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Identification of Introgressed Backcross Segregants Carrying Leaf Rust Resistance Genes *Lr24* and *Lr28* through Marker Assisted Backcross Breeding in Bread Wheat *(Triticum aestivum* L.) Variety DWR162

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ABSTRACT

Backcross lines of high yielding cultivar DWR 162 developed earlier carrying leaf rust resistance genes Lr24 and Lr28 were utilized to pyramid the two genes using marker assisted selection. Background analysis using 136 SSR markers was done to compare the genotypic background of backcrossed lines with recipient variety DWR162. Background analysis of 42 BC2F2 plants carrying both Lr24 and Lr28 was undertaken using polymorphic SSR markers to identify plants with better recovery of DWR162 background.

Key words: *Triticum aestivum L., introgression, leaf rust, foreground selection, background selection, resistance genes, molecular markers.*

INTRODUCTION

Leaf rust resistance genes named *Lr1* to *Lr71* have been characterized in bread wheat, durum wheat and diploid wheat species, as well as a number of QTL's have been identified in wheat and mapped to chromosome location and given gene designations[9]. The leaf rust *(Puccinia triticina* Eriks. Syn. *Puccinia recondita tritici* Rob. Ex Desm.) infection has been kept under control, mainly by judicious deployment of effective resistance genes in different wheat zones in India. Although several rust resistance gene(s) have been postulated in present day Indian wheat cultivars but many of them are rendered ineffective by virulent pathotypes of rust.

Not all gene combinations will yield a much greater additive effect when compared to the individual effects, but genes utilizing different resistance mechanisms or genes working at different optimal temperatures may ensure resistance among a broader range of conditions and thus achieve a greater additive effect.

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Ideally one would also combine pathotype non-specific resistance genes conveying APR with several pathotype specific genes. Such combinations have in many instances ensured complete resistance to wheat rust.

However, pyramiding of genes can prolong the effectiveness of these resistance genes. Resistance genes can be combined in a single genetic background if discriminating races are available. Nowadays with the availability of robust molecular markers linked to various rust resistance genes[2,11], two or more genes can be pyramided efficiently in a relatively short time even in the absence of virulence. Several rust resistance genes have been transferred in the genetic background of popular Indian cultivars using conventional backcross breeding[12,14]. The diverse oligo-genic alien race specific rust resistance genes *Lr9*, *Lr19*, *Lr24*, *Lr26*, *Lr28*, *Lr32*, *Lr37* which were effective against the occurring leaf rust pathotypes were deployed in the back-ground of number of popular Indian bread wheat Cultivars employing conventional back-cross method. The near isogenic lines of Kalyansona, Sonalika, UP232, PBW 226, HD 2329, HD 2285, WH 147, Lok-1, C 306, HD 2402, HD 2009, HI 1077, HS 240, J24, NI 5439,WL711 etc., carrying these genes were constituted with number of BC varying from 6-9. Out of these some of the back-crossed lines were released as commercial cultivars in the country which include Sonak (Sonalika with *Lr24*, *Sr24*), Amar (C 306 with *Lr24+Sr24*), WH 711(HD 2329 with *Lr24+Sr24*), Kurinji (PBW 226 with *Lr19+Sr25*), Kausambi (HD 2402 with *Lr19+Sr25*), MACS 6145 (C 306 with *Lr28*).

Moreover, with the availability of SSR markers covering all the chromosomes and chromosome arms in wheat, marker assisted background analysis for recovering the recurring parental genotype becomes an attractive proposition. In the present communication, we report the pyramiding of leaf rust resistance genes *Lr24* and *Lr28* using backcross lines in the background of DWR 162. Using background makers that were polymorphic between the parents background selection in BC_2F_2 population was done to identify plants with maximum recovery of recurrent parent genome.

MATERIAL AND METHODS

The bread wheat genotype DWR 162, which is susceptible to leaf rust, was selected as recipient parent for pyramiding the leaf rust resistance genes, *Lr24*, *Lr28*. 'PBW 343' near isogenic lines with *Lr24* and *Lr28* developed through marker assisted backcross breeding were used as donor parent for introgression of leaf rust resistant genes. The parents DWR 162 and NIL PBW 343, BC2F1 (generated by Arati *et al.*[1]), BC2F2, were sown and desired selections were made at All India Coordinated Wheat Improvement Project (AICWIP), Main Agricultural Research Station (MARS), University of Agricultural Sciences, Dharwad. Normal agronomical practices were followed for raising the crop.

