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Research Article

Inheritance of resistance to rice weevil (*Sitophilus oryzae* L.) in bread wheat (*Triticum aestivum* L.)

Ekhlaque Ahmad[1] and J.P. Jaiswal[1]*
[1]Department of Genetics and Plant Breeding,
G.B. Pant University of Agriculture and Technology, Pantnagar 263 145, India
***E-Mail**: jpj.gbpu@gmail.com

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Abstract

Losses of grain in storage due to insects are well-recognized problem. Infestation of store pest reduces quality of grain on one side and viability of seed on the other. Use of fumigants increases cost of the produce and makes environment unhealthy. Exploitation of available genetic variability for store pest resistance is very much essential to minimize the use of fumigants. Wheat, an important staple food, is also attacked by a number of store pest and rice weevil (*Sitophilus oryzae* L.) being the most destructive. In the present investigation, an attempt has been made to identify wheat genotypes possessing resistant to rice weevil. Crosses were attempted to generate F_1, F_2, BC_1 and BC_2 generations and genetics of resistance to rice weevil was studied. Test of allelism revealed that resistance to weevil infestation is controlled by two dominant genes and the genes interacted with each other in duplicate manner.

Key words
Bread wheat, rice weevil (*Sitophilus oryzae* L.), resistance, allelic relationship

Introduction

Use of fumigants is the most common method to save food grains from store pest. Therefore, development of crop varieties resistant to store pest could be one of the safest, most economic and environment friendly options. Insect-pests which feed mainly on crops cause 10 to 30% damage in both traded and elevator stored grains every year (Karunakaran *et al.*, 2004). It is estimated that 5-10% of world's grain production is lost due to ravages of insects. The losses may reach up to 50% in tropical countries where summer is hot and humid and storage facilities are improper and inadequate (Adams, 1998; Ahmad and Ahmad, 2002). Wheat, *Triticum aestivum* L. is the second most important cereal crop after rice in India. India is the second largest producer of wheat in world. It has harvested a record production of 97.44 million ton in acreage of 30.72 million hectares in 2016-17 (ICAR-IIWBR, 2017). During harvest season, wheat is procured by public and private agencies for storage and sale as a part of the produce. The wheat grain is retained by the farmers for their own consumption and for seed purpose. Insect infestations in grain cause quantity and quality losses and hence the crop value is lowered. The amount of damage in quality, quantity and health hazards due to insect infestation in storage when converted into monetary concerns may run into millions of rupees to national exchequer annually. Insects not only consume grain but also contaminate it with their metabolic by-products and body parts. Insects produce heat and moisture due to their metabolic activities that could lead to the development of hotspots and growth of microflora in grain. Heavily infested wheat is unfit for seed purposes and its products are unsuited for human consumption.

Wheat is heavily infested by a number of insect pests during storage, among these, rice weevil (*Sitophilus oryzae* L.); granary weevil (*Sitophilus granarius* L.); lesser grain borer (*Rhyzopertha dominicia* F.); Khapra beetle (*Trogoderma granarium* Everts); Angoumois grain moth (*Sitotroga cerealella* (Olivier) and red flour beetle (*Tribolium castaneum* Herbst.) are important (Khattak *et al.* 2000; Toews *et al.* 2000; Ebeling, 2002; Atwal and Dhaliwal, 2005; Shafique *et al.* 2006). The rice weevil is the most widespread and destructive insect-pest of stored cereals throughout the world. The name is misleading, because it may infest other grains besides rice. The rice weevil is a cosmopolite insect supposedly originated in India and spread all around the world through infested and ship-transported grains (Metcalf and Flint, 1962). It is a small reddish-brown beetle, about 1/8 inches in length, with four light yellowish spots on the back. Adults fly. Adults live 4 to 5 months, and females lay about 300 to 400 eggs in holes bored in kernels of grain. Larvae hatch inside the kernel and mature there. The period from egg to adult may be as short as 26 days. Fumigants are heavily used to save the wheat seed/grain during the storage. Concerns have been raised by farmers, regulators, and the general public about the possible increase in fumigant usage, because heavy use of fumigants is not only hazardous to human health but also pollutes environment, which is a loss in long term.

