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Anomalous behaviour of a wheat leaf rust resistance gene *Lr32*

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ABSTRACT

The rust resistance gene *Lr32* confers resistance to all leaf rust pathotypes reported from India. Anomalous segregation pattern was observed while studying inheritance of *Lr32*. The F_1's of *Lr32* were susceptible when *TcLr32* was crossed with 5 *aestivum* cultivars, viz 'Agra Local', 'PBW 343', 'HI1 077', 'HUW 234' and 'UP 2338' but the F_2 segregation ratio varied from one cross to other. Genetic background of the cross influenced the nature of resistance of *Lr32*. The expression of *Lr32* shifted from recessive to dominance in certain genetic background. A single recessive gene was responsible for this shift in gene action and, therefore, it induced dominance in *Lr32* only in homozygous condition. The degree of expression of low infection type reaction of *Lr32* was dependent on microenvironment which sometimes gave wrong impression of enhanced resistance when present in combination with *Lr23* or *Lr26* in seedling as well as adult plant tests. Differential gene action of *Lr32* may pose problems in detecting the F_2 genotype in genetic studies. However, this problem can be tackled through progeny testing.

Key words: Enhanced resistance, Environmental sensitivity, Gene action, Gene interaction, Leaf rust resistance, *Lr32*

Leaf rust *(Puccinia triticina* Jakob Erikss.) occurs throughout the wheat (*Triticum aestivum* L. emend. Fiori & Paol.) growing regions of the world. Cultivation of resistant varieties is the most effective, eco-friendly and economically viable method of combating the rust diseases (Line and Chen 1995). Incorporation of resistance is a continuous process so as to counter the ever-evolving rust pathogens. Furthermore, it is important that the genetic base of rust resistance in the cultivated varieties be diversified to protect them from pathogens. An elaborate anticipatory pre-breeding programme was initiated at the Regional Station of the Directorate of Wheat Research, Flowerdale, Shimla for the development of agronomically desirable lines with *Lr19, Lr28, Lr32, Lr39, Lr42* and *Lr45*. During transfer of *Lr32* gene into the genetic background of released varieties, certain anomalies were observed while handling the segregating populations from the crosses involving *Lr32*.

The seedling resistance genes produce characteristic infection types which are sometimes influenced by the environment such as light intensity and temperature. Certain genes, such as *Lr10*, *Lr11*, *Lr18* and *Lr37 LrPB2* express better under low temperature regime (Statler and Christianson 1993, Datta *et al.* 2009), whereas a few others, like *Lr13, Lr16, Lr17* and *Lr23* are more effective at high temperature (Statler and Christianson 1993, Brahma *et al.* 2007). The degree of resistance imparted by many genes were affected when transferred from diploid to higher ploidy level (Kerber 1987). While transferring *Lr32* in the genetic background of cultivars 'HI1077' and 'PBW343', the F_2 populations were found to contain more number of resistant seedlings than expected in a population segregating for a recessive gene, *Lr32*. The present paper describes anomalous nature of leaf rust resistance gene *Lr32*.

MATERIALS AND METHODS

The experimental material comprised F_1's of the crosses of *ThatcherLr32* ('RL6086') with 'Agra Local', 'HI1077', 'PBW343', 'HUW234', 'UP2338' and 'WH542'. The F_2 and F_3 generations of 'Agra Local'/'RL6086', 'HI1077'/'RL6086', 'PBW343'/'RL6086' were analyzed in details.

The F_2 and F_3 seedlings were raised in aluminum bread pans each containing 10 rows with the row 7th as the susceptible cultivar, 'Agra Local'. Fully expanded primary leaf of each seedling was inoculated with *P. triticina* uredospores suspended in non-phytotoxic isoparaffinic oil (soltrol). The inoculated seedlings were kept in a humid chamber for 48 hr. The seedlings were transferred to glass house benches at 22°C. Infection types (IT's) were recorded 14 days after inoculation and classified using standard method: IT's 0; (zero fleck), ; (fleck), 1 (1), 2+ and X+ were

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grouped as the resistant reaction, whereas IT's 3 (three) and 3+ (three plus) were called susceptible.

