1 and cross 2, respectively. Several factors *viz.,* cold pre-treatment, stage of the pollen during anther culture and culture medium is known to affect the frequency of albino. Calli regenerated from anthers at the early uninucleate stage of pollen induced only green plantlets and the frequency of albino increases with advancing stage of the pollen (Pande, 1997) [21]. High concentration of sucrose (9%) in the culture media is also reported to lead to the high frequency of albino regeneration (Woo and Chen, 1982) [33].

Table 6: Regeneration frequency from calli and overall anther culture response in anthers of F$_1$s of crosses among selected backcross progenies (*Pita × Pi9* and reciprocals) and (*Xa21 × Xa38* and reciprocals)

Cross	Callus regeneration		Green plantlet regeneration		Albino plantlet regeneration		Anther culture efficiency (%)
	No.	%	No.	%	No.	%	
Pita × Pi9 and reciprocals	137	6.5	41	29.9	96	70.0	0.18
Xa21 × Xa38 and reciprocals	88	5.7	20	22.7	68	77.2	0.11

Plate 2: Plantlet regeneration from induced calli of cultured anthers of F₁s of crosses among selected backcross progenies carrying different gene combinations (*Pita × Pi9* and reciprocals) and (*Xa21 × Xa38* and reciprocals)

vii. Transplantation and plantlet survival of anther culture derived plantlets

The different stages of transplantation are presented in Plate 3. Out of 41 anther culture regenerants of the cross 1 (*Pita × Pi9* and reciprocals), 38 survived till maturity with an overall plantlet survival of 92.6 per cent. Similarly, among the regenerants of cross 2, (*Pi9 × Pita* and reciprocals), 19 plants survived out of 20, with an overall survival rate of 95 per cent (Table 7). The occurrence of doubled-haploids from anther culture derived plants is a beneficial feature in rice. Both endo-reduplication and nuclear fusion have been reported to occur during rice anther culture (Segui-Simarro and Neuz, 2008) [28] and most likely to cause spontaneous chromosome doubling in the regenerants. In the present study, 47 plants exhibited fertility and produced seeds indicating occurrence of spontaneous chromosome doubling in them.

Table 7: Plant survival of anther culture derived regenerants of F₁'s of crosses among selected backcross progenies carrying different gene combinations (*Pita × Pi9* and reciprocals) and (*Xa21 × Xa38* and reciprocals)

Cross	Total number of plantlets transferred	Total number of plantlets survived	Plant survival frequency (%)
Pita × Pi9 and reciprocals	41	38	92.6
Xa21 × Xa38 and reciprocals	20	19	95.0

Plate 3: Stages of green plantlet transfer to polyhouse

Conclusion

Advances in DNA marker technology together with the idea of marker-assisted selection provide new elucidations for selecting and maintaining desirable genotypes. Foreground selection of BC₂F₂ plants of two crosses (HPR2143/DHMAS164 and HPR2143/PB1) was done successfully to ascertain the presence of blast resistance genes i.e. *Pita* and *Pi9* in these plants. Similarly, to detect the presence of bacterial blight resistance genes i.e. *Xa21* and *Xa38* in BC₂F₂ plants of crosses (HPR2143/IRBB54 and HPR2143/PR114) foreground selection was done using gene specific markers which were reported to be successful. The study efficaciously validated the protocol developed locally in our lab for induced androgenesis in *indica* rice hybrids for instant fixation of derivatives while, following it *in toto* for fixation of two genes (*Pita+ Pi9* and *Xa21 + Xa38*) achieving moderate anther culture efficiency (~0.2%) although a high percentage of albino plantlets induction was also recorded.

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