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GERMPLASM SCREENING AND INCORPORATION OF APHID RESISTANCE IN BREAD WHEAT (*Triticum aestivum* L.)

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ABSTRACT

The aphid infestation results in considerable crop losses in many crops. Aphids are emerging as potential insect pest to wheat crop and it might become a threat to the future wheat crop productivity in Pakistan. To cope with this serious problem, a large wheat germplasm comprising of 464 accessions of bread wheat were screened against aphids based on aphid population density during 2009-2010 in the experimental areas of Wheat Research Institute, Faisalabad. Out of 464 accessions, 71 showed an immune response having aphid population range 1-2. Similarly, 87 varieties/lines were found resistant having 3-4 aphids per tiller throughout the season. A large proportion of wheat accessions (127 accessions) supported 5-6 aphids per tiller on an average and was graded as moderately resistant, while 141 accessions were found tolerant. Small number of genotypes were tending towards susceptibility (17 were found moderately susceptible, 16 were found susceptible and 5 were found highly susceptible by showing aphid population range 7-8, 9-10, 11-15 and 16-20 respectively). Aphid resistance was successfully incorporated from aphid resistant lines into high yielding accessions through crossing followed by selection in segregating generations. Twenty crosses were made between aphid resistant and high yielding lines. Aphid population per tiller and grains yield per plant was assessed in F_6 generation. The crosses from two lines viz. Faisalabad 08 and BAVICORA 92 showed the highest grain yield and aphid resistance while that of V-07200 with KAL/BB showed least grain yield and high susceptibility. The results concluded that most of the germplasm accessions are resistant to tolerant against aphids and this resistance could be incorporated into the future cultivars.

Key words: Bread wheat, aphid population per tiller, aphid resistance, screening.

INTRODUCTION

The production of wheat can be increased either by bringing more area under cultivation or by increasing the yield per unit area. Currently, it is not possible to increase the area under wheat due to other competing crops (i.e adaption of BT cotton, rabi vegetables and fodder crops), less supply of irrigation water and increasing occupation of fertile land by the expanding population and industries. Therefore, the only way for increasing wheat production in the country is obtaining higher yield per unit area (Anwar *et al*. 2009). The per unit area production in Pakistan is less than other countries due to many reasons i.e., delayed planting, shortage of water, improper management, diseases and insect attack. There are number of insects which attack on wheat crop such as termites, cutworm, wheat weevil, Jassid, aphid, armyworm and *heliothis.* Among these insect pests, aphids are becoming a threat to wheat crop by causing direct or indirect losses in Pakistan.

Aphids of cereal crops are a scary threat to wheat crop. Although Aphid populations stay on wheat for a short, distinct time period but they have explosive multiplication rate and have potential to ruin the crop in few days (Jarosik *et al*. 2003; Wains *et al*. 2010a, b). Aphids are sucking insects which mainly stay on the spikes of the wheat which are directly involved in the crop yield and cause substantial yield losses by the direct effect of their feeding and as a vector of several plant viruses (Kieckhefer and Kantack 1980; Wains *et al*. 2010a). Figure 1 gives a brief view of aphid attack on wheat spikes. The spike in Figure 1A is at initial stage of infestation while B is at the last stage of infestation. The spikelets in case of Figure 1B are really not feeling well, tending towards yellowish, dull green colour as compared to Figure 1A. At the population of 15 aphids per plant, 30 to 40 percent grain yield losses have been reported by Kieckhefer and Gellner (1992).

There are different aphid species like *Schizaphis graminum* Rond., *Sitobian avenae* (F), *Rhopalosiphum rufiabdominalis* (Sasaki) and *Rhopalosiphum padi* prevailing on wheat crop in Pakistan. However, the species *Schizaphis graminum* (green bug) is found to be the most dangerous for wheat crop in the country because it affects the spikes which are direct bearer of the grains (Figure 1). The other important species is *Rhopalosiphum padi* which is usually found on wheat leaves and stems.

Different types of control measures could be employed to inhibit the aphid population below the economic threshold level. For instance, the use of chemical control is more prevalent in the country which has an enormous environmental as well as human health issues. The fluctuation in sowing date (early sowing) can be supportive for controlling aphids on wheat (Acreman and Dixon, 1985; Aslam *et al*. 2005). More recently, the use of a homeopathic medicine, 'Rigorous' has also been reported that could significantly inhibit the aphid population (Wains *et al*. 2010b). Similarly, it is found that the a toxin "indole alkaloid (i.e. Gramine)" is found to be responsible for the resistance of barley cultivars to *Schizaphis graminum* and *Rhopalosiphum padi* (Zuñiga *et al*., 1985). The other strategy to control aphid in wheat could be the development of host plant resistance through a planned plant breeding program.

