Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2015; 3(6): 360-365

Received: 04-10-2015
Accepted: 10-11-2015

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Seedling bulk test (mass screening) of fifty wheat genotypes against *Schizaphis graminum* (Rondani) (Aphididae: Hemiptera)

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Abstract
Greenbug, *Schizaphis graminum* (Rondani), (Hemiptera: Aphididae), causes heavy losses to wheat crop by directly sucking the cell sap of the plant, and indirectly, by transmitting viral diseases such as barley yellow dwarf virus. Screening of fifty wheat genotypes against *S. graminum* was conducted in the Entomology laboratory of Agricultural Research Institute, Tarnab-Peshawar, Pakistan. These genotypes were ranked resistance/susceptible at resistance rating scale (0-6) on the basis of mean number of *S. graminum* per seedling where '0' shows Immunity (I); 1, highly resistant (HR); 2, moderately resistant (MR); 3, lowly resistant (LR); 4, lowly susceptible (LS); 5, moderately susceptible (MS); and 6, highly susceptible (HS). Twelve genotypes were found highly resistant, twelve were moderately resistant while ten were lowly resistant. Ten genotypes were ranked as lowly susceptible, two were moderately susceptible while four genotypes were highly susceptible. This study provided preliminary information on the resistance/susceptibility status of tested genotypes against this aphid.

Keywords: Seedling bulk test, *Schizaphis graminum*, *Triticum aestivum* L.

1. Introduction
Wheat, *Triticum aestivum* L., is one of the most important staple foods worldwide that satisfies many human nutritional requirements. In top twenty wheat growing countries of the world more than 200 million hectares are harvested annually and about 650 million tons of wheat grains are produced [1]. In Pakistan wheat is the main crop with the largest area under cultivation and a major contributor to the economy. It was grown on 8414 million hectares produced 21749 million tons of grain with average yield of 2585 kg/ha [2]. Wheat is attacked by a number of insect pests such as termites, cutworm, armyworm, wheat weevils, Jassid, aphids and thrips which attack on wheat crop [3, 4] in the world. Wheat aphids (Hemiptera: Aphididae) are common pest of wheat which are responsible for yield loss directly by feeding injury and indirectly by transmission of plant virus [5, 6]. Aphids are soft bodied insect that suck the cell sap of their host plant. These insects having small body size ranging from 1.5 mm to 3.1 mm [7]. Cereal aphid infestations reduce wheat productivity and profitability by reductions in shoot growth, lowering chlorophyll concentrations and root growth [8].
Aphids are also becoming a potential insect pest of wheat crop in Pakistan and a threat to wheat crop productivity therefore, farmers had started pesticides spray on their wheat crop and the spray area is widening yearly [9]. Aphids can reduce wheat production by causing 35-40% yield losses by direct feeding [10, 11] and 20-80% indirectly by transmitting viral and fungal diseases [12]. Several aphid species like bird cherry oat aphid, *Rhopalosiphum padi* (L), wheat grain aphid, *Sitobion avenae* (Fabricius), corn leaf aphid *Rhopalosiphum maidis* (Fitch), Greenbug, *Schizaphis graminum* (Rondani) and Russian wheat aphids, *Diuraphis noxia* have been recorded on wheat all over the world [13, 14]. *Schizaphis graminum* is also a serious pest having a wide host range of at least 60 plant species including wheat, barley, sorghum and corn [15]. In USA, six biotypes of greenbug named A to F have been identified so for but the biotype of this species present in Pakistan is different from the biotypes found in USA so it is named as Pk-1[14]
Schizaphis graminum is elongated oval shape body size ranging from 1.5 mm to 2.0 mm, green to yellow green in color. Apterous having a green longitudinal strip moving down the middle of the dorsum. Alate with green to yellow green abdomen. Siphunculi and cauda are pale and with two pair of caudal hairs [16]. *Schizaphis graminum* (Rond.) is abundant in wheat

and reduces yield through direct damage and transmission of plant viruses [17, 18, 19]. Several crop management practices like cultural, biological, chemical and host plant resistance are available for the control of wheat aphid but host plant resistance is more important which can keep aphids population well below economic threshold level and reduced chance of biotypes development [20, 21]. Susceptibility/resistance of plants is the result of a series of interactions between plants and insects, which influence the ultimate degree of establishment of insect populations on plants. Unfavorable biophysical or biochemical plant characters interrupt one or more of insect responses, may inhabit the establishment of insect populations on a plant and render it resistant to infestation and injury [22]. Keeping in view the importance of wheat, this screening experiment was designed to evaluate the wheat genotypes against *S. graminum.*

