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GENETIC ENHANCEMENT OF LEAF RUST RESISTANCE IN BREAD WHEAT (*Triticum aestivum* L.) THROUGH MARKER-ASSISTED INTROGRESSION OF *Lr24* AND *Lr28*

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ABSTRACT

The present study was initiated with the objective of marker-assisted introgression of *Lr24* and *Lr28*, wheat leaf rust resistance genes, into an elite susceptible bread wheat genotype MP3299 from NIL-PBW 343. Marker-assisted selection alongwith screening of genotypes under epiphytotic conditions was undertaken in this study. SCAR molecular markers $SCS73_{719}$ and $SCS421_{570}$ linked to *Lr24* and *Lr28*, respectively, confirmed in segregating population of MP3299 x NIL-PBW 343, were further evaluated for leaf rust resistance under artificial leaf rust condition. In F_4 and F_5 populations, high degree of leaf rust resistance was observed in plants carrying both *Lr24* and *Lr28* genes as compared to the plants with individual leaf rust resistance gene *Lr24*, followed by *Lr28*. Significant correlation and regression were observed between most of the agronomic traits and coefficient of incidence. Six *Lr28* homozygous plants identified with high degree of leaf rust resistance were advanced for yield trials. The identified lines seem potential resistance sources and superior recombinants for future breeding programmes.

Key words: Coefficient of incidence, marker-assisted introgression, rust resistance, SCAR marker

INTRODUCTION

For a plant breeder durability of rust resistance in the cultivars being released for cultivation is more important than achieving resistance in the first phase. The objective of cultivar management, regardless of epidemic probability, is to maximize the potential durability of deployed resistance (McIntosh *et al.,* 1995). There are various pathogens causing various wheat diseases, and of these rust diseases have been a major problem for breeders, farmers and commercial seed companies. Leaf rust is probably the most important disease of wheat worldwide. *Puccinia triticina* Eriks. is a heterocious macrocylic rust fungus having *Thalictrum* spp. and rarely *Isopyrum* spp., *Anchusa* spp. or *Clematis* spp. as alternate hosts for the fungus. Within 7 to 14 days, a new generation of

urediniospores may be produced under the conditions of heavy dew, light rains or high humidity and temperatures between 15 and 22°C. Repeated urediniospore infections occur until the wheat crop matures (Samborski and Dyck, 1982). Leaf rust occurs more regularly in most world-wide regions than stem rust or stripe rust of wheat (Bolton *et al.,* 2008). Leaf rust causes severe damage to wheat varieties grown in comparatively warm and humid environment such as South Asia, North America, South East Asia and South America (McIntosh *et al.,* 1995). Yield losses in wheat from *P. triticina* infections are usually the result of decreased number of kernels per head and lower kernel weight; and it may reach up to 40% in susceptible cultivars (Knott, 1989). The management of rusts is a difficult task because of their fast multiplication and mutation abilities to change the strains which overcome the effective genes in a short time (Tiwari *et al.,* 2014). Mutations at single locus render great threat of resistance breakdown and permit the resistance to be effective for a short period. Therefore, the use of cultivars with single-gene resistance permits the selection of mutations. However, due to the selection pressure and evolution, new virulent races of fungus evolve and pose great threat and challenge to plant breeders. Hence, the use of combinations of genes has been suggested as the best method for genetic control of leaf rust (Roelfs, 1988).

More than 70 leaf rust resistance genes have been identified in wheat and some of these genes were isolated from wild relatives of wheat (McIntosh *et al.,* 2003). To increase the effectiveness of resistance trait, several alien leaf rust resistance genes have been transferred into hexaploid wheat background from their wild relatives (Sears, 1956; McIntosh *et al.,* 1982). Pyramiding is an effective approach to achieve the goal of combining resistance genes, but it becomes difficult to identify and evaluate plants in field for the expression of individual resistance genes against the background of other resistance genes. The advent of molecular marker technology has made it possible to tackle such complex problems. DNA-based molecular markers have several advantages over traditional phenotype markers. They can be used for marker-assisted selection (MAS) to improve the efficiency of selection in plant breeding because the environment does not affect the expression of molecular markers. They can also be detected at all stages of plant growth, whereas phenotypic markers often can be identified only at a specific growth stage (Singh *et al.,* 2004). The alien segment carrying linked genes *Lr24/Sr24* derive from *Agropyron elongatum* does not impose yield penalty. Similarly, the resistance conferred by *Aegilops speltoides* derived leaf rust resistance gene *Lr28* controls one of the important resistances in Indian subcontinent against the most prevalent *Puccinia triticina* pathotype 77-5 (Bipinraj *et al.,* 2010). The present study was aimed to pyramid *Lr24* and *Lr28* resistance genes in the background of MP3299 to provide high resistance against leaf rust from donor parent (NILPBW 343), a process which may be facilitated by DNA markers. We also determined the degree of relationship and regression of yield and other agronomic traits on leaf rust severity, and studied the effects of leaf rust resistance genes on common agronomic parameters and grain yield and we assessed the yield loss under diseased condition than under protected condition.