The F_1 seedlings were initially tested in winter season but subsequently repeated in summer season at different temperature regimes under controlled temperature, whereas the segregating generations were tested in winter season only. Parents and the F_1's were tested with 3 pathotypes, viz 121R63-1, 109R31-1 and 21R55. The F_2 generation was tested with either pt.121R63-1 or pt.21R55. The F_3 generation was tested with pt.121R63-1 and pt.45R35 or pt.21R55 and pt.109R31-1. Adult plant tests were conducted inside a polyhouse under temperature controlled conditions against pt.121R63-1. The characteristic infection types of the leaf rust resistance genes against the races used are given in Table 1.

The F_2's of the crosses 'Agra Local'/'RL6086' and 'HI1077'/'RL6086' were tested with race 121R63-1, whereas the F_2 population of 'PBW343'/'RL6086' was tested with race 21R55. The F_2 seedlings from single F_1 plant were scored for infection types (IT's) and transplanted in the field for the derivation of F_3 families. F_2 seedlings with identical IT's were transplanted in one block for maintaining the identity of individual F_2 phenotype. F_3 families from the cross of 'Agra Local'/'RL6086' were analyzed with race121R63-1, 'HI1077'/'RL6086' with races 121R63-1 and 45R35 and of the cross 'PBW343'/'RL6086' with races 21R55 and 109R31-1. Same set of F_3 families were tested wherever progeny testing was done simultaneously with two races and about 20 seedlings were analyzed per family. Genotype of F_2 plants were determined through progeny testing. Chi squared tests were applied to check the compliance of postulated and observed genetic ratios.

A few selected F_4 lines, with combined resistance genes, viz *Lr32+Lr23* and *Lr32+Lr26*, originating from the crosses of 'HI1077'/'RL6086' and 'PBW343'/'RL6086', respectively were analyzed for studying the combined effect of *Lr32+Lr23* and *L32+L26* genes on low infection types. Adult plant tests were done only for the line carrying *Lr32+Lr23* because the gene combination *Lr32+Lr26* was derived from the variety 'PBW343' that gives lower infection types at adult plant stage in the polyhouse condition. The resistance genes in F_4 lines were postulated on the basis of F_3 family analysis.

RESULTS AND DISCUSSION

Although seedling reaction of *Lr32* fluctuated from one testing to other and sometimes among the seedlings of the same pot, it normally varied between 1 and 2; rarely up to 2+ infection types (Table 1). The F_1's of all crosses of *Lr32*, viz 'Agra Local'/'RL6086', 'HUW234'/'RL6086', 'HI1077'/'RL6086', 'WH542'/'RL6086', 'PBW343'/'RL6086', 'UP2338/'RL6086' were susceptible (Table 2).

Table 1 Characteristic infection types of parents and *Lr* genes under study against pathotypes of leaf rust at the seedling stage at 22°C

Pathotypes	Tc*Lr23*	Tc*Lr26*	Tc*Lr32*	'PBW343'	'HI1077'	'Agra Local'
121R63-1	3+	3+	1 to 12	3+	3+	3+
21R55	3+	3+	1 to 12	3+	3+	3+
109R31-1	3+	0;	1 to 12	0;	3+	3+
45R35		3	1 to 12		0;	3+

Table 2 Seedling reaction of parents and F_1's with pt. 121R63-1, 21R55 and 109R31-1

Parents and F_1's	121R63-1		21R55		109R31-1	
	16°C	22°C	16°C	22°C	16°C	22°C
'Agra local' (AL)	3+	3+	3+	3+	3+	3+
'HUW234' *(Lr14a)*	3+	3+	3+	3+	3+	3+
'HI1077' *(Lr23)*	3+	3+	3+	3+	3+	3+
'WH542' *(Lr26)*	3+	3+	3+	3+	0;	0;
'PBW343' *(Lr26)*	;12	33+	3+	3+	0;	0;
'UP2338' *(Lr26)*	3+	3+	3+	3+	0;	0;
Tc*Lr32* ('RL6086')	1 to 2 *	1 to 2	1 to 2	1 to 2	1 to 2	1 to 2
'AL'/'RL6086' F_1	3+	3+	3+	3+	3+	3+
'HUW234'/'RL6086' F_1	3+	3+	3+	3+	3+	3+
'HI1077'/'RL6086' F_1	3+	3+	3+	3+	3+	3+
'WH542'/'RL6086' F_1	3+	3+	3+	3+	0;	0;
'PBW343'/'RL6086' F_1	-	3	3+	3+	0;	0;
'UP2338'/'RL6086' F_1	3+	3+	3+	3+	0;	0;