The use of resistant varieties of wheat against storage insect pests is a major control measure, a good alternative which is environment friendly and causes no cost to farmers. Wheat varieties behave differently to stored grain pests, resulting in the increase of research to develop grain varieties resistant to stored grain insects (Seifelnasr and Mills, 1985). Due to increasing threat of insect-pests in storage particularly rice weevil, there is a need for understanding the various dimensions of its resistance to breed the resistant genotypes. However, the major hindrance in utilizing the resistance against the pest is that resistant source is scanty. Also the nature of resistance is still not very clear because there are negligible studies on the inheritance of weevil resistance. Therefore, the success of the breeding programme lies in the identification of source of resistant genes and sound knowledge of genetic behaviour of the resistant genes. Keeping the usefulness of resistant genes in view, efforts have been made to establish the gene identity for rice weevil resistance in the present investigation.

Materials and Methods

The experimental material comprised of six bread wheat genotypes, 3 susceptible viz., PBN 51, K 76 and K 77 and 3 resistant viz., K 20, K 21 and K 50 to *Sitophilus oryzae* L. and their F_{1s}, F_{2s} and backcrosses (BC_{1s} and BC_{2s}). The pedigree information of wheat genotypes used in the study is given in Table 1. The F_{1s} were made by crossing the all six parents in half diallel fashion. All the F_{1s} were advanced to backcrosses BC_{1s} and BC_{2s} and fresh F_{1s} were also generated. The six generations including parents (P_1 and P_2), F_{1s}, $F_{2s,}$ BC_{1s} and BC_{2s} were evaluated and used to study the genetics of resistance to rice weevil. Standard package of practices was followed to raise healthy crop in the field.

Weevil culture: The experiment was conducted for screening of resistance in wheat genotypes/lines against *Sitophilus oryzae* under laboratory conditions. The experiment was performed in incubator at 27±1°C temperature and 70 ± 5 % relative humidity. Twenty seeds from each genotype of P_1, P_2 and $F_{1,}$ 60 seeds of F_2 and 40 seeds from each genotype of BC_1 and BC_2 were filled in plastic vials of 5 x 2.5 cm size and three pairs of unsexed *S. oryzae* aged 0 to 7 day old were released in each vials, then vials were closed with perforated cap and each genotype was replicated three times. The whole experiment was conducted in an incubator for their progeny development. After one month the total number of insects developed from each vials was counted. Fig. 1a, 1b and 1c depicts the susceptible parents and Fig. 1d, 1e and 1f depicts the resistant parents.

In the present study, parents (P_1 and P_2), F_1, F_2 and back cross generations of different crosses were scored according to Dobie (1974) and modified by Gudrups *et al.* (2001), Dhliwayo *et al.* (2003) and Derera *et al.* (2010) with the score of 0-3.0 as resistant and 3.1-6.0 as moderate resistant while those with a score of 6.1-8.0, 8.1-10.0 and >10.0 were considered as moderately susceptible, susceptible and high susceptible respectively. The information on the allelic relationship obtained from the present investigation is based on Chi-square test with Yates correction for continuity proposed by Yates (Panse and Sukhatme, 1957).

