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Genetic basis of rust resistance of a high yielding wheat line from northern hills zone

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Abstract

Genetic basis of rust resistance was investigated in a bread wheat line HS424 which is bred for Northern Hills Zone of India. The study showed that HS424 is resistant to leaf rust and stem rust pathotypes prevalent in India. At seedling stage it was resistant to stripe rust pathotypes except 46S119. However, at adult plant stage, HS424 showed only moderate susceptibility to pathotype 46S119. Low terminal disease against pt. 46S119 must be due to additional adult plant resistance factors in HS424. At least two resistance genes were involved in the resistance against leaf rust pathotypes wherein resistance to pathotypes 77-5 and 77-2 was governed by single dominant gene and two independent dominant genes, respectively. Stem rust resistance was governed by three genes. Resistance to pt. 40A was due to two independent dominant genes whereas single dominant gene imparted resistance to pt. 40-1. Adult plant resistance gene *Sr2* was also postulated in HS424. Based on infection type data, inheritance study, morphological -marker, genetic linkage and molecular marker analyses it is concluded that HS424 carries genes *L24+Lr26+Sr2+Sr24+Sr31+Yr9*.

Key words: Genetic basis, rust resistance, wheat, HS424

Introduction

Apart from the inherent yield potential both biotic and abiotic stresses determine the realized yield of cultivars. Among the biotic stress factors, rusts are the most important diseases of wheat in India. Though stem rust (*Puccinia graminis f.sp. tritici* Eriks. & Henn.) samples are being detected regularly from places at high altitudes as Dalang Maidan of Lahaul Valley of Himachal Pradesh, leaf rust (*Puccinia triticina* Erikss.) and stripe rust (*Puccinia striiformis* Westend. f. sp. *tritici*) are more important in the northern hills. HS424 (CPAN3004// HPW(dl)30/HS286) is an advanced bread wheat line of All India Coordinated Wheat and Barley Improvement Project. Along with other advance lines and checks it was tested with multi-pathotypes of leaf, stem and stripe rusts at seedling and adult plant stages for two consecutive years under temperature controlled glass house and polythene house at the Directorate of Wheat Research, Regional Station, Flowerdale. HS424 showed high resistance to leaf and stem rust pathotypes at seedling as well as adult plant stage. Genetic basis of rust resistance of HS424 is reported here.

Materials and methods

Plant material

The experimental material consisted of F_2 and F_3 generations derived from the cross Agra Local/HS424. Segregating generations were tested at seedling stage against predominant leaf rust pathotypes 77-2 and 121R63-1 and stem rust pathotypes 40A and 40-1.

Resistance tests

The F_2 and F_3 seedlings were raised in aluminum bread pans (trays) comprising ten rows with a susceptible check, Agra Local, at 7th row. Fully expanded primary leaves (7-8 days old) were inoculated with uredospores suspended in light, non-phytotoxic isoparaffinic oil (soltrol 170, Philips Chevron). Inoculations of leaf and stem rusts were carried out in temperature controlled glass house maintained at 22°C and 24°C, respectively. The inoculated seedlings were kept in a humid glass chamber for 48 hours and then transferred to benches. Infection types (ITs) were recorded 14 days after inoculation and classified according to standard methods [1] as follows: IT 0; (naught fleck),; (fleck) and ;2 (fleck two), were classified as the resistant reactions whereas ITs 3 (three) and 3^+ (three plus) were designated as susceptible reactions. The F_2 seedlings from the single F_1 plant were scored for infection types (ITs) and transplanted in the field for obtaining of F_3 families. About twenty five seedlings were analyzed per family. Same set of F_3 families were evaluated wherever progeny testing was done simultaneously with more than one pathotype. Chi-square test was applied to test the validity of expected genetic ratios. The stem rust resistance gene *Sr2* was postulated on the basis of microchlorosis [2], a phenotypic marker that is completely linked with the resistance gene *Sr2* which

is characterized by presence of pathotype non-specific minute chlorotic spots on the second leaf of seedlings.