Seedling test and field inoculations

Three weeks old seedlings of parental lines and segregating generations were inoculated in the field with selected pathotypes of leaf rust 77-5 (121R63-1). The pure inoculum of rust pathotypes was obtained from the Directorate of Wheat Research, Regional Station, Flowerdale, Shimla Rust severity was recorded according to the modified Cobb's scale described and was estimated on the basis of percentage area covered with pustules[10].

PCR analysis

DNA was isolated from young leaves by a modified CTAB method[5]. PCR reactions were performed in a total volume of 25 µl, containing 1× PCR buffer, 200µM of each dNTP, 20 ng of each primer, 1 U of Taq DNA polymerase (Banglore Genei Pvt. Ltd., India) and 100 ng of genomic DNA in a PTC-200 thermal cycler (MJ Research). Primers were synthesized from Sigma Aldrich Pvt. Ltd., Bangalore, India.

Molecular markers

For foreground selection of targeted genes, one SSR marker *Xwmc*313 and one SCAR marker SCS421 were used for screening of gene *Lr2*8 and two SCAR markers SCAR: SCS719 and SCS1302 for *Lr24*. For

background analysis of backcross lines of DWR162 carrying *Lr24* and *Lr28*, a total of 136 SSR markers spanning all chromosome arms were used.

PCR Amplification

For SSR markers, a touch-down PCR profile (65-55ºC) with an initial denaturation for 5 min at 94 ºC, followed by 10 touch-down PCR cycles comprising of 94ºC for 1 minute (m), 65ºC for 1 m and 72ºC for 1 m were performed. These cycles were followed by 35 cycles of 94ºC for 1 m with constant annealing temperature of 60ºC for 1 m and 72ºC for 1 m and a final extension was carried out at 72ºC for 5 min.

Agarose gel electrophoresis

The amplified products from each tube along with 2 µl of loading dye were separated on 4 percent agarose gel (2% Metaphor and 2% Agarose). 1x TAE buffer of pH 8.0 was used as electrolyte and 100 bp ladder was used as molecular weight marker. The gels were photographed using gel documentation system (Alphaimager,).

Seedling test and field inoculations

Three weeks old seedlings of parental lines and segregating generations were inoculated in the field with selected pathotypes of leaf rust 77-5 (121R63-1). The pure inoculum of rust pathotypes was obtained from the Directorate of Wheat Research, Regional Station, Flowerdale, Shimla. Rust severity was recorded according to the modified Cobb's scale described and was estimated on the basis of percentage area covered with pustules[10].

Marker assisted background analysis:

The software Graphical GenoTypes (GGT) GGT v 2.1 was used for marker assisted background analysis. The marker data generated represents the alleles, and were scored as "A"- Homozygote at marker locus as that of recurrent parent (DWR 162), "B"-Homozygote at marker locus as that of donor parent (NIL PBW 343), "H"-Herterozygote at marker locus as that of DWR 162 and NIL PBW 343, "O" - Off types showing banding pattern different from the parents, "U" missing data for the individual at the locus.

RESULTS AND DISCUSSION

Gene pyramiding of two or more race specific genes in a single genetic background can be an effective strategy to enhance the durability of major resistance genes. The present study was an attempt to pyramid the race specific leaf rust resistance genes *Lr24* and *Lr28* in the genetic background of high yielding but susceptible cultivar DWR 162.

One hundred and twenty BC_2F_2 plants were subjected to foreground selection with markers linked to leaf rust resistance genes *Lr24* and *Lr28*. Foreground selection of plants with SCAR markers SCS1302 linked to *Lr24* and SCAR marker SCS421 linked to *Lr28* showed 60 plants carrying both *Lr24* by amplifying 607 bp size band (Plate 1) and *Lr28* by amplifying 570 bp size band (Plate 2), leaf rust resistance genes, 20 plants with only *Lr24* gene, 23 plants with only *Lr28* and 11 plants showed absence of both the genes. Foreground selection with another SCAR marker SCS73719 linked to *Lr24* showed the presence of the gene by amplifying 719bp size band in all the plants carrying *Lr24* confirmed by SCS1302. The expected segregation of the SCAR markers SCS1302 and SCS421 in the ratio of 9:3:3:1 in BC2F2 generation (Table 1) was recorded indicating the goodness of fit with a calculated $\chi 2$ value of 2.76 (table $\chi 2$ value 7.82) as revealed from the results of $\chi 2$ test.

