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Genetic characterization of resistance to stripe rust, leaf rust, Karnal bunt and cereal cyst nematode in a multiple disease resistant wheat stock W8627

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Abstract

Genetic analysis of multiple disease resistance was carried out in segregating populations of bread wheat line W8627 and PBW343 against stripe rust (*Puccinia striiformis*), leaf rust (*Puccinia triticina*), Karnal bunt (*Tilletia indica*) isolates and cereal cyst nematode (*Heterodera avenae*). Seedling response of W 8627, PBW 343 and F_1 against 78S84 race of *Puccinia striiformis*, 77-5 race of *P. triticina* reflected that the wheat line W8627 possessed seedling resistance genes against both the races. Based on the segregation pattern of F_2 generation and F_3 families, two complementary recessive genes for resistance to 78S84 race of *Puccinia striiformis* and one recessive gene each for resistance to 77-5 race *P. triticina*, mixture of *Tilletia indica* isolates and Ha 41 biotype of *H. avenae* were identified. Co-segregation studies revealed no linkage between concerned resistance genes.

Key words: Bread wheat, multiple disease resistance, genetic analysis, pathogenic races

Introduction

Wheat (*Triticum aestivum* L.em.Thell.) crop is affected by a large number of pathogens like fungi, bacteria, viruses and nematodes. Amongst fungal diseases rusts being the major diseases affecting wheat causing an annual losses from Rs. 40 to 392 millions (Butler and Hayman 1906; Mitra,1931). In the North Western Plains Zone (NWPZ) yellow rust or stripe rust (*Puccinia striiformis* West) is the major concern followed by brown rust or leaf rust (*Puccinia triticina* Erikss). Being obligate parasite, significant variation exists in the pathogen population for virulence to specific resistance genes. Evolution of new virulence through migration, mutation, recombination of existing virulence and their selection is more frequent in rust. So, there is need to update the resistance genes to the elite cultivars from time to time against the prevalent virulent genes in the pathogens.

Karnal bunt (KB) caused by *Tilletia indica* Mitra is another important disease of wheat in NWPZ of India subjected to its strict quarantine importance. The disease impairs the quality of wheat and wheat products. The multiple modes of transmission (seed, soil and air) make the management of KB a difficult task. Resistance breeding is regarded as the main option for management of KB. Development and use of KB resistant varieties will open the gates for India to increase its share in the global wheat trade.

Other than the fungi, wheat is also attacked by nematodes. These nematodes cause enormous yield losses. Approximately 90 species of plant parasitic nematodes have been found associated with both wheat and barley, the most important ones are the cereal cyst nematode (CCN) (*Heterodera avenae* Wollenweber) and the ear cockle nematode (*Anguina tritici* Steinbuch) causing “molya” and “ear cockle” disease, respectively. *Heterodera avenae* was first detected on roots of wheat from the village of Nimka Thana, Sikar District, Rajasthan State. In individual fields, sometimes losses may reach to 50-66% or more depending upon on the severity of the nematode infestation (Mathur et al. 1980). It has been recognized as an endemic problem in the agroclimatic zones of Northern to Central India comprising the states of

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Punjab, Haryana, Uttar Pradesh, Himachal Pradesh, Jammu and Kashmir, Delhi and Madhya Pradesh (Uma Rao et al. 2009). CCN can be managed by cultural practices, chemicals, using CCN resistant cultivars or by integrating these approaches. However, purchase of nematicides is beyond the means of many growers in less developed regions. Cultural practices, such as the use of rotation, are possible in some cases, but use of the resistant and tolerant varieties is one of the most effective and economical method of nematode control.

It is pertinent to impart resistance in the high yielding wheat varieties to the prevalent diseases, so as to realize the full yield potential of the cultivars. Transferring resistance against such a large number of diseases in a single elite cultivar, one by one is a herculean task. So, need of the hour is the identification of "Multiple Disease Resistant Stocks", which impart resistance against many diseases simultaneously to study the mode of inheritance and utilizing them in the breeding programmes. To accomplish the above mentioned task, a bread wheat line identified by screening against rusts, KB and nematode over the years at PAU, Ludhiana was crossed with PBW 343. The segregating populations (F_1, F_2 and F_3) were subjected to artificial epiphytotics to study the genetics and co-segregation of resistance to stripe rust, leaf rust, cereal cyst nematode and Karnal bunt.

