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Genetics of durable resistance to leaf rust and stripe rust of an Indian wheat cultivar HD2009

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Abstract. The Indian bread wheat cultivar HD2009 has maintained its partial resistance to leaf rust and stripe rust in India since its release in 1976. To examine the nature, number and mode of inheritance of its genes for partial leaf rust and stripe rust resistance, this cultivar was crossed with cultivar WL711, which is susceptible to leaf rust and stripe rust. The F_1, F_2, F_3 and F_5 generations from this cross were assessed separately for adult plant disease severity under artificial epidemic of race 77-5 of leaf rust and race 46S119 of stripe rust. Segregation for rust reaction in the F_2, F_3 and F_5 generations indicated that resistance to each of these rust diseases is based on 2 genes, each with additive effects. Although the leaf rust resistance of HD2009 is similar in expression to that conferred by the gene *Lr34*, but unlike the wheats carrying this gene, cultivar HD2009 did not show leaf tip necrosis, a morphological marker believed to be tightly linked to the leaf rust resistance gene *Lr34*. Thus, the non-hypersensitive resistance of HD2009 was ascribed to genes other than *Lr34*.

Key words: durable resistance, *Puccinia striiformis*, *Puccinia triticina*, *Triticum aestivum.*

Introduction

Leaf rust and stripe rust of wheat – caused by *Puccinia triticina* (= *P. recondita* Roberage ex Desmaz f. sp. *tritici* Eriks & E. Henn.) and *P. striiformis* Westend f. sp. *tritici,* respectively – are amongst the most important foliar diseases of wheat (*Triticum aestivum* L.). Studies conducted in various wheat-growing regions of the world against different rust races suggest that the high resistance to leaf rust of wheat is primarily due to as yet undescribed genes, many of which are expressed at the adult plant stage only (Saini et al. 1988; Singh and Rajaram 1991; Shiwani and Saini 1993; Kaur et al. 2000). So far, nearly 50 genes conferring resistance to leaf rust and 30 genes conferring resistance to stripe rust have been identified (McIntosh et al. 2004). Of these, only the leaf rust resistance genes *Lr34* and *Lr46* and the linked stripe rust resistance genes *Yr18* and *Yr29*, respectively, are associated with durable resistance to the two diseases (Singh 1992a, Singh et al. 1998; Suenaga et al. 2001).

Bread wheat cultivar HD2009 (= Arjun) was released for cultivation in India in 1976 and it is still being grown in some parts of the country. This is an early-maturing (140 days) semi-dwarf spring wheat, recommended for sowing in fertile irrigated soils. The average coefficient of leaf rust and stripe rust infection of HD2009 [calculated according to Roelfs et al. (1992)] under artificial epiphytotic conditions for 1982-2002 is 18.75 and 24.0, respectively. Like Thatcher reference line RL6058 for the linked genes *Lr34* and *Yr18,* cultivar HD2009 also shows a high (compatible) seedling and adult plant reaction in glasshouse tests against the prevalent races of leaf rust and stripe rust. Despite this, HD2009 shows a low leaf rust and stripe rust severity in field tests against the same races. Its resistance cannot be ascribed to the adult plant resistance gene *Lr34* in HD2009 (Sawhney and Sharma 1997; Saini and Amita 2000). Many other cultivars resistant to

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leaf rust also appear to carry resistance that cannot be ascribed to *Lr34*, but is similar to that shown by Thatcher line RL6058 and cultivar HD2009 (Kaur et al. 2000). This type of resistance has been classified by many workers as slow rusting (Parlevliet 1988) or a non-hypersensitive type of resistance and it is believed to be durable (McIntosh 1992; Johnson 2000). The low average coefficient of infection of cultivar HD2009 and the non-hypersensitive nature of its leaf rust and stripe rust resistance even 28 years after its release, qualifies this cultivar as durably resistant to both leaf rust and stripe rust. Because only the genes *Lr34* and *Lr46* for leaf rust resistance and *Yr18* and *Yr29* for stripe rust resistance are so far known to confer such resistance (Singh 1992a; Suenaga et al. 2001), cultivar HD2009 is an additional source of as yet undescribed genes that can confer durable resistance to leaf rust and stripe rust in bread wheat. The objective of our study was, therefore, to determine the nature and number of genes that confer leaf rust and stripe rust resistance in wheat cultivar HD2009.

