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Inheritance of resistance to spot blotch disease in wheat

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Abstract

The mode of inheritance to spot blotch or Helminthosporium leaf blight (HLB) resistance was studied in segregating populations of 15 crosses involving two resistant wheat lines; a *Thinopyrum* derived CIMMYT wheat line, CIGM 84-295-1, and a Chinese line, Ning 8201. The disease reaction of F_1 and F_2 progeny of crosses involving the resistant wheat line, CIGM 84-295-1 and four susceptible lines indicated monogenic recessive resistance to HLB, whereas crosses involving Ning 8201 with the same susceptible parents showed resistance was controlled by a dominant gene. The segregation ratio of F_2 progeny derived from the cross, Ning 8201 x CIGM 84-295-1 (resistant x resistant) followed a ratio of 13 resistant: 3 susceptible as expected for genetically two independent dominant and recessive genes. The F_3 progeny testing of the F_2 susceptible plants confirmed the presence of dominant and recessive gene interaction. The progeny obtained from the crosses between the susceptible parents were all susceptible to HLB disease.

Key words: Disease resistance; Helminthosporium leaf blight; inheritance; supplementary gene interaction; *Triticum aestivum*

Spot blotch or Helminthosporium leaf blight (HLB) caused by *Bipolaris sorokiniana* (Sacc.) Shoem [syn. *Helminthosporium sativum* Pammel, King and Bakke; *Drechslera sorokiniana* (Saac.) Subram and Jain; *Cochliobolus sativus* Jain (Ito and Kuribay) (perfect stage)] is a devastating disease of wheat grown in warm and humid wheat growing regions [1, 2]. The crop loss caused by spot blotch disease increased in significance as the production of wheat expanded into non-traditional wheat growing areas. Yield reductions caused by HLB have been widely recorded and studied [3-5]. A multi-location trial to assess yield loss due to foliar blight (especially HLB) indicated 2.7 to 36.2% losses in grain yield and 0.1 to 16.3% losses in grain weight [6]. Breeding for resistance provides an economic and environmentally safe strategy to manage this disease. There are several elite cultivars that possess low to moderate levels of resistance against HLB that can be utilized in breeding and crop improvement efforts [6-10].

Lack of suitable resistance sources from within and outside India necessitate to identify potential resistance donors to the disease and to develop varieties with such build-in resistance [11, 12]. Information on the genetics of resistance is equally inadequate owing to the ambiguity in various types of disease scoring methods, large variation in disease incidence and a lack of systematic screening procedures using controlled environment. Novel sources of resistance to spot blotch were reported in some of the synthetic hexaploid wheats from CIMMYT (International Maize and Wheat Improvement Center), Mexico [13-15]. Concurrently, the crossing program at CIMMYT produced wheat lines containing germplasm from *Thinopyrum curvifolium*, that expressed high levels of spot blotch resistance [5 & 14]. In our earlier studies, we identified CIGM84-295-1, one of the CIMMYT lines and a Chinese line, Ning 8201, tested at a disease hotspot in India at Pusa, Bihar, as a potential source of spot blotch resistance [16]. Resistance in Ning 8201, one of the cultivars of present studies, when tested under field conditions in Nepal was reported to be monogenic dominant [17]. To characterize the resistance of Ning 8201 against the spot blotch pathogen in wheat growing conditions in India, we tested the wheat line against a virulent spot blotch isolate, KL-8 from Karnal, India. The present research was also undertaken to study the differences in genetic nature of resistance in these two promising spot blotch resistance sources under controlled environmental conditions.

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Materials and Methods

The wheat lines included in the present study consists of two resistant lines – CIGM84-295-1 ('Chinese Spring' /*Thinopyrum curvifolium* F_1 //'Glenson' /3/'Alondra' / 'Pavon') [18] and Ning 8201; two susceptible bread wheat lines from China – Qianfeng, Chuanmai # 18, and two lines from India – HD 2285 and WH 147. These lines were selected based on their disease reaction to a natural spot blotch pathogen in an earlier study by the authors [16]. The lines were crossed in a diallel-mating scheme without reciprocals, generating fifteen crosses. The parental and the F_1 generation of the crosses were tested under controlled environmental conditions against the monoconidial HLB isolate – KL-8, at the National Phytotron Facility (NPF), IARI, New Delhi. The F_2 populations were tested under controlled conditions in a growth chamber at NPF and the F_3 progeny families from the susceptible F_2 segregants of the control cross, CIGM 84-295-1 x Ning 8201 were also raised in NPF, New Delhi. Surface sterilized seeds were sown at a rate of 4-6 seeds/pot in sterilized media made of decomposed agropeat, sand and vermiculite in the ratio of 1:1:1 and placed in a growth chamber with a 10 ½ h photoperiod, at 280 µ Em-2/s light intensity and 21.5 ± 3.5 ^{0}C night and day temperature regime.