MATERIALS AND METHODS

Plant material

The wheat genotype Nil-PBW 343 (Chhuneja *et al.,* 2011), carrying two major leaf rust resistance genes *Lr24* on 3DL of *Agropyran elongatum* Wang. (McIntosh *et al.,* 1995) and *Lr28* on 4AL of *Aegilops speltoides* Tasusch. (McIntosh *et al.,* 1982) were used as donor parent. While genotype 'MP 3299' is a high yielding bread wheat genotype for rainfed ecosystem developed by CIMMYT through pedigree method having a parentage of DOVE/BUC/DL78 carries a major leaf rust resistance gene *Lr23+* which has been broken down by 77-5 race of *P. triticina.* The breeding programme included hybridization of both the two parents [MP3299 x NIL PBW 343] in *rabi* 2012, after crossing the parents, the segregating F_1, F_2, F_3, F_4 and F_5 generations were procured in *kharif* 2013, *rabi* 2013,

rabi 2014, *kharif* 2015 and *rabi* 2015, respectively, and confirmed through molecular tools for presence of *Lr24* and *Lr28* genes and phenotyping for rust.

DNA extraction and PCR amplification

One-month old plants of parental genotypes and all segregating generations were used for DNA isolation following a CTAB method (Dellaporta *et al.*, 1983). The two SCAR markers, SCS 73 719 for *Lr24* (Prabhu *et al.*, 2004) and SCS421 570 for *Lr28* (Cherukuri *et al.*, 2005) and one of the SSR marker wmc_{313} for *Lr28* (Annapurnalilly *et al.*, 2011) were employed for MAS to introgress in the back ground of MP 3299. The sequence details of the primers used in this study are as under:

Genes	Molecular markers	Primer sequence 5' → 3'	Amplicon size (bp)	Distance	References
Lr24	SCS73719	F: TCGTCCAGATCAGAATGTG R: CTCGTCGATTAGCAGTGAG	719	6.4 cM	Prabhu *et al.* (2004)
Lr28	SCS421570	F: ACAAGGTAAGTCTCCAACCA R: AGTCGACCGAGATTTTAACC	570	3.7cM	Cherukuri *et al.* (2005)
Lr28	Wmc313	F: GCAGTCTAATTATCTGCTGGCG R: GGGTCCTTGTCTACTATGTCT	320	5.0 cM	Annapurnalilly *et al.* (2011)

For PCR amplification of SCAR markers, 25 µL of the following reaction mixture was used: 10 mM tris HCl (pH 8.3), 0.2 mM of each dNTP, 0.25 mM of each primer, 50 ng of genomic DNA and one unit of *Taq* polymerase. PCR cycle involved an initial denaturation for 3 min at 94°C, followed by 36 cycles each with 1 min at 94°C, 1 min at 58°C, 1 min at 72°C and a final 7 min extension at 72°C. PCR products were resolved on 2.5% agarose gel for 2-3 h at 5 v/cm constant voltage and viewed under Alpha Innotech Alpha-Imager Digital gel imaging system.

Seedling reaction test for leaf rust

The seedling reaction to leaf rust under field conditions was done by planting two infector rows after every 20 rows of test material with each of 1.0 m row length. Each block was surrounded by infector row on all the four sides. Ten days-old seedlings were inoculated with pathotype 77-5 (the most virulent and predominant pathotype of leaf rust in South India, obtained from IIBWR regional station, Flowerdale, Shimla) by spraying the inoculation suspended in water fortified with tween-20 (0.75 µL mL^{-1}) at an average concentration of 20 uridiospores per microscopic field (10X x 10X). Fourteen days after inoculation, the infections were recorded using 0-4 scale (Stakman *et al.*, 1962). The data were recorded as percentage leaf area affected by leaf rust according to the modified Cobb's scale as described by Peterson *et al.* (1948).