Material and methods

Host material

Cultivar HD2009 (Lerma Rojo 64A/Nainari 60) was crossed with the susceptible cultivar WL711 (S308/Chris//Kalyansona) and the adult plants in F_1, F_2, F_3 and F_5 generations were assessed for percent disease severity to examine the nature, number and mode of inheritance of the genes controlling leaf rust and stripe rust resistance in this cultivar. The F_2 generation from the cross of HD2009 with Thatcher line RL6058, was evaluated for leaf rust severity to determine the allelic relationships between the leaf rust resistance gene(s) in HD2009 and RL6058.

To obtain the F_1, F_2, F_3 and F_5 generations, the F_1 plants were sown during the normal season 2000-01 (October 2000 to April 2001) in the experimental area of Punjab Agricultural University, Ludhiana. A part of the F_1 and F_2 seed from the cross of HD2009 with WL711 was sown during the offseason 2001 (May to September 2001) at the Wheat Research Station, Directorate of Wheat Research, Dalang Maidan, Lahaul and Spiti (H.P.), India, for advancing the generation. The F_5 generation was obtained by harvesting a random spike from each F_3 family and a random plant from each F_4 family. During the normal season 2002-2003, approximately 25–30 seeds of each F_1 and each F_3 and F_5 family were sown in 2-metre-long rows (placed 50 cm apart) to assess leaf rust severities. Two rows of each parent were planted on both sides of the F_1 plants. The susceptible cultivars WL711 and Agra Local were sown after every 20 experimental rows as well as on all sides of the experimental plot, as infector rows. The parents, F_1, F_2, F_3 and F_5 generations were tested for rust reaction simultaneously in the normal season 2002-2003.

For stripe rust experiments, the susceptible cultivars WL711 and Agra Local were sown as borders in the first week of October, which is one month earlier than the normal sowing period of wheat. The normal sowing of wheat for evaluation of parents, F_1, F_2, F_3 and F_5 generations for stripe rust reaction was done in the first week of November 2002. The sowing plan was the same as that of leaf rust experiments.

Pathogen races

We used leaf rust race 77-5 and stripe rust race 46S119, which are the most virulent and frequently identified races from the Indian subcontinent (Nayar et al. 1994). The leaf rust race 77-5 shows a high reaction on seedlings of Thatcher near-isogenic lines carrying all the known *Lr* genes owing their origin to bread wheat. Stripe rust race 46S119 is avirulent on the genes *Yr10, Yr17, Yr24, Yr26* and *Yr27* from bread wheat, which are not likely to occur in either of the parents because of their seedling susceptibility to this race. The avirulence/virulence formula for each of these two races at the seedling stage is given below:

77- 5: P *Lr9, Lr18, Lr19, Lr21, Lr24, Lr25, Lr28, Lr29, Lr32, Lr41, Lr45* / p *Lr1, Lr2a, Lr2b, Lr2c, Lr2d, Lr3, Lr3Bg, Lr3a, Lr3Ka, Lr10, Lr11, Lr12, Lr13, Lr14, Lr15, Lr16, Lr17, Lr20, Lr22, Lr23, Lr26, Lr27+Lr31* (Gatcher), *Lr33, Lr34, Lr35, Lr36, Lr37, Lr42, Lr43, Lr44, Lr46, Lr48, Lr49.*

46S119: P *Yr1, Yr5, Yr10, Yr15, Yr17, Yr24, Yr26, Yr27* / p *Yr1, Yr6, Yr7, Yr8, Yr9, Yr11, Yr12, Yr18.*

Inoculations

Creation of the leaf rust epidemic was started in mid-January. The infector rows and the experimental material was sprayed with 1 gram of leaf rust uredospores suspended in 10 litres of water with 2–3 drops of Tween 20 as dispersant solution. The field inoculations were done in the evening every alternate day and continued till the rust appeared on susceptible cultivars. The susceptible infector rows served as a source of secondary inoculum during the buildup of the epidemic.

Stripe rust epidemic was created by keeping pots containing heavily infected wheat seedlings in between infector rows and by dusting manually the uredospores from infected seedlings onto the infector rows. The disease was later spread onto the experimental material by repeated inoculation with uredospore suspension of race 46S119 in water with Tween 20. The field inoculations were done late in the evening, from mid-December to mid-January.