Joint segregation of markers for *Lr24* and *Lr28*

Since Xwmc313 is a co-dominant marker it facilitated the identification of plants as homozygous or heterozygous for leaf rust resistance gene *Lr28*. A perusal of Table 2 shows that molecular marker Xwmc313 identified 23 plants as homozygous resistant and 48 plants as heterozygous resistant carrying *Lr28*. SCAR marker SCS1302 identified 80 plants positive for leaf rust resistance gene *Lr24* out of 120 plants studied. Since, SCS1302 is a dominant marker, these 80 identified individuals included both homozygous and heterozygous resistant plants for *Lr24*. Joint segregation analysis of two

markers i.e., one co-dominant marker Xwmc313 for *Lr28* and a dominant marker SCS1302 for *Lr24* was undertaken to identify plants with different genotypic constitution with respect to the two rust resistance genes. The expected and observed frequencies of different genotypic classes are shown in the Table 2. Out of 23 plants identified as homozygous resistant for *Lr28,* 16 were observed to carry leaf rust resistance gene *Lr24* as well, either in homozygous or heterozygous condition. Remaining 7 plants, homozygous for *Lr28* did not carry *Lr24* gene. Out of 48 plants identified as carrying *Lr28* in heterozygous state, 36 also carried leaf rust resistance gene *Lr24* either in homozygous or heterozygous condition as against an expected frequency of 45. 12 plants were observed to carry only *Lr28* in heterozygous condition as against an expectation of 15. Twenty three plants were observed to carry only *Lr24* either in homozygous or heterozygous state. Out of 120 plants 11 did not carry either *Lr24 or Lr28 as* against expected value of 7.5. The six categories of genotypic classes expected from segregation of a dominant and a co-dominant marker are shown in the Table 2. A perusal of the table shows that the observed values of the six genotypic classes fits well with the expected ratio of 3:1:6:2:3:1 with a non-significant $\chi 2$ value of 5.96.

Marker Assisted Background Analysis

Out of 136 SSR markers spanning all the chromosomal regions that were used to assess parental polymorphism in the present study, 42 markers were found to be polymorphic between the parents DWR 162 and NIL PBW 343. These 42 polymorphic markers were used for background selection in BC_2F_2 generation to identify plants with maximum recovery of recurrent parent genome. Data was analyzed using software GGT v 2.1 and percent Recurrent Parent Genome (RPG) were determined and are presented in Table 3, Plate 4 to 5 and Fig. 1.

Analysis of data indicated that recovery of DWR 162 genome varied from 67.5% - 94.7 % with an average of 80.3% (Table 3). Plant BC_2F_2-5 (94.7) recorded highest RPG and lowest RPG shown was by BC_2F_2-36 (67.5). More than 87.5 % RPG at BC_2F_2 was observed in 6 plants *viz.*; BC_2F_2-5 (94.7%), BC_2F_2-16 (88.0%), BC_2F_2-19 (89.6%), BC_2F_2-29 (87.7%), BC_2F_2-30 (88.1%), BC_2F_2-32 (91.6%), BC_2F_2-39 (90.4%). Around 18 plants showed RPG between 80-87.5% and 19 BC_2F_2 plants showed recovery of 70-80% RPG.

Though not much information is available about efficiency of marker assisted background selection in wheat, both foreground and background selection has been effectively used in rice improvement (Basavaraj *et al., 2009,* Basavaraj *et al., 2010,* Joseph *et al., 2004,* Gopalakrishnan *et al*., 2008, Sundamar *et al.,* 2009).