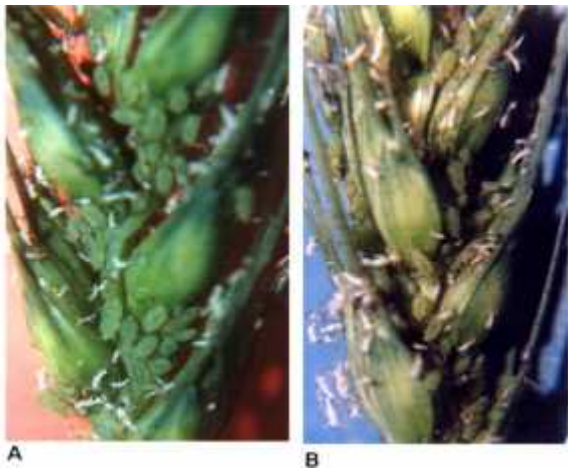

Figure 1: Green bug (*Schizaphis graminum* Rond.) attacking on wheat spikes

Among these various control strategies; host plant resistance is of eminent importance with the help of which aphid infestation could be kept below threshold level. This could also be helpful to minimize the chances of biotypes development in aphids (Lowe 1987; Riazuddin *et al*. 2004). Instead of the production of resistant varieties, moderately resistant varieties against wheat aphid have been identified because such resistance has proved easy to locate among various wheat breeding lines (Lowe, 1987). Migui and Lamb (2003) tested 41 accessions of wild and cultivated wheat belonging to 19 Triticum species in the field for resistance to three species of aphids *Rhopalisiphum padi*, *Sitobion avenae* and *Schizaphis graminum Rondani*. They reported that all three species of aphids survived and reproduced on all wheats, and reduced spike biomass compared to uninfested controls. Overall resistance to the three aphid species was observed in five to seven accessions per aphid species. Similarly, Singh *et al.* (2001) conducted a screening trial on 38 wheat strains and reported the least infestation in VL-616 and maximum in Hindi-62. Screening of wheat germplasm against aphids has been carried out in the country on small scale (Ahmad and Nasir, 2001; Ashfaq *et al*. 2007; Iqbal *et al*. 2008). However, large scale screening of wheat germplasm is necessary to select promising aphid resistant and high yielding genotypes and to manipulate them in the breeding program aimed at durable aphid resistance in wheat. The objective of this research was to screen a range of resistance in wheat from a large collection of germplasm accessions followed by the launching of a full fledge breeding program for the incorporation of aphid resistance in wheat.

MATERIALS AND METHODS

Crop husbandry: This research was carried out at Wheat Research Institute (WRI), Ayub Agricultural Research Institute (AARI), Faisalabad (31°24´ N and 73°02´ E) during the period 2009 – 2010. A total of 464 germplasm accessions of wheat were sown in December, 2009 in a Randomized Complete Block Design with two replications. The cropping scheme was wheat-fallow-wheat for the experimental field. The land was prepared by two deep ploughings followed by plankings. The fertilizer rate used for the experimental plots was kept in the ratio 120:90:0 Kg of N: P: K per hectare, respectively. First irrigation was applied to the crop after 35 days and subsequent irrigations were applied at the start of flowering, anthesis and grain filling stages. The weeds were controlled chemically (Bactril SUPER).

Screening of wheat germplasm for aphid resistance: Aphid population was counted from 10 randomly selected tillers of each line at seven days interval from mid February to mid March, 2010. The data on alate (winged) aphids (*Rhopalosiphum* and *Schizaphis* species) trapped in trays were recorded daily from 9–11 a.m. A daily record of alate aphids trapped was maintained and later on transformed into weekly basis. A criterion for aphid resistance was developed based on the number of aphids developing on per tiller of wheat. The evaluation of these varieties/lines was done on the basis of criteria given in Table-1. The data were analyzed statistically.

Table-1. Criteria for grading the aphid resistance in wheat.

