

Original Research Article

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Genetic Studies on the Effect of Slow Rusting Genes *Lr34* and *Lr68* on Minimizing Grain Yield Losses in Back Cross Segregating Populations of the Cross GW322 X Parula in Bread Wheat (*Triticum aestivum* L.)

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ABSTRACT

Achieving durability of resistance and minimizing yield losses due to Leaf rust, caused by *Puccinia triticina* Eriks, in wheat has been one of the major objective of breeding programs. The slow rusting genes that are quantitatively inherited, are known to confer durable type of resistance. In present investigation our objective was to study the effects *Lr34* and *Lr68* genes on minimizing grain yield losses using backcross segregating populations of the cross GW322 X PARULA. For this comparison of grain yield was made in protected and non-protected treatments. Experimental materials were screened with linked molecular markers to detect the presence of genes conferring resistance to leaf rust. Overall yield losses in presence of slow rusting genes *Lr34*, *Lr68* and in combination of both *Lr34* and *Lr68* were 14.98 %, 16.36 % and 13.27 % respectively which was comparatively much lower than yield losses in absence of both the slow rusting genes (33.15%). Yield losses were associated mainly with the reduction in grain yield per plant and thousand grain weight. These plants with both the genes in combination and having lower yield losses are the potential lines for further development of varieties. We conclude that although the presence of slow rusting genes which is linked with leaf tip necrosis of adult plants, causing reduction in net photosynthetic area could provide substantial protection to grain yield in high disease pressure.

Keywords

Leaf rust, Slow rusting gene, Yield losses.

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Introduction

Leaf rust, caused by *Puccinia triticina* Eriks, is considered to be the most serious disease of wheat (*Triticum aestivum* L.) and universal in occurrence. Leaf rust, also known as brown rust caused by the heteroecious basidiomycete is most common and widely distributed of the three wheat rusts and has become more serious problem of wheat causing great losses in grain yield (Huerta-Espino *et al.*, 2011).

Yield losses in wheat from *P. triticina* infections are usually the result of decreased number of kernels per head and lower kernel weight (Roelfs *et al.*, 1992; Marasas *et al.*, 2004; Kolmer *et al.*, 2005). Early infection of leaf rust on wheat generally causes higher yield losses; 60–70%, infection on the flag leaf at spike emergence may account for a yield loss of more than 30%.

More than 100 leaf rust resistance genes have been reported in wheat and its relatives, 72 of them permanently catalogued (McIntosh *et al.*, 2013). Most of these leaf rust resistance genes condition a hypersensitive reaction and interact with the pathogen in a gene-for-gene fashion. Erosion of race specific resistance genes, or their combinations, has led to search for alternative approach to resistance management.

An alternative approach is to deploy varieties that possess adult plant resistance (APR) based on combinations of minor, slow rusting genes. When present alone, APR genes do not confer adequate resistance especially under high disease pressure; however, combinations of 4-5 such genes usually result in “near immunity”. At present, among the known genes with slow rusting effect the most common are the genes: *Lr34* (Dyck, 1987), *Lr46* (Singh *et al.*, 1998), *Lr67* (Hiebert *et al.*, 2010 and Herrera-Foessel *et al.*, 2011) and *Lr68* (Herrera-Foessel *et al.*, 2012). These genes have pleiotropic effects, when pyramided in one line provide more durable and non-race specific adult plant resistance to all three rust (Singh *et al.*, 2011).

The objectives of this study was to determine the effectiveness of race non-specific and slow-rusting resistance in reducing losses in grain yield and to identify superior recombinants for yield and its component traits having both the genes when exposed to high leaf rust pressure.

Materials and Methods

The present investigation was conducted during *rabi* season 2015-16 at ICAR-All India Co-ordinated Wheat Improvement Project, Main Agricultural Research Station, University of Agricultural Science (UAS), Dharwad.