Adult plants were also tested where the parents and lines with known resistance genes were analyzed with stripe rust pathotypes 46S119 and 78S84, leaf rust pathotypes 77-5 and 104-2 and stem rust pathotypes 40A and 40-1. Adult plant resistance tests were carried out in polythene house for two seasons and scores were averaged over the seasons. Adult plant inoculations were done after the appearance of flag leaf with uredospores suspended in light weight, non phytotoxic isoparaffinic oil (soltrol 170). The polythene-house was kept humid for 48 hours with the help of humidifier. Rust severity were recorded as per the modified Cobb's scale [3].

DNA extraction and PCR conditions

Molecular marker [4] and [5] were used to detect the presence of *Lr26/Sr31/Yr9* and *Lr24*, respectively. DNA was extracted by CTAB method [6]. The polymerase chain reaction assays (PCR) for *Lr26* were carried out in 25-µl volumes in an MJ Research (Waltham, MA) thermocycler. The reaction mixture contained 0.1µM of each primer (AF1: 5' GGA GAC ATC ATG AAA CAT TTG 3' and AF2: 5' CTG TTG TTG GGC AGA AAG 3'), 0.2 mM of each dNTP, 2mM $MgCl_2$, 0.5 U *Taq* polymerase, and 50 ηg of template DNA. Standard amplification conditions were 5 min at 94°C, 38 cycles of 1 min at 94°C, 2 min at 55°, 2 min at 72°C, followed by a final extension step at 72°C for 5 min. The PCR for *Lr24/Sr24* was also carried out in 25-µl volumes. The reaction mixture contained 0.12µM of each primer (F: 5' CAC CCG TGA CAT GCT CGT A 3' and R: 5' AAC AGG AAA TGA GCA ACG ATG T 3'), 0.25mM of each dNTP, 2mM $MgCl_2$, 1U *Taq* polymerase, and 50 ηg of template DNA. Template DNA was denatured at 94°C for 3 min followed by 7 cycles of 30s at 94°C, 30s at 65°C, 40s at 72°C, primer extension temperature was reduced by 1°C per cycle for next six cycles; next 30 cycles of 30s at 94°C, 30s at 58°C, 40s at 72°C, followed by a final extension step at 72°C for 5 min. The products of *Lr26* and *Lr24* markers were analyzed using 1.5% and 2% agarose gel, respectively in 0.5X TAE buffer.

Results and discussion

Seedling reaction of HS424 against leaf rust, stem rust and stripe rust pathotypes is given in Table 1. At seedling stage it was highly resistant to all leaf rust, stem rust and stripe rust pathotypes except stripe rust pathotype 46S119. At adult plant stage, HS424 was highly resistant to leaf and stem rust pathotypes but it showed moderate susceptibility to stripe rust pathotype 46S119 (40MS terminal disease severity) as compared to Agra Local which showed 100S terminal disease severity. Moderate susceptibility along with 40 per cent infected leaf area indicated presence of adult plant resistance gene/s in HS424 against stripe rust.

Seedling resistance test with pt. 77-2 of leaf rust

The F_1's of the cross between Agra Local and HS424 were resistant. The F_2 segregated for three infection types (0; ;1 and 3+). Out of 362 seedlings, 335 were resistant and 27 were susceptible (Table 2). The data was good fit to 15 resistant: 1 susceptible (P = 0.25-0.50). Among the 97 F_3 families, 49 segregated, 7 were homozygous susceptible and 41 were homozygous resistant (Table 2). The F_3 family segregation gave good fit to 7 resistant: 8 segregating : 1 susceptible (P = 0.50-0.75).

Seedling resistance test with pt. 77-5 of leaf rust

The F_1's of the cross between Agra Local and HS424 were resistant. The F_2 segregated for two infection types (;1 and 3+). Out of 394 seedlings, 301 were resistant type and 93 showed susceptible reaction (Table 2). The data was good fit to 3 resistant: 1 susceptible (P = 0.50). Among the 93 F_3 families, 46 segregated, 28 were homozygous susceptible and 19 were homozygous resistant (Table 2). The F_3 family segregation was good fit to 1 resistant: 2 segregating: 1 susceptible (P = 0.25-0.50).