Genetic variability parameters in BC_2F_2 generation

The genetic variability studies in BC_2F_2 (Table 4) indicate high mean and wider range for all the traits under evaluation. High PCV (phenotypic coefficient of variation) and GCV (genotypic coefficient of variation) were recorded for the trait grain yield per plant and high PCV and moderate GCV for the trait, number of productive tillers per plant. Moderate PCV and GCV for number of spikelets per spike, Moderate PCV and low GCV for days to 50 per cent flowering, spike length and thousand grain weight was observed. Low PCV and GCV were observed for plant height and percent protein content. Very narrow difference between the values of GCV and PCV indicated that the environmental effect was small for the expression of these characters. The high estimates of heritability and relatively high estimates of genetic advance (as percentage of mean) were observed in the present study for number of tiller per plant. This suggests that this trait is governed by additive gene action and selection could be effective in early segregating generations for this trait. High heritability and moderate genetic advance over mean was observed for the traits, plant height, spike length and number of spikelets per spike indicating that the high heritability was due to environmental influence. Moderate heritability and high GAM for the trait grain yield per

plant indicates additive gene action for the trait and selection for such triat may be effective. Moderate heritability and moderate GAM was observed for the trait thousand grain weight indicating high environmental influence. Moderate heritability and low GAM for the traits days to 50 per cent flowering and percent protein content, indicate that these traits are highly influenced by environmental effects and selection would be ineffective. Similar findings have been reported by Dwivedi *et al*.[6] and Yousaf *et al*[15]. For leaf rust infection, high PCV and GCV, moderate heritability and high GAM was recorded, suggesting that there is high variability for this trait in BC_2F_2 population of the cross DWR 162 X NIL PBW 343.

Table 1: Observed and expected number of plants with *Lr24, Lr28* and *Lr24* and *Lr28* in BC_2F_2 generation of DWR 162 X NIL PBW 343 based on joint segregation of linked SCAR markers SCS1302 (*Lr24)* and SCS421 (*Lr28*)

Sl. No.	Genotypic class	Number of plants		Cal χ2	Tab χ2
		Observed	Expected		
1	A_ B_(9)	60	67.5	0.84	
2	A_ bb (3)	20	22.5	0.28	
3	aaB_(3)	23	22.5	0.01	
4	aabb (1)	11	7.5	1.64	
	Total	**120**	**114**	2.76	7.82

A= *Lr24*, B= *Lr28*

Table 2: Joint segregation of linked SSR marker Xwmc313 (*Lr28*) and SCAR marker SCS1302 (*Lr24)* in BC_2F_2 generation of DWR 162 X NIL PBW 343

Genotypic Frequency	Expected	No of Plants		Cal χ^2	Tab χ^2
		E	O		
AAB-	3/16	22.5	16	1.88	
AAbb	1/16	7.5	7	0.03	
AaB-	6/16	45	36	1.80	
Aabb	2/16	15	12	0.60	
aaB-	3/16	22.5	23	0.01	
aabb	1/16	7.5	11	1.63	
Genotypic ratio:3:1:6:2:3:1 A= *Lr28*, B= *Lr24*		**120**	**105**	**5.96**	**11.07**

Table 3: Background selection based on forty two polymorphic markers and recurrent parent genome recovery in the forty six BC_2F_2 plants with *Lr24* and *Lr28* genes in wheat

S NO	Genotype name	RPG%
1	BC_2F_2-1	81.9
22	BC_2F_2-2	70.0
3	BC_2F_2-3	76.8
4	BC_2F_2-4	84.7
5	BC_2F_2-5	94.7
6	BC_2F_2-6	78.3
7	BC_2F_2-7	82.9
8	BC_2F_2-8	77.8
9	BC_2F_2-9	70.1
10	BC_2F_2-10	73.9
11	BC_2F_2-11	72.3
12	BC_2F_2-12	76.8
13	BC_2F_2-13	79.2
14	BC_2F_2-14	77.0
15	BC_2F_2-15	75.0
16	BC_2F_2-16	88.0