Yield loss assessment

To ascertain wheat yield losses caused by leaf rust, the entire trial was subdivided into two experiments. One experiment was conducted completely under rust-free condition and in other experiment leaf rust epidemic conditions were created by artificial inoculation. To maintain rust free condition, Tilt fungicide (propiconazole 250 EC) @ 0.5 L ha^{-1} was applied 4 times at an interval of 15 days starting from I^{st} week of February. To assess the yield losses caused due to leaf rust, the disease severity and grain yield $plant^{-1}$ were recorded under both the conditions. To identify the relationship between leaf rust and loss in yield and 1000-grain weight (TGW), the per cent loss was calculated using the following formula (Arunkumar, 2013):

$$\text{Loss (\%)} = \frac{\text{Yp-YuP}}{\text{Yp}} \times 100$$

Where, Yp = Yield or TGW in protected treatment; YuP = Yield or TGW in artificial epiphytotic treatments

Association of yield and yield contributing traits with the leaf rust

To determine the association between yield and various yield contributing traits and leaf rust index, the coefficient of infection i.e. correlation coefficient was computed. Simultaneously the dependence of plant yield on leaf rust (CI) i.e. regression was also computed. The data was statistically computed by using SPSS 21.0v programme for regression and correlation analysis (Diaz *et al.*, 2015).

RESULTS AND DISCUSSION

The present investigation aimed for genetic enhancement of high yielding genotype MP 3299 for leaf rust resistance. In this direction two major genes *Lr24* and *Lr28* targeted to transfer in the background of MP 3299 from NIL-PBW 343 with the help of molecular markers. The resistance of *Lr24*/*Sr24* has extensively been exploited to develop commercial cultivars and found very effective against Indian leaf rust races in both seedling and adult stages. The *Lr28* is a potential gene which can be used in combination with other genes without losing yield. The present study led to the development of leaf rust resistant recombinant lines with added yield advantage. The development and identification of leaf rust resistant recombinants were carried out through marker assisted selection (MAS).

Phenotypic and molecular analysis of parents and recombinants

The seedling reaction against leaf rust pathotype 77-5 revealed a high level of resistance of donor parent NILPBW 343 with CI of 1.45 and 56 by susceptible recipient parent MP3299 (Fig. 1A & B). In wheat, the effectiveness of both major genes *Lr24* and *Lr28* has previously been demonstrated against leaf rust pathotype (77-5). A similar observation has been noticed in the present study (Table 2 & Fig. 1C). As expected, F_5 generation exhibited lower CI values than F_4 generation due to increased homozygosity. Similar results were observed for F_5 generation by Srivastava *et al.* (2009). In both generations, highest resistance was found in the plants carrying both the genes, followed by the plants with *Lr24*. Higher CI value was noted in plants with *Lr28* only than with *Lr24*. The effectiveness of *Lr24* has been demonstrated by Srivastava *et al.* (2009) in imparting resistance against leaf rust pathotype 77-5.

Fig. 1: Plants showing resistant (upper left) and susceptible (upper right) reaction; Bottom figure shows differences in infection from leaf rust pathogen in different gene combinations

Marker-assisted selections in F_1 to F_5 generations

Marker-assisted selection was followed from F_3 generation onwards with the help of already reported SCAR markers *viz.*, $SCS73_{719}$ for leaf rust resistant gene *Lr24* (Prabhu *et al.*, 2004) and $SCS421_{570}$ for *Lr28* (Chhuneja *et al.*, 2005). The donor parent NILPBW 343 confirmed the presence of both *Lr24* and *Lr28* genes followed by 16 F_1 plants for true hybridity and further segregating generations. These markers have previously been used by Revathi *et al.* (2010) and Chhuneja *et al.* (2005) in marker-assisted selection. A total of 210 F_2 plants were genotyped for *Lr24* and *Lr28* and