*Reaction of Tc*Lr32* ranged from 1 to 2 among the different seedlings of the same pot

Same results were obtained when testing was repeated in different seasons and temperature regimes. It was observed that all the F_1's of *Lr32* in various cross combinations and under different testing conditions were always susceptible (3 to 3+ infection type), thus indicating that nature of gene action of *Lr32* was not determined by environment though microenvironment may be responsible for minor variations in infection types which ranged from fleck one to fleck two.

Inheritance of Lr32 resistance in 'Agra Local'/TcLr32 (RL6086' against pt.121R63–1

The F_2 and F_3 segregation was in compliance with single recessive gene (Table 3). The recessive nature of the gene in RL6086 was conclusively proved from the fact that families were segregating in the ratio of 1resistant: 3 susceptible and importantly, segregating families have originated from the susceptible F_2 plants.

Inheritance of Lr32 resistance in 'HI1077'/Tc+Lr32 ('RL6086') against pt.121R63–1

The F_1 'HI1077'/Tc*Lr32* ('RL6086') was susceptible. In the F_2 generation, resistant seedlings were produced in excess than the expected ratio (1resistant: 3 susceptible) for a recessive gene which actually segregated in the ratio of 6 resistant: 10 susceptible (Table 3). None of the susceptible F_3 families were derived from resistant F_2 plant which authenticated proper classification of F_2 individuals. The F_3 family segregation was in compliance with 1 resistant: 2 segregating: 1 susceptible indicating that single gene conditioned resistance to pt.121R63-1.

Inheritance of Lr32 resistance in 'PBW343'/Tc+Lr32 ('RL6086') against pt. 21R55

The F_1 seedlings were susceptible. The F_2 population was good fit to 6 resistant: 10 susceptible. None of the susceptible F_3 families were derived from resistant F_2 plant which ruled out misclassification in F_2 generation. The F_3 family segregation was in compliance with 1R: 2 Segregating: 1S which indicated involvement of single gene for resistance.

In the crosses involving 'HI1077' and 'PBW343', the *Lr32* behaved in a similar manner. In the F_2, expression of *Lr32* heterozygote was dependent on genotypic constitution of the F_2 individual. When crossed with 'Agra Local' (a native land race that has very few rust resistance genes (Datta *et al.* 2008), *Lr32* behaved perfectly as a recessive gene in F_2 and F_3 generations but when crossed to 'HI1077' and 'PBW343', the F_2 segregation ratio was good fit to 6 resistant: 10 susceptible instead of 1 resistant: 3 susceptible. In the crosses of 'HI1077' and 'PBW343', phenotypic misclassification being ruled out through genetic analysis of F_3 families, it is obvious that some of the resistant seedlings were heterozygous for *Lr32* in the F_2 because they segregated in F_3 generation. However, the segregation of *Lr32* occurred in dominant as well as recessive manner and thereby indicated that the gene action of *Lr32* was conditioned by other genetic or environmental factors. Kerber (1987) in his pioneering work, mentioned that light intensity and temperature probably affects the expression of *Lr32* and consequently phenotype of heterozygotes of *Lr32* change under different testing conditions. The altered segregation ratio may be attributed either to segregation distortion or shift in gene action of *Lr32* under the influence of other gene. Segregation distortion could be ruled out because *Lr32* segregated normally when crossed to 'Agra local' and more importantly, altered F_2 segregation was obtained only in the crosses where some of the heterozygous seedlings of *Lr32* were resistant in F_2. The ratio of 6 resistant: 10 susceptible could be expected if a recessive gene is conditioning the resistance of heterozygous seedlings of *Lr32*. The phenomenon of shift in gene action from recessive to dominance and vice-versa under the influence of other host genes or races used for inoculation is rare (Chen and Line 1992, Chen *et al.* 1995).