17	BC_2F_2-17	81.3
18	BC_2F_2-18	82.1
19	BC_2F_2-19	89.6
20	BC_2F_2-20	87.2
21	BC_2F_2-21	86.0
22	BC_2F_2-22	81.4
23	BC_2F_2-23	84.6
24	BC_2F_2-24	81.0
25	BC_2F_2-25	82.8
26	BC_2F_2-26	81.3
27	BC_2F_2-27	87.1
28	BC_2F_2-28	79.2
29	BC_2F_2-29	87.7
30	BC_2F_2-30	88.1
31	BC_2F_2-31	78.6
32	BC_2F_2-32	91.6
33	BC_2F_2-33	85.3
34	BC_2F_2-34	85.8
35	BC_2F_2-35	72.1
36	BC_2F_2-36	67.5
37	BC_2F_2-37	86.2
38	BC_2F_2-38	73.5
39	BC_2F_2-39	90.4
40	BC_2F_2-40	70.3
41	BC_2F_2-41	79.5
42	BC_2F_2-42	85.2
43	BC_2F_2-43	80.5
44	BC_2F_2-44	75.1
45	BC_2F_2-45	70.6
46	BC_2F_2-46	69.5

Mean RPG - 80.3% Range of RPG- 67.5-94.7 %

Table 4: Genetic variability studies for yield and rust resistance in BC_2F_2 generation of the cross DWR 162 X NIL PBW 343

Characters	Mean	Min	Max	PCV	GCV	h2 %	GA	GAM %
Days to 50 per cent flowering	69	63	88	10.33	6.63	41.19	6.05	8.77
Plant height (cm)	92	75	106	9.56	8.02	70.38	12.75	13.86
Tillers per plant	16	4	23	20.11	16	63.30	4.20	26.22
Spike length (cm)	9.53	4	14	10.58	8.26	60.95	1.27	13.28
Number of spikelets per spike	18.75	8	23	12.54	10.22	66.42	3.22	17.16
Grain yield per plant (g)	17.6	1.2	46.5	36.6	21.5	34.51	4.58	26.02
Thousand grain weight (g)	35.97	21	55.5	13.36	8.12	36.94	3.66	10.17
Protein (%)	12	10	13.9	6.56	4.28	42.57	0.69	5.75
Average coefficient of infection of leaf rust	2.56	0	60	37.54	22.47	35.83	0.71	27.71

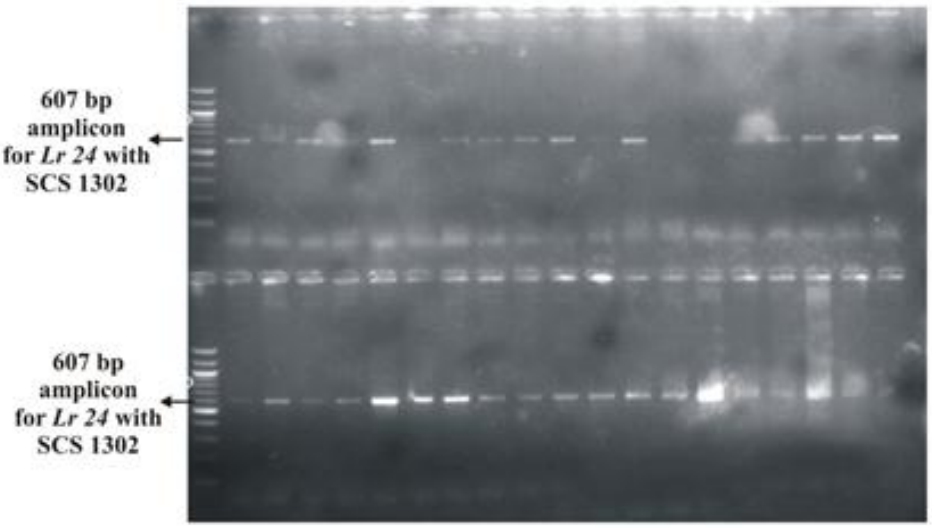

Plate 1: Molecular confirmation for the presence of *Lr24* in BC_2F_2 generations using SCAR marker SCS 1302