Materials and methods

The present investigation was conducted at the Experimental Area of the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana during 2010-11.

Plant material

The experimental material used to study the mode of, inheritance and co-segregation of resistance genes in multiple disease resistance stock (W-8627) included P_1 (W8627), P_2 (PBW343), F_1, F_2 and F_3 generations from the cross W8627/PBW343. Standard agronomic practices (Anon. 2010) were followed for raising the crop.

Pathogens

1) Stripe rust pathogen *Puccinia striiformis* f. sp. *tritici* race 78S84 and a leaf rust pathogen of *P. triticina* (77-5) were obtained from Regional Research Station, Flowerdale, Shimla. These races were multiplied on susceptible host Agra Local in separate glass houses for evaluation of infection types in seedling and adult plant stages.

2) A total of 17 isolates of Karnal bunt were maintained in the Potato Dextrose Agar (PDA) culture slants.

3) Sick plot were maintained by mixing nematode cysts in the soil, ensuring an initial population of two cysts/500cc of soil.

Evaluation of resistance against stripe rust and leaf rust at seedling stage

Seedlings of W8627 and PBW 343 and F_1 were raised in disposable glasses filled with well composed sandy loam soil. In each glass, 6-8 seeds of each parents and F_1 were sown in two replications for each of the race 78S84 and 77-5. The temperature in the glass houses was maintained in the range between 15-20^oC. Seven to ten days old seedlings were inoculated with homogenous urediospore suspension of both the races prepared separately for two sets, keeping the inoculums density of 5 to 6 spores per low microscopic field, with the help of sprayer. The inoculated material was then placed for incubation in a polythene chamber maintained at 100 per cent relative humidity and temperature from 15-20^oC for 48 h. for successful infection process. The host response for seedling infection types was recorded 14 days after inoculation, following the modified scale by McIntosh et al. (1995) for stripe rust and Stakmen et al. (1962) for leaf rust, respectively.

Genetic characterization of resistance to different diseases

Parents, F_1, F_2 and F_3 families from the cross W8627 x PBW343 were evaluated for different diseases and nematode infestation under simulated epiphytotics to study the genetics and mode of inheritance of resistance to rusts, KB and CCN.

The parents and segregating generations from the cross W8627 x PBW343 of experimental materials were sown in the field of the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, during last week of November, 2010. Five rows of P_1 and P_2, 2 rows of F_1, 10 rows of F_2 and 100 progenies with 20-25 plants per progeny in two rows of F_3 were planted in 1.5 m row with a row to row distance of 20 cm and plant to plant distance of 15 cm. Rust epidemic was created by spraying urediospores of *P. striiformis* race 78S84 and *P. triticina race* 77-5 in water, on the experimental material, thrice in a week starting from December, 2010 till the first

week of February, 2011. Infector rows were planted along the experimental wheat material which constituted a mixture of susceptible varieties, Agra Local, Kharchia, WL711 and C306. The terminal disease severity was considered as appropriate for recording as there is no visible further increase in intensity of disease. The adult plants of F_1, P_1 and P_2 and individual plants of F_2 and F_3 were observed for percentage of leaf area covered with rust according to Cobb's Scale as described by Peterson et al. (1948).

The parents, F_1, F_2 and F_3 generations from the cross W8627 x PBW343 were also evaluated for percent KB infection against the mixture of isolates. The cultures were propagated using standard techniques. A piece of sporulating inoculum was placed on the top of the yeast potato dextrose agar (YPDA) slant in a test tube. The inoculations were done under aseptic conditions. The inoculated tubes were kept in upright position at 20±1°C so that the sporidia were showered all over the slant. Seven to ten days old cultures were used for inoculations. Equal numbers of well growing culture tubes of all isolates were used for preparation of inoculum. The inoculum was diluted in sterilized tap water. The concentration of inoculum was adjusted to 4-6 sporidia per microscopic field (10 x 10 magnification). This equaled 5 x 10^3-10^4 sporidia/ml of inoculum. An intensive inoculation scheme was undertaken to ensure that there was no disease escape. Inoculations were started from second week of February and continued till second week of March during the year 2010-2011. Inoculations were done as per the procedure standardized by Aujla et al. (1982). Using hypodermic syringe, 2-3 ml of inoculum suspension was put at the boot leaf stage. Two ears per plants were inoculated with 2 ml of sporidial culture. In case of parental lines and F_1s, two spikes each of randomly selected 10 plants were inoculated, while in case of F_2 and F_3, two spikes of every single plant were inoculated.