2. Materials and Methods

This experiment was conducted in Entomology laboratory at Agricultural Research Institute Tarnab-Peshawar, Khyber Pakhtunkhwa, Pakistan during 2011. *Schizaphis graminum* (Rondani) and plant culture was maintained in control environmental condition at a temperature of 20 ± 7 ^{O}C, 60 ± 5 % relative humidity and photoperiod of 16:8 h L:D [14]. Culture of *Schizaphis graminum* was maintained on fresh seedlings of mixed seeds of wheat and oat varieties other than the selected wheat genotypes for the laboratory study. Seeds were grown in four plastic pots already filled with substrate mixture in ratio of (2 parts field soil:1 part Sand:1 part well rotten farm yard manure), which also contained urea and phosphorous fertilizer applied in the field soil [23]. Water was applied to substrate mixture and pots were placed in plastic plates. Mixture of wheat and oat seeds were sown at 1.5 cm depth from the surface of the substrate soil in each pot. On emergence seedling were daily checked for water requirement and fresh water was added to the plastic plates to keep the soil of every pot moist through pores at the bottom of each pot and pots with plastic plates were placed individually inside the rearing caged. To start the aphid colony in laboratory, few alate *S. graminum* when first appeared in January 2011, on wheat in the field, were collected in test tubes and sifted to laboratory and individually caged on already grown fresh wheat seedlings. Aphid which successfully started reproducing their offspring were shifted to wheat seedling in rearing cages. Plastic pots with wheat seedling were replaced as needed by adding new pots with fresh seedling and letting aphids move from the old dried seedling to the newly grown seedling in other pots. Dried seedling were removed from the plastic pots, put them in plastic bags and were discarded outside the laboratory.

Fifty wheat genotypes were screened in seedling bulk test for knowing their resistance against *S. graminum.* Seeds of the wheat genotypes (Table-1) were obtained from the wheat section of Cereal Crop Research Institute (CCRI) Pirsabak, Nowshera, Khyber Pakhtunkhwa province with pedigree/parentage information. For convenience fifty genotypes were divided in groups and each group was consist of five genotypes. Five seedlings of each genotypes was sown in a tray 45 cm long, 30 cm wide and 6 cm deep in straight lines keeping 5 cm distance between lines and plant to plant distance was maintained at distance of 7 cm. Trays were already filled with a mixture of field soil, farm yard manure and sand in ratio of (2:1:1) field soil was supplied with DAP and urea fertilizer.

A white triangular filter paper piece was placed near the base of each seedling when it was in one leaf stage and 10-12 cm height. Five apterous of *S. graminum* were released on every filter paper from aphid culture which was already maintained in the laboratory. The number of aphids (adults and nymphs) on each seedling was counted at two days interval after aphids released. The data stopped when the plant attained the size of 18-20 cm height as seedling started logging after 5-6 days after aphids release. The data presented as mean number of aphid per seedling adopted from [24] and resistant and susceptible genotypes were ranked on rating scale (0-6) according to [25, 26] where '0' shows immunity, 1' highly resistant 2' moderately resistant, 3' lowly resistant, 4' lowly susceptible, 5' moderately susceptible and '6' highly susceptible.

3. Results

The initial mass screening (Table-1) indicates that genotypes are significantly different at (F 198; df 49, 249; $P<0.05$) in respect of mean number of *S. graminum*/seedling at resistance rating scale (0-6). Twelve genotypes were found to have higher resistance (1) against *S. graminum*. The mean number of aphids on these genotypes ranged from (1-2.80) included Iqbal-2000, Zargoon-79, PR-104, PR-102, Bau's-Bagula, Baviacora, Cham-04, AS-2002, Pirsabak-2004, Kauz's', PAR-173, Chakwal-50.

Whereas, moderately resistance was further shown by twelve genotypes including SA-42, Hashim-2008, Attahabib-2010, Shalimar-88, Sehar-2006, KT-2000, PR-103, Tatara-97, Pirsabak-2008, Maxipak-65, Kohistan-97, Siran-2010 at resistant scale (2) with mean number of *S. graminum*/seedling ranged from (3-4.80).