Results and Discussion

Figures 2a to 2f show the performance of F_{1S} in resistant x resistant and susceptible x susceptible crosses. The results obtained with weevil infestation test on parental lines, F_1, $F_{2,}$ BC_1 and BC_2 generations are presented in Table 2, 3 and 4. The inheritance pattern of weevil resistance in different susceptible x resistance crosses was observed under controlled experimental conditions. The genetic analysis on resistance to rice weevil (*Sitophilus oryzae* L.) was carried out in nine susceptible x resistant crosses involving three each susceptible wheat genotypes viz., PBN 51, K 76 and K 77, and resistant genotypes, K 20, K 50 and K 51 (Table 2). F_{1s} in all the nine crosses were observed possessing resistance to weevil infestation. In case of F_2 population of five crosses *viz.*, PBN 51/K 20, PBN 51/K 21, PBN 51/K 50, K 76/K 20 and K 77/K 20 the exhibited frequency of resistance and susceptible seeds was 49 and 11, 47 and 13, 50 and 10, 47 and 13, 48 and 12, respectively. This provides a good fit to the expected 13R:3S ratio revealing dominant recessive interaction. In BC_1 the expected ratio in all the above mentioned crosses was 1R:1S. The above results suggested that resistance to weevil is governed by two genes with dominant and recessive interaction. In case of F_2 population of cross K 76/K 21 the expected frequency of resistance and susceptible out of 60 seeds, 54 were resistant and 6 were susceptible. This provides a good fit to the expected ratio 15R:1S (dominant recessive interaction) (χ^2 =1.099, P=0.20-0.30). In BC_1 generation of this cross, out of 40 seeds screened, 30 appeared resistance and remaining 10 were susceptible, indicating the expected ratio 3R:1S (χ^2 =0.423, P=0.30-0.50). The results obtained from F_1, F_2 and backcross generations revealed the digenic nature for resistance with duplicate dominant interaction. However, in case of three crosses, namely, K 76/K 50, K 77/K 21 and K 77/K 50 the expected frequency of resistance and susceptible seeds out of 60 seeds in each F_2 population was 45 and 15 which reveals good fit to the expected 3R:1S ratio revealing the monogenic nature of resistance. Further segregation in BC_1 progenies fitting well to the expected ratio 1R:1S in all the above three crosses.

Three resistant x resistant crosses viz., K 20/K 21, K 20/K 50 and K 21/K 50 were studied for finding out the allelic relationship between the parents and segregating generations (Table 3). In case of cross K 20/K 21, all the 20 seeds evaluated in the F_1 generation were resistant. In F_2 out of 60 seeds, 55 showed resistant reaction and the rest 5 seeds exhibited susceptibility reaction. This provides a good fit to the expected 15R:1S ratio (χ^2 =0.268, P=0.50-0.70). In BC_1 generation, 37 seeds were found resistant. However, in BC_2 generation 38 out of 40 seeds screened were resistant and the rest were susceptible. Thus results suggested that the two resistant parents, K 20 and K 21 have different genes conditioning resistance to weevil infestation. In case of cross K 20/K 50, 19 seeds out of 20 screened for weevil resistance in the F_1 generation, 19 showed resistant reaction (Table 3). In F_2 out of 60 seeds evaluated, 54 were resistant and 6 seeds were susceptible. This mode of segregation fitted well to the expected ratio 15R:1S ratio (χ^2 =0.073, P=0.70-0.80). In BC_1 generation, 37 seeds showed resistance reaction, whereas out of 40 seeds screened in BC_2 generation 38 appeared resistant. Thus results obtained from this cross suggested that the two resistant parents have different genes conditioning resistance to weevil infestation. In case of cross K 21/K 50, in F_1 generation, out of 20 seeds 19 exhibited resistant reactions and remaining one seed was susceptible (Table 3). The F_2 population comprised of 60 seeds, out of which 53 were resistant and remaining 7 susceptible. The segregation pattern observed in F_2 generation provides a good fit to the expected ratio 15R:1S ratio (χ^2 =0.574, P=0.30-0.50). In BC_1 generation, 36 seeds showed resistance reaction. On the other hand in BC_2 generation, 36 seeds out of 40 exhibited resistant and whereas 4 were susceptible. Thus results obtained from different generations of the cross K 21/K 50 indicate that both of resistant parents differed with respect to genes conditioning the resistance. The segregation frequency in F_2 populations of all the three crosses, K 20/K 21, K 20/K 50 and K 21/K 50 had good fit to the expected ratio of 15R:1S. The appearance of susceptible seeds and segregation in F_2 into 15R:1S ratio confirmed that the two dominant genes are governing resistance and the resistant parents have dissimilar gene constellation. Thus showing non-allelic interaction among each other.