Seedling resistance test with 40A of stem rust

The F_1's of the cross between Agra Local and HS424 were resistant. The F_2 segregated for two infection types (2 to 2⁻ and 3+). Out of 302 seedlings, 284 were

Table 1. Seedling reaction of HS424, a susceptible cultivar Agra Local and near isogenic lines for the genes *Lr9, Lrl9, Lr24 Lr26* against selected pathotypes of leaf, stem and stripe rust

Line/cultivar	Leaf rust pathotypes						Stem rust pathotypes				Stripe rust pathotypes		
	77-1	77-2	77-5	104-2	77-7	77-8	21A-2	40A	40-1	117-2	N	46S-119	78S-84
TcLr9	0;	0;	0;	0;	3+	0;	3+	3+	3+	3+	3+	3+	3+
TcLr24	;1	;1	;1	;1	;1	;1	2-	2-	3+	2-	3+	3+	3+
TcLr26	3+	0;	3+	3+	3+	0;	2-	2-	2-	2-	0;	3+	3+
TcLr19	;-	;-	0;	;-	;-	3+	2	2	2	2-	3+	3+	3+
HS424	**;1-**	**;1**	**;1**	**;12**	**;1**	**;1**	**2-**	**2-**	**2-**	**2-**	**;**	**3**	**0;**
Agra Local	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+

Table 2. Segregation of seedling reaction against leaf, stem and stripe rust pathotypes in F_2 and F_3 generations from cross of HS424 with Agra Local

Pathotypes and generation	Number of seedlings/families			Expected ratio	χ^2 value	P range
	Resistant	Segregating	Susceptible			
Leaf rust						
Pathotype 77-2						
F_2	335		27	15R:1 S	0.73	0.25-0.50
F_3	33	44	6	7R:8seg:1S	0.58	0.50-0.75
Pathotype 77-5						
F_2	301		93	3R:1 S	0.45	0.50
F_3	19	46	28	1R:2seg:1S	1.75	0.25-0.50
Stem rust						
Pathotype 40A						
F_2	284	76	18	15R:1 S	0.09	0.75-0.90
F_3	41	54	5	7R:8seg:1S	0.74	0.50-0.75
Pathotype 40-1						
F_2	293		86	3R:1S	1.14	0.25-0.50
F_3	29	52	17	1R:2Seg:1S	3.30	0.10-0.25

resistant and 18 were susceptible (Table 2). The data gave good fit to 15 resistant: 1 susceptible ratio (P = 0.75-0.90). Among the 100 F_3 families, 54 segregated, 5 were homozygous susceptible and 41 were homozygous resistant (Table 2). The F_3 family segregation was good fit to 7 resistant: 8 segregating : 1 susceptible (P = 0.50-0.75).

Seedling resistance test with 40-1 of stem rust

The F_1's of the cross between Agra Local and HS424 were resistant. The F_2 segregated for two infection types (2 to 2^- and 3^+). Out of 379 seedlings, 293 were resistant and 86 were susceptible (Table 2). The data gave good fit to 3 resistant: 1 susceptible ratio (P = 0.25-0.50). Among the 98 F_3 families, 52 segregated, 17 were homozygous susceptible and 29 were homozygous resistant (Table 2). The F_3 family segregation was good fit to 7 resistant: 8 segregating : 1 susceptible (P = 0.10-0.25).