Inoculum multiplication and inoculation procedure

The monoconidial isolate *Bipolaris sorokiniana* (KL-8, Karnal) was obtained from the Division of Plant Pathology, Indian Agricultural Research Institute (IARI), New Delhi. The spot blotch isolate was cultured and maintained following the method of Gilchrist [19]. The spore suspension contained 6 x 10^4 spores/ml[20] and a surfactant, Tween-20 @ 1 ml/100 ml of spore suspension, which was added to allow a uniform spray [25]. Four days post spraying of the inoculum, 0.5-1ml of spore suspension/plant was injected with a hypodermal syringe into the mid-rib of leaves at boot leaf stage to avoid disease escapes. The inoculated plants were maintained in dark for 24 hours at 22-24^{o}C with a relative humidity of 95%. Thereafter, they were maintained with 10½ hours photoperiod at 22-24^{o}C with a relative humidity of 80% and the disease reaction of the plants was scored three times at weekly intervals beginning seven days after the inoculum injection.

Disease scoring and statistical analyses

The disease reactions were scored using the double-digit scoring method [20]. The disease score was based on the percentage leaf area damaged on top leaves i.e., flag leaf and flag leaf-1. As the disease progressed from the bottom to the top of the plant, the diseased area on the leaf below the flag leaf (flag-1) is greater than that on the top leaf. The double digits represent the percentage leaf area damaged on the flag leaf and flag-1 leaf respectively. For instance, if the percentage damage on a flag leaf is 10 and corresponding percentage damage on flag-1 is 30, the first digits of these numbers represent a scale of 1,3 which is characterized as 'resistant'. The two values thus obtained were considered together to arrive at a score. Based on these scores, the lines were characterized as 'immune' (0,0), 'resistant' (<1,3), 'moderately resistant' (<3,4), 'moderately susceptible' (<5,6), 'susceptible' (>5,7) and 'highly susceptible' (>7,8) [21]. The disease scores per plant were recorded by averaging the scores on all tillers of every inoculated plant. The parental performance (± SE) was used to define the cut-off points to categorize the segregating progenies into disease groups. The segregation analysis was carried by testing the goodness of fit of the observed data from the expected segregation ratios through Chi-square tests.

Results and discussion

To test the consistency of disease reaction in the parental lines with those observed in a screening study under natural epidemic conditions [16], the wheat lines were challenged with the isolate KL-8 (Karnal, India) under controlled conditions. The wheat lines, CIGM 84-295-1 and Ning 8201 were resistant to this isolate with a disease score as low as 1,2 whereas other lines; Qianfeng, Chuanmai # 18, WH147 and HD 2285 were susceptible with disease scores ranging from 6,8 to 7,9 (Table 1). The data represents averages of three replicates ± SE. Spot blotch disease reaction of the wheat lines was similar to that detected in the earlier studies [16] suggesting that there was no variation in the pattern of disease response observed in these wheat lines when tested under different conditions. The wheat lines were crossed in a diallel-mating scheme, which allows a reliable drawing of inferences from the F_2 populations due to the simultaneous need for all possible combinations of crosses to fully coincide with gene postulations.

All F_1 progeny of crosses between the resistant parent CIGM 84-295-1 and four susceptible parents were fully susceptible (Table 2) suggesting recessive nature of the alleles controlling resistance to spot blotch disease. The F_2 progenies segregated as 1 resistant: 3 susceptible indicating the presence of a single gene with recessive alleles in the wheat line, CIGM 84-295-1, that impart resistance to spot blotch disease.

Table 1. HLB disease reactions of the resistant and four susceptible plants

Parents	Disease reaction	Disease score range		Disease score
		Flag leaf	Flag-1 leaf	
CIGM 84-295-1	Resistant	5-10	10-15	1,2
Ning 8201	Resistant	5-15	10-20	1,2
Qianfeng	Susceptible	50-65	60-95	6,8
Chuanmai#18	Susceptible	55-70	75-90	6,8
HD 2285	Susceptible	60-80	70-95	7,8
WH 147	Susceptible	65-85	75-95	7,9