Aphid pop. Range	Response of accessions
1-2	Immune
3-4	Resistant
5-6	moderately resistant
7-8	Tolerant
9-10	moderately susceptible
11-15	Susceptible
16-20	highly susceptible

Incorporation of aphid resistance to elite wheat lines through shuttle breeding: The incorporation of aphid resistance to elite wheat lines was done by adopting the process of crossing between aphid tolerant/resistant varieties/lines with high yielder wheat varieties/lines and selection of high yielding plus aphid resistant plants in the subsequent generations. For this purpose wheat germplasm were screened against aphid and crossed with high yielder varieties/lines. During 2009-10, 32 crosses were developed (Aphid tolerant vs high yielder), out of these twenty (20) selected crosses (Table-2) were sown at Kaghan to raise F_1 generation through shuttle breeding. "Shuttle breeding" is a technique for growing a crop which involves growing two successive plantings in one year to make the breeding process faster. For instance, one planting in the summer season in a location where growing conditions are favorable (i.e. Kaghan) and second planting in another location during the winter season where winter conditions are favorable to plant growth (i.e. WRI, Faisalabad).

Table-2. Crossing plan for incorporation of aphid resistance in wheat.

Female (Aphid tolerant)	Male (high yielding)	
Acc. #1 AS-2002	Acc. #171,	FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ
	Acc. #86	Ketanna
Acc. #11	SR-3	
Faisalabad 2008	Lu-26	
Acc. #11	Acc. # 98	PAVON-76
Faisalabad 2008	Acc. #174	WBLL1*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ
	Acc. #245	SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ
Acc. #61	Acc. #11	Faisalabad 2008
BAVIACOR M 92	Acc. #29	Naeem 82
	Acc. #70	GALVEZ
Acc.#264	Acc. #11	Faisalabad 2008
FRET-2	Acc. #186	CHIBIA//PRL /CM65531/3/FISCAL
Acc. #440	Acc. #299	KAL/BB CM26992
V-07200	Acc. #303	MRNG/BUCS//BLO/PSNS
Acc. #15	Acc. #42,	SEHER -06
GA-2002	Acc. #50	V-87094
	Acc. #51	SINDH-81
Acc.#81	Acc. #22	LASANI-08
HARTOG=	Acc. #45	SHALIMAR-88
HTG.(PAVON)	Acc. #409	AARI-11

The seed of F_1 generation raised at Kaghan were sown at Faisalabad as F_2 generation and filial generations were advanced up to F_6 generations keeping in view the objectives of crosses. The odd generations (F_1, F_3 and F_5) were grown at Kaghan through shuttle breeding while the even generations like F_2, F_4 and F_6 were grown at WRI, Faisalabad. The yield (yield per plant in grams) and aphid data (aphid population per tiller) was recorded only in the even generations up to F_6. In total, 187 out of 400 single head rows of twenty crosses in F_6 generation were sown at Faisalabad and were sent for preliminary yield trials, regular yield trials and were included in the varietal development program in future. During the process of development of filial generations, the resulting genotypes were characterized repeatedly for aphid tolerance and grain yield at WRI, Faisalabad.

RESULTS AND DISCUSSION

Germplasm screening against aphid attack: The germplasm consisting of 464 accessions resulted in lot of variability for aphid resistance. The full germplasm data containing aphid population per tiller and grain yield per ten tillers is given in Table-3. The germplasm lines were grouped into 11 different groups based on different characters given as names of the groups in Table-3 along with average aphid population and grain yield. Based on the scaling of aphid tolerance given in Table-1, a wide range of different groups of genotypes were found ranging from immune to susceptible which are shown in Table-4. Aphid population was recorded on 464 germplasm accessions for 4 weeks starting from 3rd week of February, 2010 until 2nd week of March. Aphid population ranging from 1-2 aphids per tiller was recorded on 71 accessions which show response as immune. Similarly, 87 accessions were found resistant having 3-4 aphids per tiller throughout the season. 127

accessions supported 5-6 aphids per tiller on an average and showing moderately resistant response. A great proportion of germplasm i.e. 141 accessions were found tolerant because they carried 7-8 aphids per tiller. Moreover, 17 were found moderately susceptible, 16 were found susceptible and 5 were found highly susceptible by showing aphid population range 9-10, 11-15 and 16-20 respectively. The recent cultivars like Faisalabad 08, Lasani-08, Seher-06, Shafaq-06, AARI-11, Punjab-11, Millat-11, V-08173 and V-07096 etc. ranged between resistant to tolerant behavior against aphids along with high yield. Mainly the accessions in the germplasm belonging to the groups which were classified as resistant against rusts, septoria leaf blotch and hessain fly were showed high degree of resistant against aphids. This might be due to the accumulation of resistance genes, that code for the proteins which are not preferred by the aphids and Hessian fly. This was the first time that a huge germplasm of wheat was subjected to screening against aphid attack. Early on, screening of wheat germplasm against aphids has been carried out in the country but only on a small scale (Ahmad and Nasir 2001; Ashfaq *et al*. 2007; Iqbal *et al*. 2008).