In F_1 of the cross PBN 51/K 76, all the 20 seeds observed were susceptible to rice weevil. The F_2 population comprises of 60 seeds, out of which 53 seeds were susceptible and 7 resistant. The segregation pattern observed provided a good fit to the expected ratio of 15S:1R ratio (χ^2 =0.742, P=0.30-0.50). In BC_1 generation, 39 seeds were susceptible out of 40 screened, however out of 40 seeds screened in BC_2 generation, 35 were susceptible and 5 seeds were resistant. This shows that these two susceptible parents differed from each other with respect to genes for susceptibility. In case of cross PBN 51/K 77 out of 20 seeds evaluated in the F_1 generation, 17seeds (85%) showed susceptible reaction while 3 seeds (15%) were resistant. The F_2 population consisted of 60 seeds, out of which 38 seeds were susceptible and remaining were resistant. This provides a good fit to the expected ratio of 7S:9R ratio (χ^2 =0.746, P=0.30-0.50). Out of 40 seeds screened in $BC_{1,}$ 21 exhibited susceptible reaction whereas 19 were resistant. This provides a goodness of fit to the expected ratio 1R:1S (χ^2 =0.357, P=0.50-0.70). In BC_2 generation, out of 40, 39 seeds were susceptible to weevil infestation. The results revealed that PBN 51 and K 77 have dissimilar genes conditioning susceptible reaction. In the F_1 generation of 3rd cross K 76/K 77, out of 20 seeds screened, 19 exhibited susceptible reaction. The F_2 population consisted of 60 seeds in which 37 were susceptible and the remaining 23 were resistant. This provided a good fit to the expected ratio of 7S: 9R ratio (χ^2 =0.107, P=0.70-0.80). In BC_1 generation, 38 seeds out of 40 showed susceptible reaction. However, out of 40 seeds evaluated in BC_2 generation, 36 were susceptible and 4 seeds appeared resistant (Table 4). Findings revealed that PBN 51 and K 77 have dissimilar genes conditioning susceptible reaction. The observation revealed that K 76 and K 77 have dissimilar genes conditioning susceptible reaction.

The available literature on inheritance for resistance to rice weevil in wheat is very meager. The findings of present investigation are in partial agreement with Singh and Bhatia (1978) who reported that in the resistant x resistant crosses all the seeds in F_1, reciprocal F_1 and back crosses were almost free from weevil infestation, however in F_2 generation seed showed weevil infestation in these crosses, which was indication of the presence of non allelic interaction among them. Dissimilar genes for resistance in the parental lines have also been suggested, on the basis of appearance of susceptible plants in F_{2s} of resistant x resistant crosses. Some aspects on allelic relation in Russian Wheat Aphid (RWA) has been reported by Asad and Dorry (2001) and Estakhr and Assad (2002) who observed two different genes conditioning resistance to RWA in two wheat resistant lines and segregated independently. In present study, susceptible seeds were obtained in BC_1 also which further supported the non-identical nature of the gene(s) conditioning resistant to rice weevil.

From the breeders' perspective, this study opens up the choice of diverse materials for utilization in wheat improvement for rice weevil resistance**.** It is expected that the finding of the present study would be useful as all the resistant parents can provide alternative source of resistance, which may be exploited in breeding programme by utilizing them in various combination for developing weevil resistant variety of wheat.

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Table 1. Parentage of resistant and susceptible genotypes used in the study

Genotypes	**Parentage**	**Reaction to *Sitophilus oryzae* L.**
PBN 51	BUC'S'/FLK'S'	Susceptible
K 76	VL 616/HD 2857	Susceptible
K 77	UP2467/HD 2590/UP 2556	Susceptible
K 20	HD 2857/UP 2556	Resistant
K 21	HPW 89/PBW 343	Resistant
K 50	K 9107/GW 9909	Resistant

Table 2. Segregation patterns for reaction to weevil infestation in different generations of susceptible x resistance crosses