Percentage infection of F_1, P_1, P_2 and of individual plants in F_2 and F_3 was calculated by using the formula:

$$\frac{\text{Number of infected grains}}{\text{Total number of grains}} \times 100$$

The parents, F_1 and F_2 generation from the cross W8627 x PBW343 were grown in CCN infested field (sick plots). The plants were uprooted with sufficient soil around the roots and then cysts from the soil of the each plant were extracted following standard Cobb's decanting and sieving technique. The roots were washed free of soil, the cysts were collected on a 60 mesh sieve (pore size of 250 µm) and the final population was recorded, under the stereomicroscope. The responses of plants to the nematode were rated as highly resistant (HR) having 0.0, resistant (R) having 0.1-4, moderately resistant (MR) having 4.1-9, susceptible (S) having 9.1-20 and highly susceptible (HS) having >20 cysts per plant respectively as suggested by All India coordinated Wheat and Barley Improvement Project. Simple chi-square analysis was applied to test the fitness of genetic ratios in segregating populations, obtained from the cross between MDR stock x PBW343. Similarly chi-square test for co-segregation of different diseases (χ^2 for independence of traits) was applied to scrutinize the linkage between above mentioned diseases. The χ^2 value was calculated as per the standard procedure.

Results

Seedling reaction of P_1, P_2 and F_1 against stripe rust and leaf rust

The results explicitly indicated that PBW343 and F_1 are seedling susceptible while W8627 is resistant at seedling stage against race 78S84 and race 77-5, respectively (Table 1). This implicated that the resistance in the multi disease resistant (MDR) stock is controlled by seedling resistance gene (hypersensitive type of disease reaction) which imparted resistance at the adult plant stage also.

Nature and inheritance of resistance to stripe rust, leaf rust, cereal cyst nematode and Karnal bunt

To elucidate the nature of gene(s) imparting resistance to stripe rust, leaf rust, CCN and Karnal bunt, disease reaction of F_1 was keenly observed. The disease reaction of P_1, P_2 and F_1 is presented in Table 1.

The resistant parent W8627 showed disease free reaction against 78S84 race of stripe rust and 77-5 race of leaf rust at adult plant stage under field conditions. This was further confirmed that the resistance against stripe rust and leaf rust in MDR stock is hypersensitive type of resistance. The F_1 exhibited high stripe rust and leaf rust severity (60S) at adult plant stage under field conditions, which indicated that the nature of gene(s) imparting resistance against these two diseases is recessive. Similarly, susceptible reaction had been observed in F_1 against cereal cyst nematode and KB which further depicted that the resistance against the cereal cyst nematode and KB is also controlled by recessive gene(s).

Table 1. Disease reaction of P_1, P_2 and F_1 against different diseases at seedling and adult stages

Parents and F_1	Disease reaction			
	78S84 (Stripe rust)	77-5 (Leaf rust)	Karnal bunt (%)	Cereal cyst nematode (No. of cysts)
At seedling stage				
W 8627	0	0	-	-
PBW 343	3	3	-	-
F_1	3	3	-	-
At adult stage				
W8627	TR (R)	TR (R)	4 .00 (R)	4 (R)
PBW343	40S (S)	40S (S)	19.00(S)	7 (S)
F_1	60S (S)	60S (S)	28.00 (S)	10 (S)

R = Resistant; S = Susceptible

Inheritance of resistance against stripe rust, leaf rust, Karnal bunt and cereal cyst nematode

To examine the inheritance of resistance to various diseases, the segregation pattern for resistance and susceptibility in F_2 was studied and segregation pattern in F_3 progenies was also examined to confirm the results of F_2 generation as presented in Table 2.

Stripe rust

The F_2 generation contained 17 resistant and 178 susceptible plants against the race 78S84 of stripe rust depicting a good fit (p = 0.05) for expected ratio of 1(R):15(S) which indicated that the resistance is controlled by two complementary recessive genes while F_3 progenies segregated in a ratio of 7 homozygous resistant, 91 segregating and 7 homozygous susceptible. This observed segregating ratio gave a good fit (p = 0.05) for an expected ratio of 1(HR):14(S): 1(HS), confirming F_2 results. Thus, resistance in MDR stock against the stripe rust race 78S84 is controlled by complementary recessive genes.