All F_3 families resistant to pathotype 77-5 were also resistant to pathotype 77-2 but many of the families that were resistant to pathotype 77-2 were susceptible to pathotype 77-5. Therefore, it was concluded that the gene imparting resistance against 77-5 was also operating against 77-2 but the second gene is effective only against pathotype 77-2. Among the designated leaf rust resistance genes (*Lr1-Lr56*), genes *Lr9, Lr19, Lr24, Lr25, Lr28, Lr29, Lr32, Lr39, Lr41, Lr42* and *Lr45* confer resistance to pathotype 77-5 [7&8]. All these genes are also resistant to pathotype 77-2. Three genes namely, *Lr15, Lr20, Lr26* are resistant to 77-2 but susceptible to 77-5. Therefore, one of the two independent dominant genes of HS424 that imparted resistance to pathotype 77-2 is likely to be either *Lr15* or *Lr20* or *Lr26*, whereas the other gene must be among the genes that are resistant to 77-5 since the second gene was effective against both pathotypes. The inheritance pattern of HS424 against stem rust pathotypes 40A and 40-1 indicated presence of *Sr24/Lr24* which is effective against pathotype 40A only whereas resistance against both the pathotypes 40A and 40-1 may be ascribed to *Sr31*. Conclusive evidence of the presence of *Lr24* and *Lr26* came from molecular marker study (Fig. 1). A specific 500bp fragment was amplified from the DNA of HS424 and Tc*Lr24* which confirmed presence of *Sr24/Lr24* in HS424. Similarly, a 1.5kb specific fragment was amplified in HS424 and other lines carrying *Lr26* which confirmed presence of rye chromatin in HS424. Pedigree information substantiated that rye chromatin was due to 1BL.1RS translocation and hence, it was concluded that HS424 also carry *Lr26*. Therefore, the two genes for stem rust resistance in HS424 must be *Sr24* and *Sr31* as these

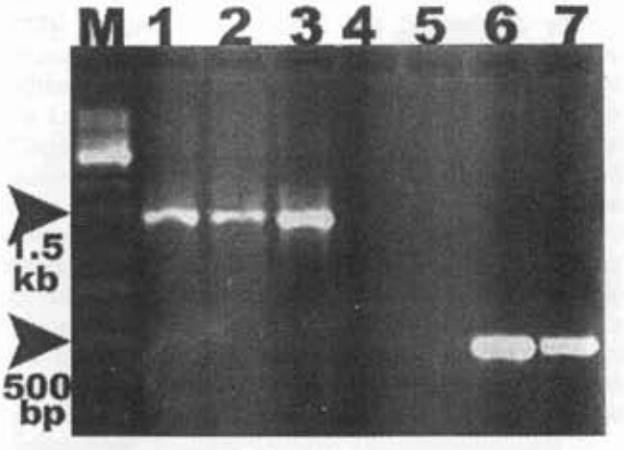

Fig. 1. Lanes 1-4 : PCR product of *Lr26* marker and lanes 5-7 : PCR product of *Lr24* marker; M: Marker; 1: Tc*Lr26*; 2: PBW343; 3: HS424; 4: Agra Local; 5: Agral Local; 6: Tc*Lr24*; 7: HS424

genes are completely linked with *Lr24* and *Lr26*, respectively. HS424 also carries *Yr9* as it is completely linked with *Lr26*. *Yr9* is resistant to all pathotypes of stripe rust except 46S119 and 78S84. Therefore, resistance to pathotype 78S84 must be due to additional seedling resistance gene. It is likely that the additional seedling resistance gene is *Yr2* as it is resistant to pt. 78S84 and prevalent in Indian wheat lines. However, its presence can not be proved conclusively from the present study. Though the seedling of HS424 was susceptible to pt. 46S119 but the adult plant has shown less terminal disease severity (40MS) as compared to the susceptible check Agra Local (100S). Low terminal disease must be due to additional adult plant resistance factors in HS424. A slow rusting stem rust resistance gene, *Sr2*, was also postulated on the basis of microchlorosis, a morphological marker associated with *Sr2* [2&9]. Based on infection type data, inheritance study, morphological marker, genetic linkage and molecular marker analyses it is concluded that HS424 carries genes *L24+Lr26+Sr2+Sr24+Sr31+Yr9*. In addition, it also has adult plant stripe rust resistance gene/s. The rust resistance of varieties released for Northern Hills Zone (NHZ) is mainly dependent on *Lr26+Sr31+Yr9* gene complex [10]. Resistance of HS424 is not solely dependent on 1BL.1RS translocation, hence, it will add additional rust resistance genes in the NHZ.

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