The F_1 progeny of crosses involving the resistant parent, Ning 8201 and susceptible parents were resistant indicating dominance of resistance. The F_2 progeny of the four crosses segregated in the ratio of 3 resistant: 1 susceptible (Table 2), indicating the presence of a single dominant gene controlling resistance. The F_1 and F_2 progenies of the six possible crosses between susceptible lines, were susceptible and showed no segregation for HLB disease reaction suggesting that these lines carried no alleles for spot blotch resistance (Table 2). Segregation of the F_2 progeny of cross, CIGM 84-295-1 x Ning 8201 was consistent with a ratio of 13 resistant: 3 susceptible as expected for joint segregation of a dominant gene and a recessive gene for resistance. The F_3 progeny testing of the F_2 susceptible group, comprising of ten plants from the control cross, CIGM 84-295-1 x Ning 8201 segregated as 10 resistant: 42 susceptible plants, fitting into a ratio of 1 resistant: 5 susceptible as expected from the segregation of three F_2 susceptible classes confirming the dominant and recessive gene interaction.

The wheat line, CIGM-84-295-1 was found to have a recessive gene for resistance. In another CIMMYT wheat line, Chirya-3, Ragiba *et al*. [21] found that resistance was conferred by two recessive genes located on chromosomes, 7B and 7D [22]. Digenic recessive inheritance was also reported by Singh et al. [23] from a field-based study where the disease complex comprised a mixture of *Bipolaris sorokiniana* and *Alternaria tritici.* The present studies did not clearly confirm that both genes specifically conferred resistance to spot blotch. Identification of digenic recessive resistance in Chirya-3 reported by the authors earlier

Table 2. HLB disease reaction recorded on F_1 and F_2 plants of the 15 diallel crosses under controlled environmental conditions

Cross (female x male)	Reaction (F_1)	F_2		F_3		Ratio		P-value
		R	S	R	S	F_2	F_3	
CIGM84-295-1 x Ning 8201*	R	57	10			13R:3S		0.2014
				10	42		1R:5S	0.6199
CIGM84-295-1 x Qianfeng	S	35	6			1R:3S		0.1254
CIGM84-295-1 x Chuanmai#18	S	10	35			1R:3S		0.6671
CIGM84-295-1 x WH 147	S	15	33			1R:3S		0.3173
CIGM84-295-1 x HD 2285	S	9	52			1R:3S		0.0647
Ning 8201 x Qianfeng	R	64	14			3R:1S		0.1504
Ning 8201 x Chuanmai#18	R	102	27			3R:1S		0.2857
Ning 8201 x WH 147	R	67	21			3R:1S		0.8065
Ning 8201 x HD 2285	R	51	21			3R:1S		0.7973
Qianfeng x Chuanmai#18	S	0	69			All S		
Qianfeng x WH 147	S	0	98			All S		
Qianfeng x HD 2285	S	0	79			All S		
Qianfeng x WH 147	S	0	68			All S		
Chuanmai x HD 2285	S	0	62			All S		
WH 147 x HD 2285	S	1	44			All S		

*F_3 progeny of 10 F_2 susceptible plants segregated into 1R:5S; R: Resistant S: Susceptible

[21] was a first report and these resistance genes were located through monosomic analysis on the wheat chromosomes – 7B and 7D [22]. Molecular tagging of the resistance genes would be a viable option to aid in marker-assisted breeding of wheat for HLB resistance. In one other study Ragiba et al [24] identified four putative RAPD markers associated with the recessive resistance genes in Chirya-3 which could be used in marker assisted HLB resistance breeding program. Identification of markers associated with the dominant and recessive HLB resistant genes in Ning 8201 and CIGM-84-295-1 respectively would provide additional set of molecular markers for early selection in breeding programs.

Spot blotch resistance in Ning 8201 was controlled by a dominant gene. This is agreed with an earlier field-based study [7 & 17]. Based on the comparison of segregation patterns of resistance genes in CIGM84-295-1 and Ning 8201, the wheat lines constitute two different sources of resistance. The disease reaction of the two potential sources of resistance to spot blotch under natural epidemic conditions [16] and against the monoconidial isolate, KL-8 under controlled conditions indicated that resistance was consistent irrespective of environmental conditions and race flora.

The typical expression of supplementary epistasis involving a dominant and a recessive allele for the spot blotch disease resistant trait was obtained. These genes along with the complementary recessive genes identified in an earlier study by the authors [22] that confer resistance to HLB could be successfully utilized in breeding HLB resistant lines. The resistant lines Chirya-3, CIGM84-295-1and Ning 8201 could be used as donors for gene pyramiding against HLB disease. A resultant population with combined genome of the resistant lines Chirya-3, CIGM84-295-1 and Ning 8201 with diverse resistant sources would be able to provide defense mechanisms in a way that the yield loss as a result of HLB infection could be significantly minimized.

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