Leaf rust

The disease reaction of 165 F_2 plants and 102 F_3 families against leaf rust race 77-5 was recorded. The F_2 generation consisted of 33 resistant and 142 susceptible plants indicating a good fit (p=0.05) for the expected ratio 1(R):3(S). The F_3 families segregated in a ratio of 19 homozygous resistant (HR), 50 segregating (Seg) and 33 homozygous susceptible (HS). This segregating ratio gave a good fit (p = 0.05) for an expected ratio of 1(HR):2 (Seg): 1(HS) and thus suggesting the involvement of a single recessive gene controlling resistance against the leaf rust race 77-5.

Karnal bunt

In case of Karnal bunt, the F_2 generation segregated into 118 susceptible and 47 resistant plants against a mixture of isolates of KB indicating a good fit (p = 0.05) for the expected ratio of 1(R):3(S). The F_3 generation consisting of 27 homozygous resistant, 50 segregating and 23 homozygous susceptible plant progenies. The observed frequency of segregating families fit well to an expected ratio of 1(HR):2 (Seg.): 1(HS) (p = 0.05), thus suggesting that the resistance is governed by a single recessive gene. The resistance is easy to handle in a breeding programme.

Table 2. Segregation pattern of F_2 progeny and F_3 families against stripe rust (78S84), leaf rust (77-5), cereal cyst nematode and Karnal bunt

Race or disease	Generation and segregation pattern										
	F_2					F_3					
	No. of plants			Postulated ratio	χ^{2*}	No. of plants			Total	Postulated ratio (HR: Seg: HS)	χ^{2*}
	R	S	Total	(R:S)		HR.	Seg	HS			
Stripe rust (78S84)	17	178	195	1:15	1.92	7	91	7	105	1:14:1	0.08
Leaf rust (77-5)	33	132	165	1:3	3.55	19	50	33	102	1:2:1	3.87
Karnal Bunt	47	118	165	1:3	0.80	27	50	23	100	1:2:1	0.32
Cereal Cyst Nematode	30	84	114	1:3	0.11						

*p = 0.05, Seg = Segregating, HS = Homozygous susceptible, HR = Homozygous resistant S = Susceptible, R = Resistant, HR = Homozygous recessive, HS = Homozygous susceptible

Cereal cyst nematode

The F_2 population of the cross W8627 x PBW343 segregated in a ratio of 30(R): 84(S) indicating a good fit (p = 0.05) for the expected ratio 1(R):3(S). This explicitly indicates that the resistance in MDR stock against the cereal cyst nematode is controlled by a single recessive gene.

To scrutinize the linkage between different diseases, co-segregation data of these diseases was recorded. The data for linkage detection is presented in Table 3. The χ^2 test for the independence of traits was carried out between stripe rust and leaf rust, stripe rust and CCN and between leaf rust and CCN. Tabulated χ^2 ($p \leq 0.05$) with 1 d.f. is 3.84 which is less than the calculated value of χ^2 in case of leaf rust and stripe rust; stripe rust and CCN, indicating association between them, while tabulated χ^2 ($p \leq 0.05$) with 1 d. f. is 3.84 which is more than the calculated value of χ^2 in case of leaf rust and CCN indicating no association between them.

Discussion

To examine the inheritance of resistance to various diseases, the segregation pattern of F_2 was studied and segregation pattern of F_3 progenies was examined to confirm the results of F_2 generation (Table 2).

The resistance in MDR stock against the stripe rust race 78S84 is controlled by complementary recessive genes. Newly evolved stripe rust race 78S84 which led to the breakdown of resistance gene *Yr9* in elite cultivar PBW 343 (Nayar et al. 1996) has led the plant breeders to search for new resistant sources. Many seedling resistance genes (*Yr5, Yr7, Yr10, Yr15* and *Yr17*) have been identified and found effective against the race 78S84. Gerenchter et al. (1989) identified a new dominant gene*Yr15* imparting resistance against the race 78S84 in *Triticum dicoccoides.* Inheritance studies carried out by Zhang et al. (2009) in two resistant stocks, *Triticum aestivum* sub sp. *spelta* var. *album* and Lee against stripe rust race, 78S84 showed presence of a single dominant gene*Yr5* and *Yr7*, respectively, responsible for resistance. A new hypersensitive gene had been identified in Australian cultivar Cook effective against the races, 46S119 and 78S84 by Khan et al. (2011). Most of the resistance genes imparting resistance against the race, 78S84 are of dominant nature while in present study the resistance is imparted by two recessive genes showing complementary epistasis. So, these two recessive genes could be diverse.