Disease assessment

Field assessments of leaf rust and stripe rust severity were based on a modified Cobb scale (Peterson et al. 1948), which is expressed as percent leaf area covered with rust. In the field experiments, every plant was scored and – based on these observations – each F_3 and F_5 family was classified as resistant, segregating or susceptible. Plants showing disease severity equal to or higher than the susceptible parent WL711 were classified as susceptible. All other plants were considered resistant.

Statistical analysis

The χ^2 test was applied to assess the goodness of fit of observed ratios to theoretical expectations.

Results

Leaf rust experiments

Cultivars HD2009 and WL711 showed leaf rust severity of 30% and 80%, respectively, in 2002-2003 and the F_1 plants from the cross of HD2009 with WL711 displayed up to 40% leaf rust severity. In the F_2 generation from this cross, out of the 494 plants studied, 471 were resistant and 23 were susceptible. The segregation ratios of resistant and susceptible F_2 plants showed digenic inheritance of leaf rust resistance in this cross (Table 1). The segregation for severity in F_3 and F_5 generations in the cross of HD2009 with WL711 closely fitted a two-gene ratio, 7 resistant : 8 segregating : 1 susceptible and 3 resistant : 1 susceptible respectively, thereby confirming the hypothesis deduced from the evaluation of the F_2 generation. The cross between HD2009 and RL6058 (*Lr34*) did not contain any susceptible segregant in a population of 342 F_2 plants (Table 1).

Stripe rust experiments

Cultivar HD2009 showed stripe rust severity of 20% and the susceptible cultivar WL711 displayed 80% stripe rust, while F_1 showed 30% severity in 2002-2003. The F_2 population derived from the cross of HD2009 with WL711 segregated as 441 resistant and 40 susceptible plants, which

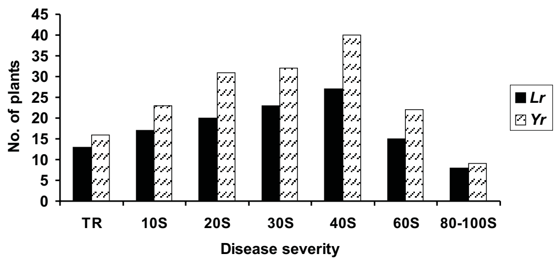

Figure 1. Distribution of F_5 families showing different severity levels in the cross of HD2009 with WL711
TR = trace resistance; S = susceptibility (in %)

gave a good fit in 15 resistant : 1 susceptible ratio (Table 1), thereby suggesting digenic inheritance of stripe rust resistance in the cross of HD2009 with WL711. The segregation pattern of F_3 and F_5 (Figure 1) generations of this cross confirmed the digenic ratios (7 resistant : 8 segregating : 1 susceptible and 3 resistant : 1 susceptible, re-

Table 1. Segregation for leaf rust severity in F_2, F_3 and F_5 generations of two wheat crosses against leaf rust race 77–5 and stripe rust race 46S119

Rust/Cross	Generation	No. of plants with reaction				Expected χ^2ratio	
		Resistant	Segregating	Susceptible	Total		
Leaf rust							
HD2009 × WL711	F_2	471	–	23	494	15:1	2.14^{ns}
	F_3	85	70	9	164	7:8:1	4.35^{ns}
	F_5	101	–	23	124	3:1	2.75^{ns}
RL6058 × HD2009	F_2	342	–	0	342	255:1	–
Stripe rust							
HD2009 × WL711	F_2	441	–	40	481	15:1	3.50^{ns}
	F_3	77	112	17	206	7:8:1	4.02^{ns}
	F_5	125	–	33	158	3:1	1.43^{ns}

ns = non-significant at 5% level of significance spectively). These results confirmed the hypothesis deduced from F_2 tests that two genes control the inheritance of stripe rust resistance of cultivar HD2009.