Parent/Cross/ Generation	Observed Frequencies		Expected Frequencies		Expected segregation ratio (R : S)	χ^2 Value	P value
	R	S	R	S			
Cross (PBN 51/K 20)							
P_1 (PBN 51)	0	20	-	All	-	-	-
P_2 (K 20)	20	-	All	-	-	-	-
F_1(PBN 51/ K 20)	20	-	All	-	-	-	-
F_2	49	11	48	12	13:3	1.270	0.20-0.30
BC_1(PBN 51/ K 20//PBN 51)	21	19	20	20	1:1	0.216	0.50-0.70
BC_2 (PBN 51/ K 20//K 20)	37	3	All	-	-	-	-
Cross (PBN 51/K 21)							
P_1 (PBN 51)	-	20	-	All	-	-	-
P_2 (K 21)	18	2	All	-	-	-	-
F_1(PBN 51/ K 21)	19	1	All	-	-	-	-
F_2	47	13	48	12	13:3	2.620	0.05-0.10
BC_1(PBN 51/K 21//PBN 51)	22	18	20	20	1:1	0.670	0.30-0.50
BC_2 (PBN 51/K 21//K 21)	36	4	All	-	-	-	-
Cross (PBN 51/K 50)							
P_1 (PBN 51)	-	20	-	All	-	-	-
P_2 (K 50)	18	2	All	-	-	-	-
F_1 (PBN 51/ K 50)	19	1	All	-	-	-	-
F_2	50	10	48	12	13:3	1.656	0.10-0.20
BC_1(PBN 51 /K 50//PBN 51)	21	19	20	20	1:1	0.545	0.30-0.50
BC_2 (PBN 51 /K 50//K50)	35	5	All	-	-	-	-
Cross (K 76/K 20)							
P_1 (K 76)	1	19	-	All	-	-	-
P_2 (K 20)	20	0	All	-	-	-	-
F_1(K 76/K 20)	19	1	All	-	-	-	-
F_2	47	13	48	12	13:3	1.024	0.20-0.30
BC_1(K 76/K 20//K76)	23	17	20	20	1:1	0.419	0.30-0.50
BC_2(K 76/K20//K20)	36	4	All	-	-	-	-
Cross (K 76/K 21)							
P_1 (K 76)	1	19	-	All	-	-	-
P_2 (K 21)	19	1	All	-	-	-	-
F_1 (K 76/K 21)	19	1	All	-	-	-	-
F_2	54	6	54	6	15:1	1.099	0.20-0.30
BC_1 (K 76/K 21//K76)	30	10	30	10	3:1	0.423	0.30-0.50

BC$_2$ (K 76/K 21//K21)	35	5	All	-	-	-	-
Cross (K 76/K 50)							
P$_1$ (K 76)	1	19	-	All	-	-	-
P$_2$ (K 50)	18	2	All	-	-	-	-
F$_1$(K 76/K 50)	19	1	All	-	-	-	-
F$_2$	45	15	45	15	3:1	1.242	0.20-0.30
BC$_1$ (K 76/K 50//K 76)	24	16	20	20	1:1	0.598	0.30-050
BC$_2$(K 76/K 50//K 50)	34	6	All	-	-	-	-
Cross (K 77/K 20)							
P$_1$ (K 77)	1	19	-	All	-	-	-
P$_2$ (K 20)	20	0	All	-	-	-	-
F$_1$ (K 77/K 20)	19	1	All	-	-	-	-
F$_2$	48	12	48	12	13:3	1.636	0.20-0.30
BC$_1$ (K 77/K 20//K 77)	21	19	20	20	1:1	0.428	0.30-0.50
BC$_2$ (K 77/K 20//K 20)	35	5	All	-	-	-	-
Cross (K 77/K 21)							
P$_1$ (K 77)	1	19	-	All	-	-	-
P$_2$ (K 21)	19	1	All	-	-	-	-
F$_1$ (K 77/K 21)	19	1	All	-	-	-	-
F$_2$	46	14	45	15	3:1	0.450	0.50-0.700
BC$_1$ (K 77/K 21//K 77)	23	17	20	20	1:1	0.427	0.50-0.70
BC$_2$ (K 77/K 21//K 21)	35	5	All	-	-	-	-
Cross (K 77/K 50)							
P$_1$ (K 77)	1	19	-	All	-	-	-
P$_2$ (K 50)	18	2	All	-	-	-	-
F$_1$ (K 77/K 50)	19	1	All	-	-	-	-
F$_2$	46	14	45	15	3:1	2.052	0.10-.020
BC$_1$ (K 77/K 50//K77)	22	18	20	20	1:1	0.634	0.30-0.50
BC$_2$ (K 77/K 50//K 50)	33	7	All	-	-	-	-