The resistance against the leaf rust race, 77-5 is controlled by a single recessive gene. Resistance genes imparting resistance against 77-5, a variant of leaf rust race 77, have been reported from various sources of wild relatives of wheat (Tomar and Menon 2001). Leaf rust resistance gene *Lr19* transferred from *Agropyron elongatum* into wheat (*Triticum aestivum* L.) imparts resistance to all the Indian pathotypes of leaf rust (Bhardwaj et al. 2005). Similarly, wheat-*Aegilops triuncialis* introgression T2BS·2BL-2L (0.95) carrying leaf rust resistance gene *Lr58* is a novel source of resistance against many isolates of the rust pathogen in US and India Kuraparthy et al. (2007). These two available resistance sources are dominant in nature, pointing to, that resistance gene in MDR stock against the isolate 77-5 is novel and diverse.

Short life span of the resistance genes has compelled the plant breeders to switch to “Gene pyramiding”. Novel genes against the prevalent stripe rust and leaf rust races are pre-requisite, to accomplish this task. So, there is need to designate the above identified genes. The multiple resistance may be exploited for the wheat improvement purpose.

The KB resistance in the W8627 is conferred by a single recessive gene, which is easy to handle in a breeding programme. Although, most of the studies have indicated partial dominance of KB resistance. It is controlled by 2-3 major genes with additive effects. Earlier, Bag et al. (1999) reported the mode of inheritance of Karnal bunt (*Neovossia indica*) resistance in some Indian bread wheat lines and cultivars, *viz*., HP1531, HD29 and W485 against Delhi isolates which is conditioned by a single recessive gene. However, this was not the case in the present study as the resistance against mixture of isolates of KB is also controlled by a single recessive gene.

Cereal cyst nematode

The resistance in MDR stock against the cereal cyst nematode is also controlled by a single recessive gene. Population of cereal cyst nematode has been increasing at the appreciable level, since 1990. It can be a potential menace in near future. Therefore, the efforts are being directed to explore resistance genes against it. Barley, durum, rye, triticale and wild grasses are the treasures of resistance to Karnal bunt, while very few resistant sources are available in bread wheat. All the available sources of resistance reported against cereal cyst nematode to date feature single-gene

Table 3. Chi-square test for co-segregation of different diseases

Disease and classes		Stripe rust			
		Resistant	Susceptible	Total plants	Total χ^2*
Leaf rust	Resistant	4 (2)	21 (23)	25	
	Susceptible	5 (7)	84 (82)	89	
	Total plants	9	105	114	
	Total χ^2				4.79
		Cereal cyst nematode			
		Resistant	Susceptible	Total plants	
Stripe rust	Resistant	5 (2)	4 (7)	9	
	Susceptible	25 (28)	80 (77)	105	
	Total plants	30	84	114	
	Total χ^2				12.22
		Cereal cyst nematode			
		Resistant	Susceptible	Total plants	
Leaf rust	Resistant	8 (7)	17 (18)	25	
	Susceptible	22 (23)	67 (66)	89	
	Total plants	30	84	114	
	Total χ^2				1.08

*p = 0.05

inheritance and most of these genes are dominant in nature. Similarly single-gene inheritance has been reported in Australian cultivars, Loros and AUS 10894, where single dominant gene, *Cre1* (formerly *Ccn1*) on chromosome 2BL is known to impart resistance against the cereal cyst nematode (Majnik et al. 2003). The same gene *Ccn1* was reported in cultivar Katyil. Likewise, a gene *Cre8* on chromosome 6BL in cultivar Festiguay is responsible for resistance against cereal cyst nematode (Williams et al. 2003). However, the gene imparting resistance in Festiguay is a single recessive gene, which could be a novel resistance source.

Co-segregation studies gave a superficial idea, that the resistance genes against leaf rust, stripe rust and CCN are present in form of gene cluster on a single chromosome in MDR stock. Similar gene cluster was identified by Tanguy et al. (2005) on chromosome 2AS of the wheat line VPM1. To make co-segregation studies more transparent, there is need to have more elaborated study on linkage relationships. Tight linkages between various resistance genes may be very beneficial, as it becomes possible to transfer multiple resistance against different diseases simultaneously to elite cultivars.

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