Table-3. The germplasm accessions in different groups and average aphid population and grain yield

Group No.	Group name	No. of accessions	Av. Aphid pop./ tiller	Av. Yield /10 tillers
G-1	National Source and Approved Varieties	127	4.72	31.44
G-2	International Source	138	3.58	26.91
G-2A	International Source: Septoria Leaf Blotch and Rust (Yr,Lr,Sr) Resistant Lines	4	3.85	33.75
G-2B	International Source: Hessain Fly Resistant Lines	26	3.60	29.27
G-3	Long Heads	5	3.36	29.4
G-4	More No. of Grains / Spike	3	3.73	36.33
G-5	High Grain Weight	19	3.44	28.52
G-6	High Tillering	8	3.5	27.5
G-7	Early Maturity	3	8.63	31.33
G-8	Resistant to Different Disease	24	3.42	30.34
G-9	High Protein	8	5.3	35.71
G-10	Potential Donors	7	3.84	30.28
G-11	Advance Lines Developed at WRI, Faisalabad	92	4.05	34.88
Total		**464**		

The aphid population was relatively less in the year of screening which might be due to harsh environmental factors, as it is already known that the aphid dynamics are significantly affected by the temperature and humidity (Ashfaq *et al*. 2007; Aheer *et al*. 2008; Wains *et al*. 2010a). As far as the aphid dynamics is concerned in the screening year, the aphid population increased gradually from 19th January to 19th February every year (data not shown) and afterwards decreased because wheat was producing tillers in its early stage and aphid does not reproduce rapidly on the early growth stages of wheat (Kieckhefer and Gellner, 1998; Ahmad and Nasir, 2001). This might be due to the low quality of food (sap) available in the early stages of the wheat. Changes in quality and quantity of the food occur with life of the plant and its growth stages, which ultimately affect the survival, longevity, distribution, reproduction and speed of development of insects (Yazdani and Agarwal, 1997).

Table-4. Screening of Wheat Varieties/lines against Aphids.

Aphid population Range	Response of different varieties/lines against aphids recorded at weekly interval				Means
	18-02-2010	25-02-2010	04-03-2010	11-03-2010	
1-2	69	73	80	61	71
3-4	88	84	77	96	87
5-6	127	115	127	137	127
7-8	140	150	144	130	141
9-10	18	190	15	18	17
11-15	15	18	17	15	16
16-20	7	5	4	7	5

The germplasm accessions were divided into 11 different groups based on the origin, resistance to different diseases and insects, various quality characters and yield traits. On an average the accessions belonging to national sources showed tolerant to resistant behavior against natural incidence of aphid attack. Similarly, the accessions which were classified as resistant against different diseases, septoria leaf blotch and hessain fly were also found to be resistant against aphids (Table-3). This might be due to the accumulation of resistance genes in those lines which could also be important for aphid resistance.

Incorporation of aphid resistance to elite wheat lines through shuttle breeding: The aphid resistance was incorporated into the high yielding wheat lines by making various crosses as mentioned in Table-2 followed by a series of successive filial generations until F_6.

The performance of different crosses was evaluated for aphid resistance and grain yield in F_6 generation at WRI, Faisalabad location. The development of aphid resistant wheat is an underdeveloped research area which could be an integral part of wheat integrated pest management mainly based on improving grain yield coupled with development of aphid resistance. Many scientists have emphasized the importance of development of aphid resistant wheat cultivars (Lowe 1987; Migui and Lamb, 2003; Smith *et al*., 2004; Riazuddin *et al*. 2004; Wains *et al*. 2010a). In addition to screening of a large germplasm, the objective of this research was to incorporate resistance against aphids in high yielding cultivars of wheat. This is why crosses were made between aphid resistant accessions and high yielding lines which resulted in very promising transgressive segregants selected in F_6 generation showing both these characters as shown in Table-5. The head to row selection was used for selecting high yielding and aphid resistant lines until F_6. Most of the crosses showed high yield and good degree of aphid resistance except the crosses from AS2002, GA-2002, V-07200 and HARTOG=HTG which showed the degree of resistance tending towards moderate susceptibility. However, on the other hand, the descendents from the crosses of Faisalabad 2008 and BAVICORA M92 showed the response tending towards high yield coupled with high degree of resistance. Moreover, the segregants in F_6 from the crosses of Faisalabad 2008 showed maximum yield and best resistance response against aphids.