Table 2: Rust reaction of parents and the segregating generations

Lines	Rust reaction (CI)	
NILPBW 343	1.45	
MP3299	56.0	
	F_4	F_5
Plants with *Lr24*	19.5	12.0
Plants with *Lr28*	26.86	20.00
Plant with *Lr24* and *Lr28*	0.05	0.00
Plants without both the genes	55	70

Table 3: Frequency of plants with different genes in different segregating generations

Generation	Total plants (No.)	*Lr24* gene	*Lr28* gene	Both *Lr24* & *Lr28* genes present	Both *Lr24* & *Lr28* genes absent
F_1	45	12 (26.0%)	9 (20.0%)	16 (35.0%)	8 (17.0%)
F_2	210	43 (20.4%)	44 (20.5%)	108 (51.4%)	15 (7.1%)
F_3	150	31 (20.6%)	32 (21.3%)	61 (40.6%)	26 (17.3%)
F_4	250	55 (22.0%)	53 (21.3%)	85 (34.1%)	57 (22.8%)
F_5	24	7 (29.1%)	5 (20.8%)	10 (41.4%)	2 (8.3%)

108 plants identified as carrying both the genes (Fig. 2A). Because of dominant nature of SCAR markers, homozygous plants were not identified. In each generation, plants were confirmed both genotypically and phenotypically. In every generation, plants carrying both the genes were in higher percentage and lowest for absence of both genes (Table 3).

Yield loss assessment

To assess the efficiency of both major genes *Lr24* and *Lr28* on yield and control of disease severity, yield loss was measured by considering the yield under epiphytotic and controlled condition. The data revealed significant difference between infected and protected treatments in relation to rust severity. El-Shamy *et al.* (2011) reported significant correlation between mean disease severity and percentage loss for 1000 kernel and grain yield plant^{-1}. Similarly, in our study high degree of negative