The materials of present experiment comprised of 180 lines of different segregating populations of the cross GW322 X PARULA. These advanced backcross segregating lines were evaluated in Augmented Block Design. These lines were sown in protected condition and artificial epiphytotic condition in 6 blocks, each block consist of 30 lines with 4 checks viz., UAS304, HD2189, GW322 and Parula having spacing of 20 x 20 cm. Genomic DNA extraction was extracted from fresh leaves using cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1987) with little modifications. Polymerase Chain Reactions (PCR) were performed by using a protocol appropriate for pair of primers. DNA amplification was performed in 20 µl reaction mixture. The PCR products were mixed with 2 µl of loading dye (0.25% bromophenol blue with 40% sucrose) and were loaded into each well and separated on 2 per cent agarose gel using 1X TAE buffer of pH 8.0 containing ethidium bromide. After electrophoresis gel viewed under UV-transilluminator (JH-Bio). The tightly linked marker for the respective gene of interest specific amplicon size was observed on the agarose gel (Plate 1). The allele specific marker *cssfr-5* for *Lr34* gene was codominant in nature, amplified 751 bp for the presence and 523 bp for the absence of gene. The STS marker *csGS* for *Lr68* was dominant in nature, amplified 385 bp PCR product for the presence and no product amplification for the absence of the gene.

Susceptible checks were planted after every ten genotypes and all around the experimental plots using the universal susceptible varieties like Lal Bahadur, Agra Local, and Local Red. At days to 50 per cent flowering of the crop, field was maintained under irrigation and the suspension of mixture of pathotypes of leaf rust was sprayed on the genotypes at continuous interval.

Randomly five plants were selected and observations were recorded for the traits like plant height (cm), days to 50 per cent flowering, days to maturity, no of productive tillers per plant, spike length (cm), number of spikelet per spike, no of seeds per spike, thousand grain weight (g) and grain yield per plant (g).

In this investigation, the difference between grain yield per plant was considered between the protected and epiphytotic plots in presence of different gene (*Lr34*, *Lr68*) and their combination (*Lr34 + Lr68*) and per cent loss was calculated as the difference among the protected and infected treatments using the following equation adopted by Calpouzou *et al.*, (1976).

$$\text{Loss (\%)} = \frac{Y_h - Y_d}{Y_h} \times 100$$

Where,

Y_d = yield of disease plants (infected treatment);

Y_h = yield of healthy plant (protected treatment).

Correlation studies were also done. The correlation analysis between grain yield per plant and coefficient of infection was done using the SPSS 16.0 version.

Results and Discussion

Leaf rust pressure was high and uniform throughout the experiment in artificial epiphytotic condition. The fungicide-protected plots remained free from leaf rust during the entire crop season. Plants carrying slow rusting genes viz., *Lr34* and *Lr68* displayed immune to compatible type of rust

reaction. Recurrent parent GW322 displayed moderately susceptible to susceptible type of reaction throughout the experiment, while donar parent Parula displayed immune to partially immune type of reaction. The grain yield per plant and thousand grain weight was comparatively higher for fungicide-protected plots than leaf rust inoculated plots. The difference in yield was expected because leaf rust infection was initiated at different plant development stage in the leaf rust inoculated plots compared with the protected plots. The correlation analysis between grain yield per plant and coefficient of infection was done using the SPSS 16.0 version. The results (Table 1) indicated that both the traits are significantly correlated with each other at 0.01 levels (2-tailed) with a value of -0.318**.

In the BC₂F₄ population (Table 2) plants with *Lr34 + Lr68* combination recorded least reduction in grain yield per plant (13.72%) followed by plants with only *Lr34* gene (14.9%). Plants with *Lr68* gene alone recorded reduction in grain yield per plant upto 22.21 per cent. However plants lacking both the genes recorded very high percentage of reduction in yield (33.15%).

Progeny 4-14-98 with *Lr34 + Lr68* gene recorded lowest reduction in yield with only 5.06 per cent whereas progeny 7-39-87 lacking both the genes recorded highest reduction in yield with 42.25 per cent of loss. In the BC₃F₃ population (Table 3) presence of *Lr68* gene alone recorded less reduction in grain yield per plant (10.33%) compared to plants with *Lr34* (10.67%) whereas plants with *Lr34 + Lr68* recorded 12.16 per cent reduction in yield. However plants lacking both the genes recorded very high percentage of reduction in yield (33.74%). Progeny 21-27-157 with *Lr34* recorded lowest reduction in yield with only 3.07 per cent of loss.