Table-5. Performance of different crosses for aphid resistance and grain yield in F6 generation

Sr. No	Parentage/Pedigree	Aphid pop/ Tiller	Yield(g)/ Plant	STAUTS
1	AS-2002/Kentanna	6.5	67.4	T-LY
2	AS-2002/FRET2*SNITRAP 1 /KAUZ*2/TRAP/KAUZ	5.8	122.6	MR-HY
3	Faisalabad 2008/SR-3	4.2	166.2	R-HY
4	Faisalabad 2008/Lu-26	5.3	103.5	MR-LY
5	Faisalabad 2008/SNI/TRAP1 /3/KAUZ*2/TRAP//KAUZ	1.6	190.4	R-HY
6	Faisalabad 2008/WBLL1*2/4/SNI/TRAP1/3 /KAUZ* 2 /TRAP// KAUZ	1.7	167.9	R-HY
7	Faisalabad 2008/PAVON-76	1.9	129.1	R-HY
8	BAVIACORA M92/Faisalabad 2008	2.5	102.8	R-LY
9	BAVIACORA M92/Naeem-82	3.6	135.5	R-HY
10	Fret-2/Faisalabad 2008	3.2	111.8	R-HY
11	Fret-2/CHIBIA/PRL11/CM65531/3/FISCAL	7.3	108.9	T-HY
12	V-07200//KAL/BB	13.3	92.8	S-LY
13	V-07200/MRNG/BUC///BLO/PSNS	11.7	139.2	S-HY
14	GA-2002/V-087094	8.7	119.5	MS-HY
15	GA-2002/Seher-06	7.2	106.8	T-LY
16	GA-2002/SINDH-81	8.9	102.1	MS-LY
17	HARTOG=HTG/SHALIMAR-88	6.7	104.3	T-LY
18	HARTOG=HTG/LASANI-08	5.1	110.5	MH-HY
19	HARTOG=HTG/AARI-11	5.0	86.5	MR-LY
20	BAVIACORA M92/GALVEZ=JUNCO	3.5	116.0	R-HY

Whereas, resistant and high yielder (R-HY), moderately resistant and high yielder (MR-HY), moderately resistant and low yielder (MR-LY), tolerant and high yielder (T-HY), tolerant and low yielder (T-LY), susceptible and high yielder (S-HY), susceptible and low yielder (S-LY), moderately susceptible and high yielder (MS-HY), moderately susceptible and low yielder (MS-LY).

Mainly the crosses in which the cultivar 'Faisalabad 2008' was involved (whether as pollinator or otherwise), revealed the highest resistance against the aphids coupled with the highest yield per plant. Similar

results were shown by the crosses of BAVICORA 92 with the other varieties. This showed that these two lines have very good scope for both the aphid resistance and high yield and could be utilized to launch breeding program aimed to improve both these characters. Especially the cultivar 'Faisalabad 2008', which might have the genes for aphid resistance in its genetic architecture, can be studied in detail for these genes. Previously, 22 greenbug resistance genes have been characterized in various member of family Gramineae; for instance in wheat, rye, *Aegilops tauschii* and *Aegilops speltoides* (Dubcovsky *et al*., 1998; Smith *et al*., 1999; Castro *et al*., 1999; Flinn *et al*., 2001). The crosses of germplasm accession 'V-07200' showed the highest susceptibility and the lowest grain yield as well. Both the cultivar 'Faisalabad 2008' and 'V-07200' could be utilized as contrasting parent to work out the inheritance of aphid resistance in wheat. Mornhinweg *et al*. (2002) worked on the inheritance of Russian wheat aphid resistance in spring barley germplasm line STARS-9577B. So it can be stated that at the end we were able to incorporate some degree of aphid resistance in wheat. All crosses of Faisalabad 2008 (Faisalabad 2008/SR-3, Faisalabad 2008/Lu-26, Faisalabad 2008/SNI/TRAP1 /3/KAUZ*2/TRAP //KAUZ, Faisalabad 2008/WBLL1*2/4/ SNI/TRAP1/ 3/KAUZ* 2 /TRAP//KAUZ, Faisalabad 2008/PAVON-76, Fret-2/Faisalabad 2008) and BAVICORA 92 (BAVIACORA M92/Faisalabad 2008, BAVIACORA M92/Naeem-82, BAVIACORA M92/ GALVEZ= JUNCO) are target crosses which can be used for further development of aphid resistance in wheat (Table-4).

Conclusion and future Prospects: Most of the germplasm accessions have good degree of aphid resistance in wheat. There is genetic variation for aphid resistance in different accessions and selection for aphid resistant cultivars is possible. The contrasting lines could be crossed to develop segregating generations to investigate quantitative trait loci (QTLs) involved in aphid resistance coupled with high yield. This could also be step forward towards breeding for ideotype plants in wheat.

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