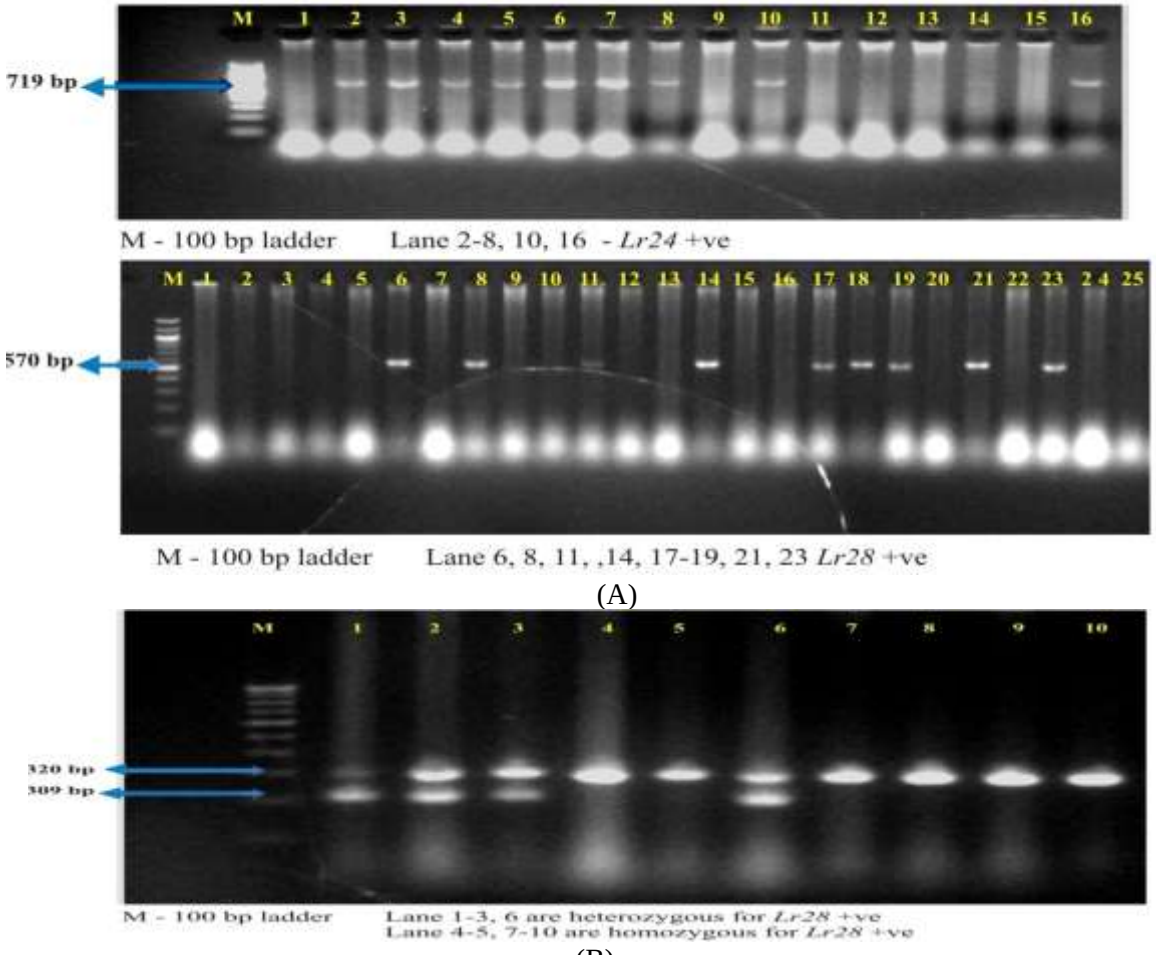

Fig. 2: Screening of rust resistance genes with SCAR marker SCS73719, SCS421570 and SSR marker Wmc313 (A) Marker screening for *Lr24* gene with 719 bp and *Lr28* gene with 570 bp (B) Marker screening for *Lr28* gene with SSR marker with 320 bp

Table 4: Yield loss assessment of segregating generation of the cross MP3299 x NILPBW 343

Progeny	CI		Reduction in 1000-grain weight (%)		Reduction in yield (%)	
	F_4	F_5	F_4	F_5	F_4	F_5
Plants with *Lr24*	19.50	12.0	25.45	19.86	23.75	23.36
Plants with *Lr28*	26.86	20.0	36.65	22.75	34.56	25.26
Both *Lr24* & *Lr28*	0.05	0.0	32.24	10.58	29.19	12.34
Without both	55.00	70.0	65.00	45.26	59.98	56.25

correlation (r = -0.64) between yield and leaf rust index (CI) confirmed the effect of rust incidence on yield levels of genotypes. Genotypic differences were found in grain yield in presence of leaf rust, under potential yield conditions and also in protected fields. The yield loss assessment of F_4 and F_5 generation indicated that the percent reduction in 1000-grain weight and grain yield was highest in plants without both the genes with 65.00 and 59.98% in F_4, respectively, and 45.26 and 56.25% in F_5 generation whereas plants with both the genes had lowest reduction levels with 32.24 and 29.19% and 10.58 and 12.34%, respectively, in F_4 and F_5 (Table 4). At the same time the plants with either of the genes showed lower 1000-grain weight and yield reduction than the plants without both the genes but higher reduction than the plants with both the genes. As expected, the percent reduction in 1000-grain weight and yield was less in presence of both the genes rather than individual genes. In this kind of interaction, the combination of genes provides high levels of resistance and higher levels of agronomic performance than when present separately (Kolmer *et al.*, 1991). *Lr24* is more effective than *Lr28* which recorded lower CI value in both F_4 and F_5 generations and the higher CI values for *Lr28* can be substantiated as due to presence of a virulent race 77-10 (377R60-1) as reported by Bharadwaj *et al.* (2010).

The formula used to determine the yield reduction was effective in determining the yield and 1000-grain weight as it was successfully used by Hasan *et al.* (2012). The results show that the two leaf rust resistance genes *Lr24* and *Lr28* are involved in higher 1000-grain weight and grain yield in disease condition (Ochoa *et al.*, 2007) where yield loss correlated strongly with disease severity.

Association of yield and yield contributing traits with leaf rust

The statistical analysis for correlation between the yield, yield contributing traits and leaf rust severity (CI) in F_4 and F_5 generations indicated that the traits like tillers plant^{-1} and spike length spikelets spike^{-1} and yield exhibited significant negative correlation to leaf rust severity at (P = 0.05) level (Table 5). Whereas, the traits like plant height and thousand grain weights showed positive but insignificant correlation to leaf rust severity.