Further genetic analysis was carried on the assumptions that the microenvironment was not playing role in deciding

Table 3 Segregation of the F_2 and F_3 generations to leaf rust races in the seedling test

Cross	Number of seedlings/families Resistant	Segregating	Susceptible	Expected ratio	χ^2	p
'Agra Local'/'RL6086'						
F_2 (121R63-1)	99		334	1R: 3S	1.01	0.31
F_3 (121R63-1)	28	54	21	1R: 2Seg: 1S	1.19	0.55
HI1077/Tc*Lr32*						
F_2 (121R63-1)	242		351	6R: 10S	2.68	0.10
F_3 (121R63-1)	19	52	26	1R: 2Seg: 1S	1.50	0.47
F_3 (45R35)	45	49	3	7R: 8Seg: 1S	1.70	0.42
'PBW343'/Tc*Lr32*						
F_2 (21R55)	91		119	6R: 10S	2.91	0.09
F_3 (21R55)	29	47	25	1R: 2Seg: 1S	0.82	0.66
F_3 (109R31-1)	42	55	4	7R: 8Seg: 1S	1.35	0.51

R, Resistant; S, susceptible

the gene action of *Lr32* because F_1's were always susceptible under different testing conditions and the F_2 generation, some heterozygotes of *Lr32* were resistant while others were susceptible and thus phenotype of *Lr32* heterozygote was dependent on genetic background and also F_1 heterozygotes were always susceptible, the gene responsible for shift in gene action of *Lr32* is recessive and effective only in homozygous condition.

Based on the above assumptions, studies were targeted on *Lr23*, which is present in 'HI1077' and *Lr26*, that is present in 'PBW343' to find out their role, if any, in the expression of *Lr32*. Accordingly, F_3 families of 'HI1077'/Tc*Lr32* and 'PBW343'/Tc*Lr32* were also tested with pt.45R35 and pt.109R31-1 respectively for determining the role of *Lr23* and *Lr26* in altering gene action of *Lr32*. The gene that is affecting the nature of *Lr32* must be present in homozygous state in the F_2 plants that were resistant to pt. 121R63-1 or pt. 21555 but segregated in F_3. This was determined in the following manner: Step1. Identification of those F_2 individuals in which *Lr32* behaved as a dominant gene in fact these were the F_2 seedlings that were resistant to pt.121R63-1 but segregated for *Lr32* in the F_3 step 2. Determination of the genetic constitution (with respect to *Lr23* or *Lr26*) of these F_2 individuals. The genotypic constitution of F_2 individuals were determined from the F_3 families. For example, in the cross 'HI1077'/Tc*Lr32* if a F_3 line is homozygous resistant (0; infection types) to pt. 45R35 in the F_3, this indicated that it was derived from a F_2 with genetic constitution *Lr23–Lr23*.

Inheritance of leaf rust resistance in 'HI1077'/TcLr32 ('RL6086') against pt. 45R35

The set of F_3 families were same as that of testing with pt.121R63-1. The F_3 family segregation which was in compliance with 7: 8: 1 confirmed involvement of two genes, *Lr32* (from 'RL6086') and *Lr23* (from 'HI1077'), in the control of resistance against pt. 45R35. The characteristic IT's of 'HI1077' *(Lr23)* and 'RL6086' *(Lr32)* were distinct which enabled further classification of F_3 lines in to three categories *viz* homozygous for *Lr23*, heterozygous for *Lr23* and devoid of *Lr23*. The F_3 progenies which were derived from the F_2 seedlings that were resistant to pt.121R63-1 but segregated for *Lr32* in the F_3, were not necessarily homozygous for *Lr23* (2 homozygous resistant, 6 segregating and 3 homozygous susceptible) and a few of them even devoid of it.