Discussion

The seedlings and adult plants of HD2009 showed a high reaction (compatible) to leaf rust race 77-5, so the resistance to leaf rust race 77-5 cannot be ascribed to any race-specific gene (Kaur et al. 2000). Stripe rust race 46S119, used for the present work, showed also a high seedling and adult plant reaction in HD2009, thus indicating that no race-specific resistance gene is involved in conferring resistance against stripe rust. In the absence of hypersensitive resistance against race 77-5 of leaf rust and 46S119 of stripe rust, the low leaf rust and stripe rust severity in HD2009 can be ascribed to gene(s) that confer only non-hypersensitive resistance. Rubiales and Niks (1995) reported that cultivars with resistance gene(s) like *Lr34* do not evoke hypersensitive response, show a long latency period, small uredinia and other similar attributes, which restrict pathogen growth without forcing selection pressure on it. In an earlier study, Saini and Amita (2000) also ascribed the partial leaf rust resistance of cultivar HD2009 to a long latency period. McIntosh (1992) suggested this type of resistance to be useful for developing wheat cultivars with durable resistance. In the present study, the leaf rust and stripe rust resistance in a cross of HD2009 with WL711 has been ascribed to 2 genes each, which confer non-hypersensitive resistance. The distribution of F_5 families showing different severity levels in the cross of HD2009 with WL711 (Figure 1) were in accordance with the ratios expected for segregation of 2 independently inherited loci each for leaf rust and stripe rust. The F_5 families showed continuous variation for leaf rust and stripe rust reaction, which suggests that the genes conferring non-hypersensitive leaf rust and stripe rust resistance of cultivar HD2009 have additive effect.

Our conclusion that the partial resistance of HD2009 to leaf rust involves additive genes is in agreement with reports published earlier on additive nature of genes conferring such resistance (Kuhn et al. 1980; Bjarko and Line 1988; Broers and Jacobs 1989; Singh and Rajaram 1992; Singh et al. 1998; Suenaga et al. 2001). Shiwani et al. (1990) and Pretorius and Kloppers (1996) reported two genes for leaf rust resistance in the Thatcher reference line RL6058 carrying the gene *Lr34*. The second gene in RL6058 was not detected by Canadian workers earlier (Dyck and Samborski 1982) because of differences in pathogen cultures prevalent in Canada, India and South Africa. The partial resistance of HD2009 is similar in expression to that conferred by the gene *Lr34*, but unlike the wheats carrying this gene, cultivar HD2009 did not show leaf tip necrosis, a morphological marker tightly linked to the leaf rust resistance gene *Lr34* (Singh 1992b). Thus, partial resistance of HD2009 appears to be based on gene(s) other than *Lr34*. Consequently the F_2 hybrids between HD2009 and RL6058 should segregate for 4 genes (255 : 1). Therefore, the non-segregation of this F_2 population for susceptible plants is ascribed to the small population size (342 plants). Sawhney and Sharma (1997), Saini and Amita (2000) also reported that adult plant resistance of HD2009 is not based on the gene *Lr34*. Despite testing of cultivar HD2009 over a number of environments within India, this cultivar did not show leaf tip necrosis, further suggesting that HD2009 does not have *Lr34* and the linked stripe rust resistance gene *Yr18*. Loci unlinked to leaf tip necrosis, affecting both leaf rust and stripe rust response, are already known to occur (Singh et al. 2004; Navabi et al. 2005,). One such durable leaf rust resistance gene, *Lr46* linked to a stripe rust resistance gene *Yr29*, has also been reported to be present in cultivar Pavon 76 (Singh et al. 1998). As this gene is not effective in India, therefore cultivar HD2009 has not been tested for *Lr46*.

In addition to the two genes for non-hypersensitive leaf rust resistance reported in the present study, cultivar HD2009 carries the hypersensitive seedling resistance genes *Lr10* and *Lr13* (Singh and Gupta 1991; Sawhney et al 1992; Kaur et al 2000), while cultivar WL711 carries only *Lr13* (Gupta et al. 1984). Both the genes *Lr10* and *Lr13* are ineffective against leaf rust race 77-5, which was used for the present study (Saini et al. 1998). Therefore, only 2 undescribed genes showing additive effects are responsible for the non-hypersensitive leaf rust resistance of cultivar HD2009.

Like the present results, the study by Singh et al. (2004) strongly supports that diverse partially effective adult plant resistance genes associated with durable resistance are widely prevalent in bread wheat. High levels of resistance, reaching near immunity, have already been obtained by intercrossing various wheats with many such unknown resistance genes (Singh et al. 2004). Therefore, it is important to assess critically the utility of such diversity and precisely identify the loci responsible for the high resistance, to ensure its systematic utilization in breeding for durable resistance to leaf rust and stripe rust.

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