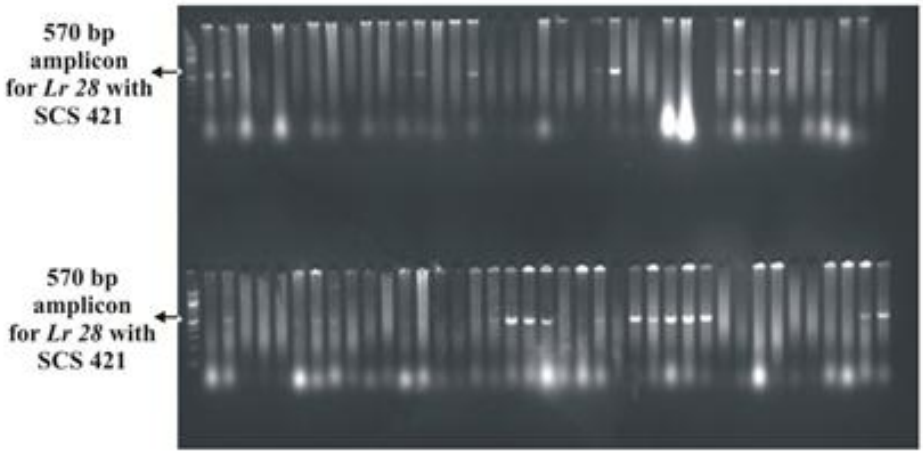

Plate 2: Molecular confirmation for the presence of *Lr28* in BC_2F_2 generations using SCAR marker SCS 421