Furthermore, based on resistant rating scale (3) ten genotypes e.g. Nuwyt-3, Fakhr-e-Sarhad, Kohinoor-83, Kohsar-95, MPT-2011-2012, Barsat-2010, Faisalabad-83, Satluj-86, Zam-2004, Bathore-2008 having mean aphid seedling-1 (5-6.8) were declared as lowly resistance.

The Lowly susceptible ten genotypes, at resistant scale (4) with mean aphid (7-8.8) seedling-1 were included Pewees, Bakhtawar-92, Auqab-2000, Saleem-2000, Dera-98, SA-75, Daman-98, Faisalabad-2008, PR-103, Pirsabak-2005. This pattern was followed by moderately susceptible at resistant rating scale (5) included Fasalabad-85, Khyber-87 with (9-10) *S. graminum*/seedling. Lastly four out the fifty tested genotypes (Zamindar-80, Gomal-2008, Punjab-96, Lasani-2008) were ranked as highly susceptible at resistance scale (6) with more than ten aphids/seedling.

Table 1: Initial mass screening of wheat genotypes against *S. graminum* during, 2011

Genotypes	Pedigree/Parentage	*S. graminum*/ seedling	RS	Ranking
---, ----, -----		0	0	I
Iqbal-2000	BURGUS/SORT 12-13//KAL/BB/3/PAK 81 PB 21912-11A-0A-0A-59A-0A-0A	1.60 u	1	HR
Zargoon-79	CC-Inia/Tobari-C.fon/BB CM8237-G-1M-3Y-2M-4Y-0M	1.80 tu		
PR-104	--------	2.20 st		
PR-102	------	2.40 rs		
Bau's-Bagula	TTR'S'/JUN'S' CM59123-3M-1M-3Y-1M-3M-2Y-1M-0Y-54Y-0M	2.40 rs		
Baviacora	CM 92066-J-0Y-0M-0Y-4M-0Y	2.60 qrs		
Cham-04	FLK/HORK CM39816-1S-1AP-0AP-0SYR	2.60 qrs		
AS-2002	KHP/D31708//CM74A370/3/CIAN079/4/RL6043/*4NAC	2.80 pqr		
Pirsabak-2004	KAUZ/STAR	2.80 pqr		
Kauz's'	JUP/BJY'S' JUP/BJY'S'/URES CM7458-4Y-1M-3Y-1M-4Y-0B	2.80 pqr		
PAR-173 Blue bird	CNO//SN64/KLRE/3/8156 II23584-303M-0Y-111A-1A-1436-0PAK	2.80 pqr		
Chakwal-50	ATTILA/3/HUI/CARAC//CHEN/CHTO/4/ATTILA	2.80 pqr		
SA-42	C271/LR 64//SON 64	3.0 pq	2	MR
Hashim-2008	JUP/ALD'S'//KLT'S'/3VEE'S'/6/BEZ/TOB/815 6/4/ON/3/6*TH/KF//6*LEE/KF/51CW91-0321-2AP-0TS-1AP-2AP-0L-0AP	3.0 pq		
Attahabib-2010	INQALAB91*2/TUKURU	3.0 pq		
Shalimar-88	PB81/HD2182//PB81	3.20 p		
Sehar-2006	CHIL/2*STAR/4/BOW/CROW//BUCP/PVN/3/--CMSS9Y0045-100Y-200M-17Y-10M-0Y	3.20 p		
KT-2000	GEN#WHETON	3.80 o		
PR-103.	-------	3.80 o		
Tatara-97	JUP/AMB"S"//KLT"S"/3/VEE"S"	4.20 no		
Pirsabak-2008	KAUZ/PASTOR	4.20 no		
Maxipak-65	PJ62/GB55 II.8156-0PAK	4.40 n		
Kohistan-97	V-1562//CHRC'S'/HORK/3/KUFRA-1/4/CARP'S'/BJY'S'(PB.24883B-1A-0A)	4.60 n		
Siran-2010	PBW343*2/KUKUN	4.60 n		
Nuwyt-3	----------	5.20 m	3	LR
Fakhr -e- Sarhad	PFAU'S'/SERI/BOW'S'	5.20 m		
Kohinoor-83	OREF1-58/FDL//MFN/2*TIBA63/3/COC CM37987-I-1Y-5M-0Y-0PAK	5.40 m		
Kohsar-95	PSN/BOW CM69560-1M-1Y-1M-2Y-0M-0PAK	5.20 m		
MPT-2011-2012	MILAN/KAUZ//PASTOR/3/PASTOR	5.40 m		
Barsat-2010	FRET2	6.0 l		
Fasalabad-83	FURY/KAL/BB CM37138-48Y-1M-5Y-1M-4V-5Y-0A-0PAK	6.0 l		
Satluj-86	CMT/YR//MON'S'	6.40 kl		
Zam-2004	KAUZ* 2/OPATA//KAUZ	6.60 jk		
Bathore-2008	URES/JUN//KAUZ CM 96818-i- 0Y-0M-0B-2Y-1Y-0M	6.80 jk		
Pirsabak-2005	MUNIA/CHTO//AMSEL	7.0 ij	4	LS
Pewees	INIA66/7C//MAYA/3/PCI/TRMCM31630	7.60 h		
Bakhtawar-92	JUP/BJYG/URES	7.40 hi		
Auqab-2000	CROW'S'/NAC//BOW'S'PB22138-3A-0A-0A-234A-0A	7.60 h		
Saleem-2000	CHAM-6//KITE/PGO	7.80 gh		
Dera-98	F12-71/COC//CNO79 CM76688-9Y-03M-02Y-2B-0Y	7.80 gh		
SA-75	NA1 60/CB 151//S 948/3/MXP	7.80 gh		
Daman-98	BOW'S'/3/CAR853/COC//VEE'S'	8.20 fg		
Faisalabad-2008	PBW-65/2*PASTOR	8.60 ef		
PR-103	----------	8.80 e		
Fasalabad-85	MAYA/MON//KVZ/TRM CM44083-N-3Y-1M-1Y-1M-1Y-OB	9.60 d	5	MS
Khyber-87	KVZ/TRM//PTM/ANA-CM43930	10 cd		
Zamindar-80	RON/CHA//BB/NORCM5484-F-5Y-4M-1Y-1M-1Y-0M-0PAK	10.40 c	6	HS
Gomal-2008	ATTILA CM85836-4Y-0M-0Y-14M-0Y-5M-0Y-15J-0Y-0AP	11.80 b		
Punjab-96	SA 42 *2/4CC/INIA//BB/3/ INIA/HD832 PB1352-B-4K-36A-0A	13.40 a		