Fig.1 Overall yield loss assessment in backcross segregating generation of the cross GW322 X PARULA

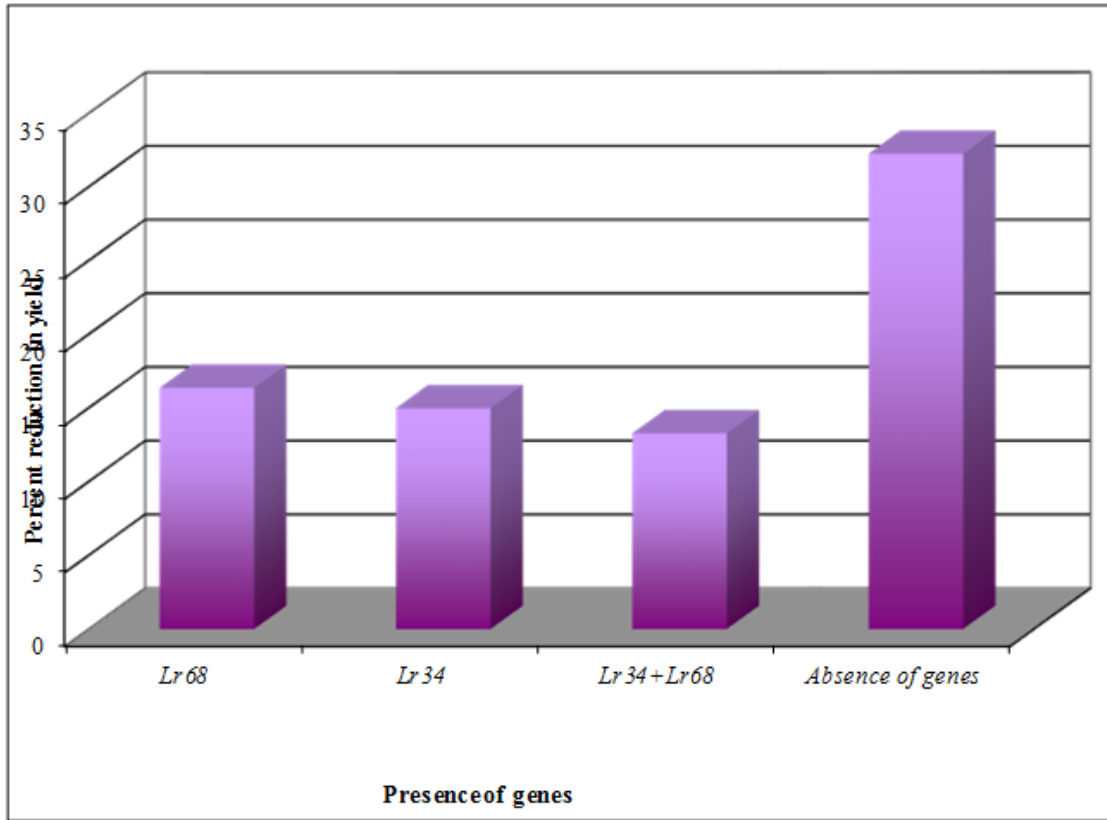
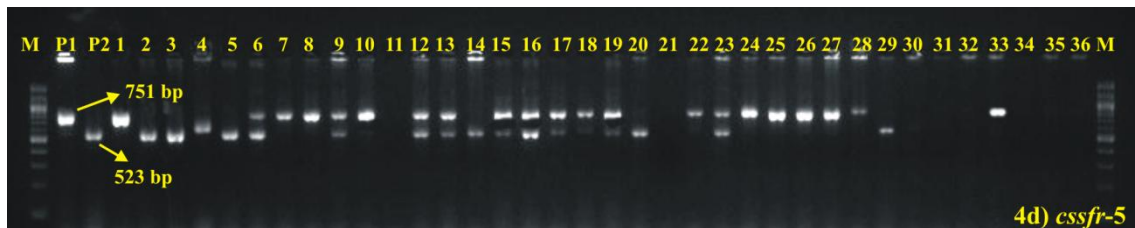
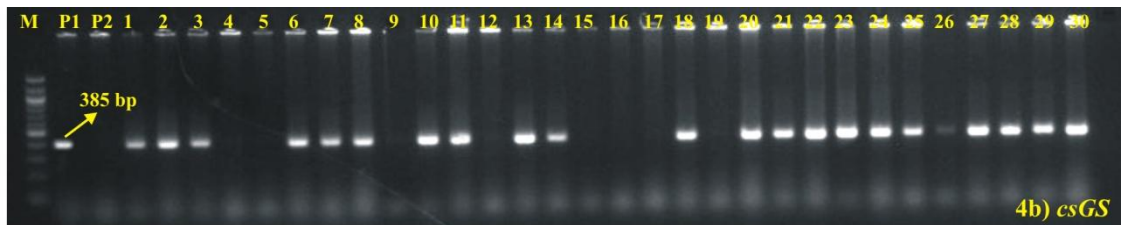