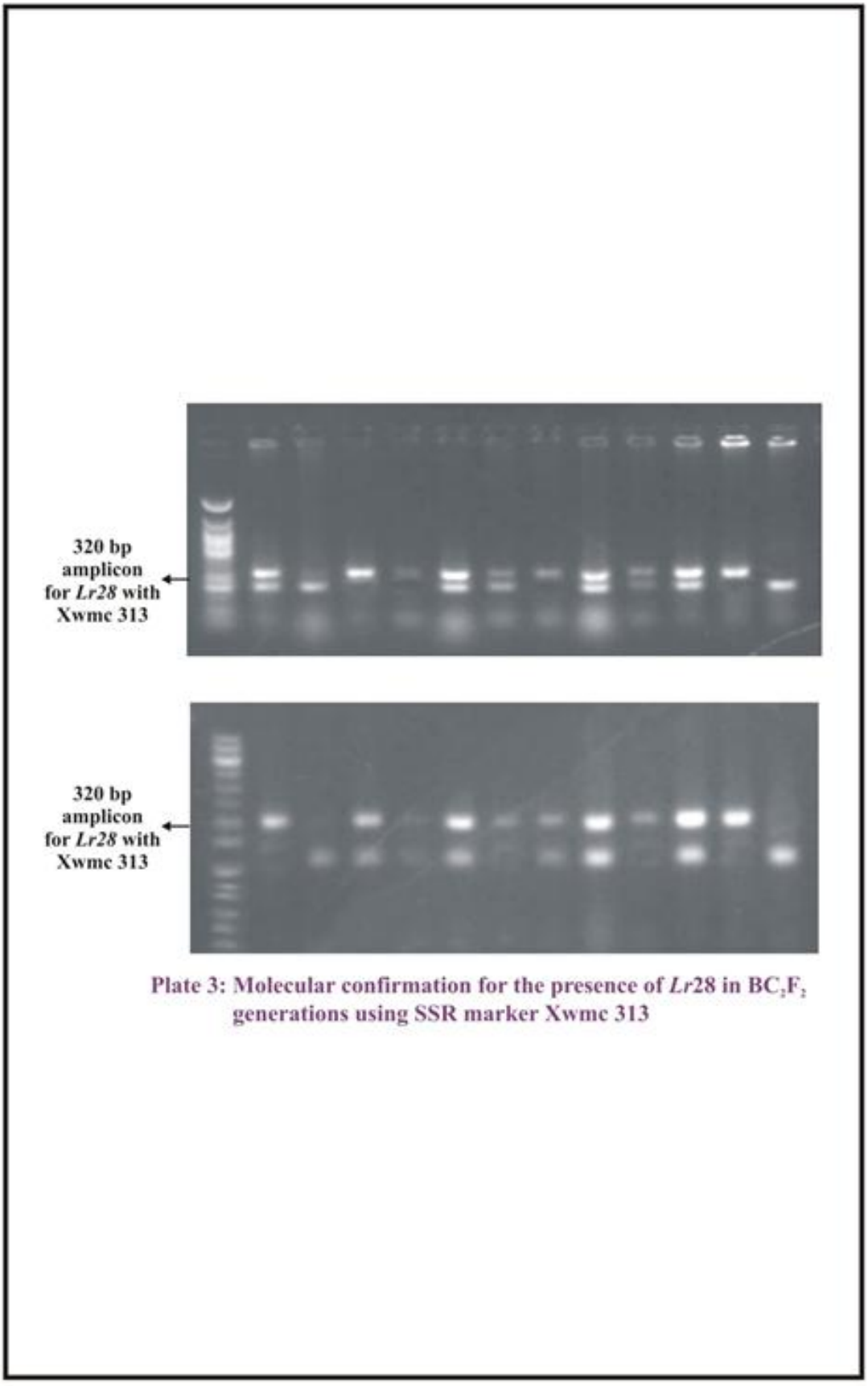

Plate 3: Molecular confirmation for the presence of *Lr*28 in BC_2F_2 generations using SSR marker Xwmc 313

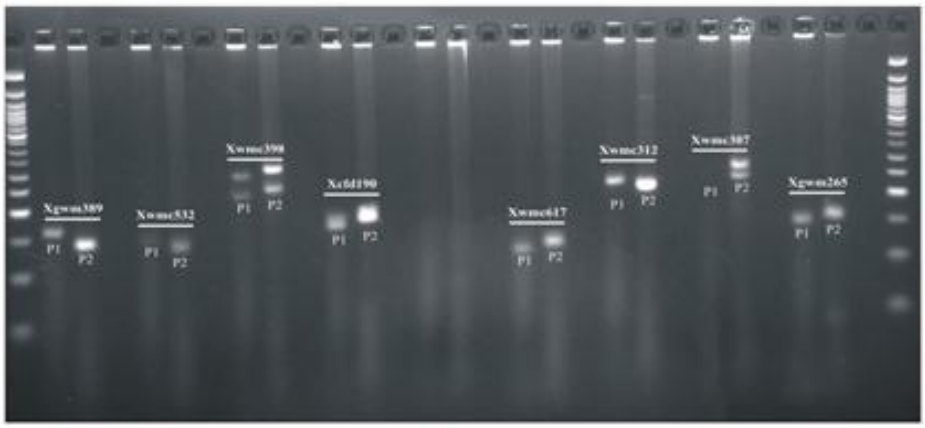

Plate 4: Polymorphic markers between recurrent parent DWR162 and donor parent NIL PBW 343