The significant correlation of yield and yield contributing traits indicated that leaf rust severity leads to the reduction in yield. At the same time regression analysis showed dependence of grain yield on leaf rust severity and revealed high degree of regression of yield on leaf rust (Table 6). The regression analysis presented the beta value for yield i.e. -0.35 and -0.15 for F_4 and F_5, respectively. The beta indicates per unit change in yield with change in leaf rust severity. The present results are in accordance with finding of Zhang *et al.* (2007). The correlation and regression analysis of yield with leaf rust severity showed the influence of leaf rust severity on yield levels. With this view, the present investigation was initiated with the objective of development of leaf rust resistant varieties through pyramiding of two major genes.

Effect of Lr genes on yield and yield contributing traits

Plants with both genes showed higher values for the traits like tillers plant^{-1}, 1000-grain weight and

Table 5: Correlation between yield and yield contributing traits with CI in segregating generations

Generations		Plant height	Tillers plant^{-1}	Spike length	Spikelets spike^{-1}	Grains spike^{-1}	1000-grain weight	Grain yield
CI	F_4	0.18	-0.62*	-0.40*	-0.53*	-0.39	0.10	-0.52*
	F_5	0.01	-0.40*	-0.30*	-0.25*	-0.06	0.13	-0.54*

*: Significant at P = 0.05

Table 6: Regression between yield and CI in segregating generations

Generations	R	R^2	Beta	Standard error of estimate	Significance F
F_4	0.818	0.669	-0.351	9.53	0.009
F_5	0.525	0.276	-0.152	5.49	0.040

Table 7: Mean performance of plants with different gene in F_5 generation and their parents

Plants with gene (s)	DFF	PH (cm)	PTP	SL (cm)	SP/P	G/S	TGW (g)	GY/P (g)	CI
Lr24	58.0	62.2	17.0	8.0	13.0	50.0	35.65	28.5	12
Lr28	65.0	62.7	14.7	11.0	22.7	59.7	37.70	18.8	20
Both *Lr24* and *Lr28*	63.5	69.9	18.7	10.8	21.6	57.3	44.80	28.0	0
None of *Lr24* or *Lr28*	55.0	62.3	9.0	8.3	15.0	50.0	36.20	5.4	70
MP3299	45.0	65.2	8.8	10.0	16.3	65.0	27.46	25.9	56
NILPBW 343	76.9	62.3	11.0	10.6	20.0	47.0	32.50	12.3	1.45

DFF: Days to 50% flowering, PH: Plant height, PTP: Productive tillers plant^{-1}, SL: Spike length, SPP: Spikelets spike^{-1}, GS: Grains spike^{-1}, TGW: 1000-grain weight, GYP: Grain yield plant^{-1}, CI: Coefficient of infection

grain yield plant^{-1} with CI values of 0.0 (Table 7). One interesting observation was that the plants with *Lr28* gene showed higher values for traits like spike length and spikelets spike^{-1} than both the parents as well as plants with both the genes. Similar kind of positive effect of *Lr28* gene in enhancing yields through yield components has been reported by Kumar and Raghavaiah, (2004). The increase in yield and yield contributing traits directly indicated the positive effects of both the leaf resistance genes. For traits like days to 50% flowering and grains spike^{-1} the recipient parent (MP3299) had more desirable values which were found to have slightly lower values in plants with both the genes but these were better than donor parent (NILPBW 343). Molecular marker screening for plants in F_5 generations resulted in 10 plants having both the genes and out of these plants six were homozygous for *Lr28* when screened for wmc_{313} marker (Fig. 2B). All these plants had higher 1000-grain weight and grain yield of 35-38 and 28-32 g plant^{-1}, respectively.

Conclusion: We have successfully transferred two leaf rust resistance genes into the wheat cultivar MP3299 from the recipient parent NILPBW 343 using two SCAR marker and one SSR marker. Although co-dominant markers are preferred, however in present study two dominant markers were successfully utilized as earlier used by Chhuneja *et al.* (2011). We also undertook the correlation and regression analysis so as to understand the dependence and effect of disease on yield and other traits and found a strong degree of relationship between disease, yield and other traits. All the plants with both genes showed high level of rust resistance and these newly introduced lines/plants do not have any kind of linkage drag, instead presence of *Lr28* presented positive effect on grain yield of the plant. Homozygous plants with both the genes can be advanced to release as a variety.

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