Plate.1 Molecular confirmation of plants for presence of *Lr34* and *Lr68* genes



M - 100 bp ladder, P1 (PARULA), P2 (GW 322), Lane 1, 4,,6-10, 12, 13, 15-19, 22-28, 33 *Lr34* +ve



M - 100 bp ladder, P1 (PARULA), P2 (GW 322), Lane 1-3, 6-8, 11-14, 18, 20-22, 24-25, 27-30 *Lr68* +ve

Table.1 Correlation between grain yield and coefficient of infection (CI) in back cross segregating generations of the cross GW322 x PARULA in bread wheat

		CI	Yield
CI	Pearson Correlation	1	-0.318**
	N		68
Yield	Pearson Correlation	-0.318**	1
	N	68	

** . Correlation is significant at the 0.01 level (2-tailed).

Table.2 Grain yield loss assessment in the BC₂F₄ progenies of the cross GW322 x PARULA in bread wheat

Generation	Progeny	Minor gene screened	Percent reduction in yield	
BC ₂ F ₄	7-47-85	<i>Lr68</i>	14.76	
	7-38-80-249	<i>Lr68</i>	14.81	
	7-38-80-250	<i>Lr68</i>	26.70	
	7-45-82-258	<i>Lr68</i>	18.00	
	7-45-82-259	<i>Lr68</i>	36.35	
		Mean		22.21
		7-28-74	<i>Lr34</i>	5.81
		7-31-75	<i>Lr34</i>	19.35
		7-33-76	<i>Lr34</i>	16.95
		7-34-77	<i>Lr34</i>	11.98
		7-35-78	<i>Lr34</i>	15.71
		7-40-81	<i>Lr34</i>	14.86
		95	<i>Lr34</i>	11.71
		4-14-93	<i>Lr34</i>	6.51
		47-149-104	<i>Lr34</i>	14.51
		47-147-105	<i>Lr34</i>	21.41
		47-139-107	<i>Lr34</i>	26.55
		Mean		15.03
		7-37-79	<i>Lr34+Lr68</i>	17.08
		7-40-81	<i>Lr34+Lr68</i>	14.86
		7-45-82-260	<i>Lr34+Lr68</i>	9.07
		92-96	<i>Lr34+Lr68</i>	13.90
		4-15-97-338	<i>Lr34+Lr68</i>	16.91
		4-14-98	<i>Lr34+Lr68</i>	5.06
		4-13-99	<i>Lr34+Lr68</i>	14.20
		4-11-100	<i>Lr34+Lr68</i>	18.44
		47-131-108	<i>Lr34+Lr68</i>	13.50
		Mean		13.72
		7-39-87	Without minor gene	42.25
		7-41-88	Without minor gene	37.37
		7-42-89	Without minor gene	28.38
		7-43-90	Without minor gene	24.60
		Mean		33.15

Table.3 Grain yield loss assessment in the BC₃F₃ and BC₃F₄ progenies of the cross GW322 x PARULA in bread wheat