Mean number of *S. graminum*/seedling followed by the same letters are not significantly different at 5 % level of significance (LSD-test)
RS: Resistance scale (0-6)
I: immunity, HR: Highly resistant, MR: Moderately resistant, LR: Lowly resistant, LS: Lowly susceptible, MS: Moderately susceptible, HS: Highly susceptible.

4. Discussion

Resistance is the genetically inherited traits in a genotypes resulting in less damage than in other (susceptible) genotypes individuals which lack these genetic characteristics. In this way, plant resistance is conditioned by the presence of certain genes that express the presence or absence of certain chemical or morphological traits that interfere with the ability of an herbivore to utilize a plant, and the plant to tolerate the attack [27]. Researchers have identified several genes from resistant germplasm and were able to transferred genes into wheat cultivars have conferred resistance against biotic stress such as insect attack, powdery mildew, stem rust, leaf rust and yellow rust [28]. Different researchers have reported resistant gens which included Gb1, Gb2, Gb3, Gb4, Gb5, Gb7/Gbx2, Gba, Gbb, Gbc, Gbd, Gbx1 and Gby which, have proved resistant against different biotypes of greenbug [29]. Mass screening is a technique, which allows the researchers to evaluate the resistance of different genotypes against aphids at seedlings stage [27]. Weibull (1987) screened 27 lines of barley in seedlings stage against *Rhopalosiphum padi* and plants were grouped as resistant and susceptible on the basis of aphids number/plant. Similarly [30] screened 133 triticale line against Russian wheat aphids. Assad *et al.* (1999) evaluated 76 barley genotypes through initial mass screening test for reaction to Russian wheat aphid. Wang *et al*. (2011) and Li *et al*. (2013) evaluated resistance in 22 and 292 wheat germplasm respectively against *S. avenae.* Different scales (1-6), (0-9) and (0-6) were used to ranked the resistant and susceptible wheat genotypes against aphids on the basis of visual observation of plant damage rate or leaf chlorosis [32, 14, 31, 25, 26] but [24] ranked genotypes resistant or susceptible as mean number of aphids seedling^{-1}.