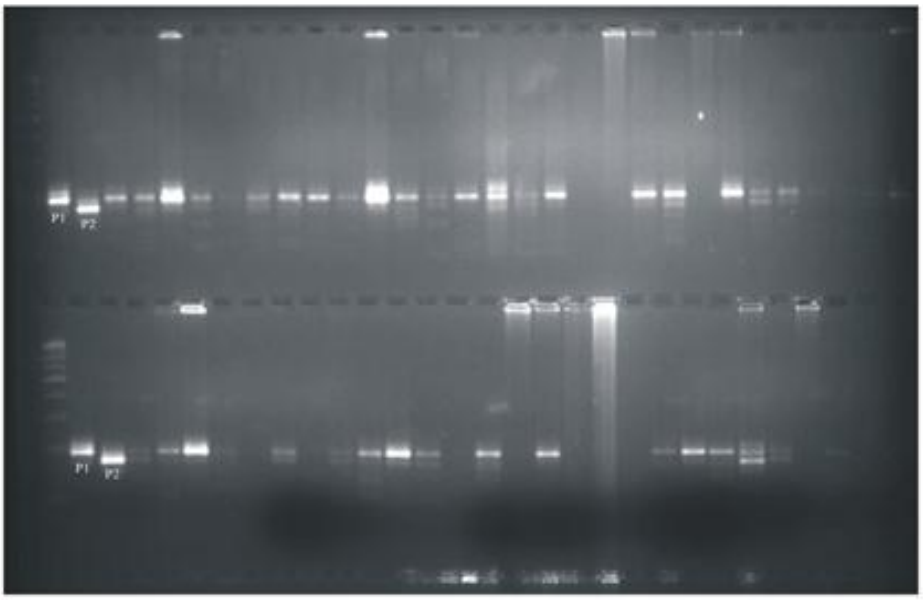

Plate 5: Background selection using polymorphic markers in BC_2F_2 generation

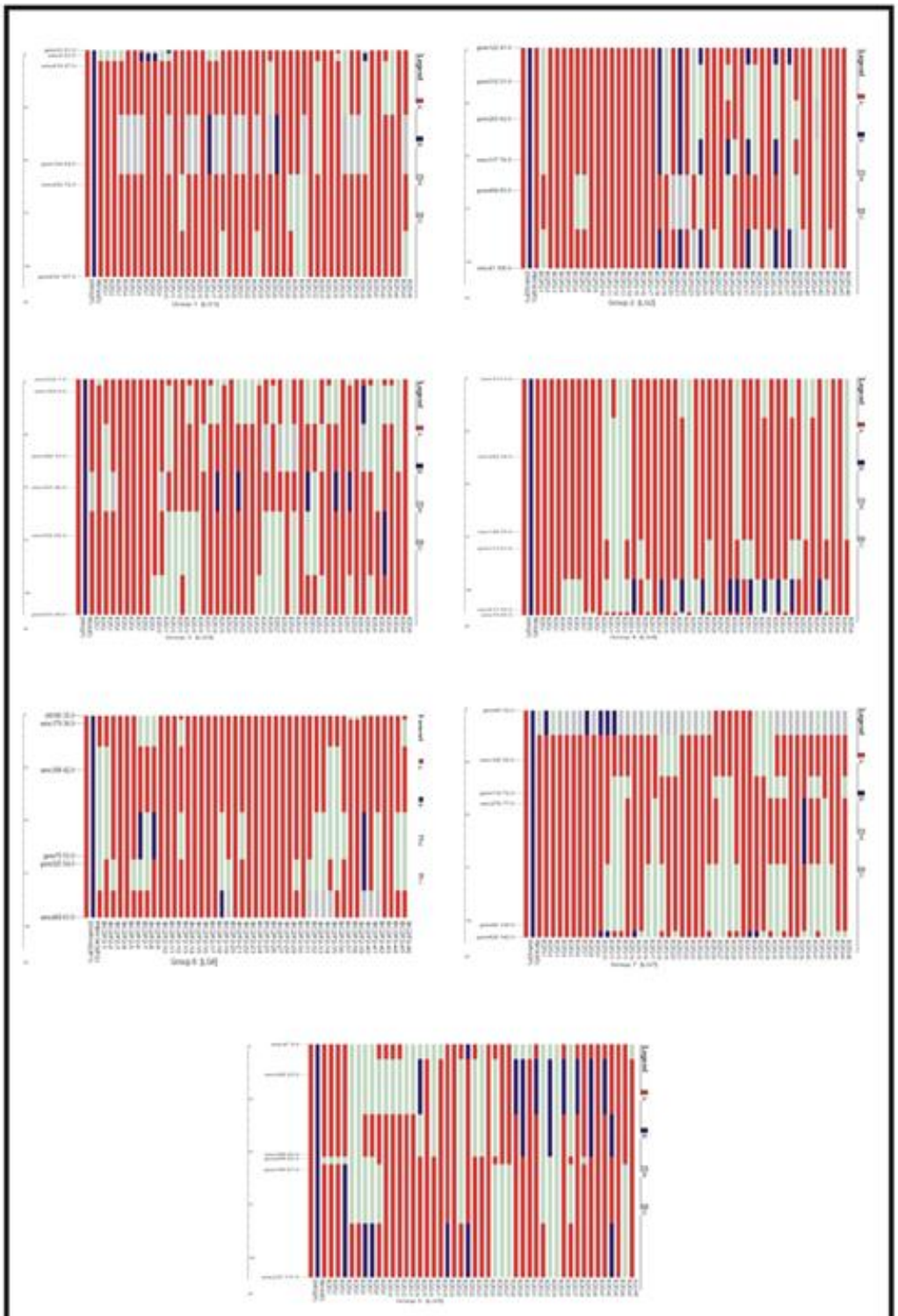

Fig. 1: Graphical genotype of BC_2F_2 plants based on marker assisted background selection

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