R: Resistant, S: Susceptible

Table 3. Segregation patterns for reaction to weevil infestation in different generations of resistance x resistance crosses

Parent/Cross/ Generation	Observed Frequencies		Expected Frequencies		Expected segregation ratio (R : S)	χ^2 Value	P value
	R	S	R	S			
Cross (K 20/K 21)							
P_1 (K 20)	20	0	All	-	-	-	-
P_2 (K 21)	19	1	All	-	-	-	-
F_1 (K 20/K 21)	20	0	All	-	-	-	-
F_2	55	5	54	6	15:1	0.268	0.50-0.70
BC_1 (K 20/K21//K 20)	37	3	All	-	-	-	-
BC_2 (K 20/K 21//K 21)	38	2	All	-	-	-	-
Cross (K 20/K 50)							
P_1 (K 20)	20	0	All	-	-	-	-
P_2 (K 50)	18	2	All	-	-	-	-
F_1 (K 20/K 50)	19	1	All	-	-	-	-
F_2	54	6	54	6	15:1	0.073	0.70-0.80
BC_1 (K 20/K50//K 20)	37	3	All	-	-	-	-
BC_2 (K 20/K 50//K 50)	38	2	All	-	-	-	-
Cross (K 21/K 50)							
P_1 (K 21)	19	1	All	-	-	-	-
P_2 (K 50)	18	2	All	-	-	-	-
F_1 (K 21/K 50)	19	1	All	-	-	-	-
F_2	53	7	54	6	15:1	0.574	0.30-0.50
BC_1 (K 21/K50//K 21)	36	4	All	-	-	-	-
BC_2 (K 21/K 50//K 50)	37	3	All	-	-	-	-

R: Resistant, S: Susceptible

Table 4. Segregation patterns for reaction to weevil infestation in different generations of susceptible x susceptible crosses

Parent/Cross/ Generation	Observed Frequencies		Expected Frequencies		Expected segregation ratio (R : S)	χ^2 Value	P value
	R	S	R	S			
Cross (PBN 51/K 76)							
P_1 (PBN 51)	0	20	-	All	-	-	-
P_2 (K 76)	1	19	-	All	-	-	-
F_1(PBN 51/ K 76)	0	20	-	All	-	-	-
F_2	7	53	6	54	1:15	0.740	0.30-0.50
BC1(PBN 51/ K 76//PBN 51)	1	39	-	All	-	-	-
BC_2 (PBN 51/ K 76//K 76)	5	35	-	All	-	-	-
Cross (PBN 51/K 77)							
P_1 (PBN 51)	0	20	-	All	-	-	-
P_2 (K 77)	1	19	-	All	-	-	-
F_1(PBN 51/ K 77)	3	17	-	All	-	-	-
F_2	22	38	26	34	7:9	0.746	0.30-0.50
BC_1(PBN 51/ K 77//PBN 51)	19	21	20	20	1:1	-	-
BC_2 (PBN 51/ K 77//K 77)	1	39	-	All	-	-	-
Cross (K 76/K77)							
P_1 (K 76)	0	20	-	All	-	-	-
P_2 (K 77)	1	19	-	All	-	-	-
F_1(K 76/K77)	1	19	-	All	-	-	-
F_2	23	37	26	34	7:9	0.107	0.70-0.80
BC_1(K 76/K77 //K 76)	2	38	-	All	-	-	-
BC_2 (K 76/K77 //K 77)	4	36	-	All	-	-	-

R: Resistant, S: Susceptible

Fig. 1a. Parent 1: PBN 51 (Susceptible)

Fig. 1b. Parent 2: K 76 (Susceptible)

Fig. 1c. Parent 3: K 77 (Susceptible)

Fig. 1d. Parent 4: K 20 (Resistant)

Fig. 1e. Parent 5: K 21 (Resistant)

Fig. 1f. Parent 6: K 50 (Resistant)

Fig. 1 a to 1f : Response of parental genotypes under controlled condition