In our studies total fifty genotypes were tested for resistant to green bug under a laboratory conditions in a (mass screening test) and the response was rated as high resistant with resistant rating scale (1) followed by moderately resistant (2) and lowly resistant (3) where as the susceptibility was recorded with resistant rating scale as lowly susceptible (04) followed by moderately susceptible (5) and highly susceptible (6).

Among the tested entries only twelve (Iqbal-2000, Zargoon-79, PR-104, PR-102, Bau's-Bagula, Baviacora, Cham-04, AS-2002, Pirsabak-2004, Kauz's', PAR-173, Chakwal-50) genotypes responded as highly resistant, which was followed by moderately resistant (SA-42, Hashim-2008, Attahabib-2010, Shalimar-88, Sehar-2006, KT-2000, PR-103, Tatara-97, Pirsabak-2008, Maxipak-65, Kohistan-97, Siran-2010) and ten (Nuwyt-3, Fakhr-e-Sarhad, Kohinoor-83, Kohsar-95, MPT-2011-2012, Barsat-2010, Faisalabad-83, Satluj-86, Zam-2004, Bathore-2008) of the tested entries were rated as lowly resistant. Furthermore the susceptibility behavior was grouped as lowly susceptible (Pewees, Bakhtawar-92, Auqab-2000, Saleem-2000, Dera-98, SA-75, Daman-98, Faisalabad-2008, PR-103, Pirsabak-2005) two of the tested entries Fasalabad-85 and Khyber-87 behaved as moderately susceptible and four (Zamindar-80, Gomal-2008, Punjab-96, Lasani-2008) were rated as highly susceptible. (Inayatullah *et al*. 1993) who tested 439 different native wheat germplasm against *Schizaphis graminum* in control condition. presented more or less similar result for genotypes Kohinoor-83, Zargoon-79, Faisalabad-83, Khyber-87 and Zamindar-80 were in susceptible group while Satluj-86 showed intermediate response. While, in our studies Zargoon-79 proved highly resistant, Khyber-87 and Zamindar-80 were also susceptible while, other three genotypes were lowly resistant. Slight differences with [14] in the resistant status for these genotypes may be due to the difference in resistant rating scales. Our finding are more identical to[33] who screened different genotypes in field against *S. graminum* identified Tatara-97 as highly resistant followed by Saleem-2000, Dera-98, FakhreSarhad-99, Bakhtawar-92, While in our studies on the base of resistant rating scale for these genotypes Tarata-97 also proved resistant followed in ascending order by FakhreSarhad-99, Bakhtawar-92, Saleem-2000 and Dera-98. However, [34] screened twelve varieties/lines of wheat in field, which included Inqilab-91, Iqbal-2000, MH-97, BWP-97, PND-1, Punjab-96, 2333, 2210, 2049 and 2460 against *S. graminum* (R.). In that screening experiment variety Iqbal-2000 showed intermediate response while, Punjab-96 proved second best in respect of resistant to this aphid. In our studies genotypes Iqbal-2000 and Punjab-96 responded as highly resistant and moderately susceptible respectively. Changes in the resistance status of a genotypes in field is attributed to different biotic and a-biotic factors [35]. Host plant resistance is more important but in some cases host plant own defense may also affect the performance of natural enemies [36, 37]. For example, waxes or hair on leaf surface (glandular trichomes) can hinder the searching ability of natural enemies [38]. On one side insect resistance cultivars increase the performance of biological agents but in different situation high level of plant succulence in cultivars have detrimental effect on the performance of natural enemies [39]. Some insect resistance cultivars having volatile compounds influence the performance of bio-control agents [40]. Plant genotypes affect the performance of insect herbivores and the community structure of predators and parasitoids [41]. Screening and identification of resistant germplasm is fundamental and continuous process for sustainable production [22].

5. Conclusion and Recommendation

This study provided the preliminary information about the resistance/susceptibility of used genotypes against *S. graminum.* The genotypes which were resistance should be tested for their performance against aphid in presence of different biotic and abiotic factors. The genotypes which show resistance in the field condition, further should be subjected to the categorization of resistance factors studies and their genetic base of resistance should be explored. This information could serve as a basis to develop integrated management of aphid on wheat.

6. Acknowledgments

These studies were completed with the financial help of Higher Education Commission, Islamabad Pakistan studies under Ph. D fellowship for 5000 Scholars (Indigenous) Scheme, Batch-VI an award for Ph.D.

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