Generation	Progeny	Minor gene screened	Percent reduction in yield
BC ₃ F ₃	21-27-156	<i>Lr68</i>	11.11
	21-27-158	<i>Lr68</i>	9.55
		Mean	10.33
	21-27-155	<i>Lr34</i>	12.68
	21-27-157	<i>Lr34</i>	3.07
	18-69	<i>Lr34</i>	16.62
		Mean	10.67
	21-27-154	<i>Lr34+Lr68</i>	12.94
	21-27-159	<i>Lr34+Lr68</i>	11.39
		Mean	12.16
	31	Without minor gene	32.38
	32-34	Without minor gene	35.11
	Mean	33.74	
BC ₃ F ₄	31-18-102-356	<i>Lr68</i>	17.76
	31-18-102-337	<i>Lr34</i>	9.72
	18-122-110	<i>Lr34</i>	15.28
	18-137-111	<i>Lr34</i>	4.79
		Mean	9.93
	31-83-102	<i>Lr34+Lr68</i>	8.28
	31-81-103	<i>Lr34+Lr68</i>	11.59
		Mean	9.94
	31-81-103-358	Without minor gene	17.82
	31-81-103-359	Without minor gene	46.25
	Mean	32.03	

Table.4 Overall yield loss assessment in back cross segregating generation of the cross GW322 x PARULA in bread wheat

Presence of genes	Percent reduction in yield
<i>Lr68</i>	16.36
<i>Lr34</i>	14.98
<i>Lr34+Lr68</i>	13.27
Absence of genes	32.25

In BC₃F₄ generation (Table 3) plants with *Lr34* alone and plants with *Lr34* + *Lr68* recorded lower reduction in yield upto 9.94 per cent. Plants with *Lr68* alone recorded high reduction in grain yield per plant (17.76%). However, plants lacking both the genes recorded very high amount of reduction in yield (32.03%).

The idea of this investigation was to identify the effect of slow rusting genes (*Lr34* and *Lr68*) on yield and also to identify superior recombinants for yield and its component traits having both the genes. In backcross segregating populations of the cross GW322 x PARULA viz., BC₂F₄, BC₃F₃ and BC₃F₄ percent reduction in yield was assessed in

presence and absence of slow rusting genes. In this analyses the yield losses were comparatively lower for most of the progenies with slow rusting genes in their background. However, high yield losses were observed in some progenies that were immune to the leaf rust having both slow rusting resistant genes in their background. Sayre *et al.*, (1998) found that leaf rust caused losses irrespective of the level of resistance possessed by the cultivars.

The plants respond to inoculation with energy-demanding physiological processes, probably defense reactions, using stored host energy that otherwise would go to growth and seed production. In addition, a reduction in photosynthetic leaf area due to leaf tip necrosis pleiotropically associated with *Lr34* also can cause yield reductions.

Overall yield loss assessment considering all backcross segregating generation of the cross GW322 x PARULA was carried out (Table 4 and Fig. 1). Plants carrying *Lr34* gene exhibited 14.98 per cent reduction in yield while it was only 13.27 per cent in presence of both *Lr68* and *Lr34* genes. In the absence of both genes overall reduction in yield was 32.25 per cent. These finding were agreement with Singh *et al.*, 1997, who reported in the presence of *Lr34* reduction in yield was only 15 per cent. In the absence of *Lr34* reduction in yield were substantially higher and ranges between 42.5 per cent to 84 per cent depending upon planting date and year.

In presence of *Lr68* alone reduction in yield was recorded 16.36 per cent while in presence of both *Lr68* and *Lr34* was 13.27. Similar results was reported by Singh *et al.*, (2001) who reported that cultivars with *Lr34* and two to three additional genes have shown a stable environmental response and final disease ratings lower than five percent under high disease pressure and yield losses less upto 6-10 per cent.

These losses can be further minimised by introgression of other additive, slow rusting genes like *Lr67* gene in the background of these advanced backcross segregating populations having *Lr34* and *Lr68* gene in their background, to form the *Lr34*- complex (Singh and Rajaram, 1992). Increasing the frequency of these genes and introgression through marker assisted selection should be a useful strategy for controlling leaf rust disease and minimizing yield losses. These plants with both the genes in combination and superior in performance are the potential lines for further development of varieties, keeping in view the durability of resistance which will provided by the plants with the genes in combination.

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