Inheritance of leaf rust resistance in 'PBW343'/TcLr32 ('RL6086') against pt.109R31-1

The F_3 family segregation which was in compliance with 7: 8: 1 confirmed involvement of two genes governed resistance, one of them being *Lr32* and the other must be from 'PBW343' *(Lr26)*. The characteristic infection type of *Lr26* is 0; which enabled postulation of *Lr26* in F_3 generation and their predecessor F_2.

Out of 14 F_3 families that were derived from F_2 seedlings resistant to pt.21R55 but segregated for *Lr32* in the F_3, 4 were homozygous for *Lr26* and the rest were either heterozygous or lacking *Lr26* (8 segregating and two homozygous susceptible).

The gene that has conditioned the expression of *Lr32* was effective only in homozygous condition because F_1's were always susceptible. Had the known genes of 'PBW343' and 'HI1077' influenced the gene action of *Lr32* they must be in homozygous state in the plants that were resistant as well as heterozygous for *Lr32* in F_2 generation. However, genotypic constitution of all such F_2 plants were not necessarily homozygous for *Lr23* or *Lr26* and hence their role in conditioning the gene action of *Lr32* was ruled out.

Enhanced resistance

Some of the F_3 lines of 'HI1077'/*TcLr32* and 'PBW343'/*TcLr32* produced lower infection types (IT's) than the parental line Tc*Lr32* ('RL6086') under the same set of testing conditions. Interestingly, all these lines carried either *Lr23* or *Lr26* in addition to *Lr32* which led to suspect enhanced resistance in gene combinations *Lr32+Lr23* and *Lr32+Lr26*. Since characteristic infection types of *Lr32* varied from one testing to other it was necessary to validate this observation in the F_4 lines. None of the 20 fixed F_4 lines originating from the crosses of 'HI1077'/RL6086 (10 lines) and 'PBW343'/RL6086 (10 lines), that were homozygous for gene combinations *Lr32+Lr23* or *Lr32+Lr26*, conferred enhanced resistance by lowering the IT's at the seedling stage (Table 4).

Table 4 Seedling reaction of gene combination lines (F_4)

Parents and F_4 lines	Genes postulated	pt.121R63-1	pt.121R127	pt.21R55	pt.45R35	pt.109R31-1
'Agra local'		3+	3+	3+	3+	3+
'HI1077'	*Lr23*	3+	3+	3+	0	3+
'PBW343'	*Lr26*	1 to 3+	3+	3+		0
'RL6086'	*Lr32*	1 to 2	1 to 2	1 to 2	1 to 2	1 to 2
'HI1077'/Tc*Lr32* F_4	*Lr32*	1 to 2	1 to 2	1 to 2	1 to 2	1 to 2
'PBW343'/Tc*Lr32*F_4	*Lr32*	1 to 2	1 to 2	1 to 2	1 to 2	1 to 2
'HI1077'/RL6086 F_4	*Lr32+Lr23*	1 to 2	1 to 2	1 to 2	0	1 to 2
'PBW343'/Tc*Lr32* F_4	*Lr32+Lr26*	1 to 2	1 to 2	1 to 2		0

Same set of F_4 lines (except 'PBW343'/Tc*Lr32* lines) were also tested at adult plant stage with pt. 121R63-1 at about 22°C in the polythene house. In comparison to the lines carrying *Lr32* alone, genotypes with *Lr32+Lr23* neither lowered the infection types nor did it reduce the rust severity which remained 15 MR. It was concluded that the low infection types that appeared in the F_3 progenies were due to unknown environmental factors. As compared to the lines carrying only *Lr32,* the fixed lines with gene combinations, namely *Lr32+Lr23* and *Lr32+Lr26* produced lower infection types in the F_3 families. However, when the seedling tests were repeated in the F_4 families, resistance conferred by the above gene combination lines were same as that of lines with only *Lr32*. Enhanced resistance was not detected in adult plant test as well. The variation in infection types conferred by *Lr32* may be attributed to microenvironment which played major role in determining the extent of hypersensitive reaction. The degree of low infection type was dependent on light intensity and temperature which sometimes gave wrong impression of enhanced resistance when present in combination with *Lr23* or *Lr26* in seedling and adult plant tests.

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