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Study on Different Components of Resistance in Wheat Genotypes to Green Bug (*Schizaphis graminum)* (Rondani)

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ABSTRACT

The green bug (*Schizaphis graminum*) (Homoptera: Aphididae) is a major aphid species attacking wheat crop in Pakistan, and is reported as a major wheat pest across the world. Host plant resistance is a strong pillar in Integrated Pest Management (IPM) for reducing the impact of this pest. Several green bug resistant genes have been identified and introduced in commercial high yielding cultivars of wheat to manage green bug. Three different experiments were conducted on ten cultivars (MPT-V13, MPT-V3, MPT-V6, PR-104, MPT-V17, MPT-V26, MPT-V5, MPT-V33, MPT-V28 and PR-102) to determine categories of resistance. During first experiment 'antixenosis' none of the genotype proved resistant while in second experiment 'tolerance' the genotype 'MPT-V33' had shown good and significant vigor compared to all the tested genotypes. The third experiment antibiosis resistance was identified in genotype 'MPT-V5', where green bug took significantly longer time (8.5 days) to produce 31.3 nymphs compared to the tested genotypes and susceptible check.

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Authors' Contribution

SAK designed and supervised the experiments. KJ conducted the experiment and wrote the article. ZK maintained plant and insect culture. SRAS statistically analyzed the data. IK co-supervised the study.

Key words

Green bug, wheat, IPM, resistance.

INTRODUCTION

The cereal aphid Green bug (*Schizaphis graminum*) (Aphididae: Homoptera) is an important insect pest of wheat, and causes damage from early fall until late spring before the crop matures. This aphid is often the most significant factor limiting profitable wheat production (Burton *et al*., 1985; Kieckhefer and Kantack, 1988; Webster, 1985; Riedell *et al*., 1999; Kindler *et al*., 2002). Several species of aphid can reduce yields of small grains and increase production costs. (Hussain *et al*., 2015) Different species of aphids including, green bug, inject toxins into host plants and cause serious plant damage (Clifford *et al*., 2004).

The green bug is one of the major wheat aphid species in KPK province of Pakistan (Khan *et al*., 2006). It is a serious threat to farmers in developing countries in which chemical control is often not an option and usually considered the most important option for green bug management (Kolbe and linke, 1974). Green bugs feed exclusively on the aerial portions of the plant but cause biomass reductions in both shoot and root systems, which is ultimately expressed as reductions in yield (Kieckhefer and Kantack, 1988; Burton *et al*., 1985; Pike and Schaffner, 1985).Genetic resistance of cereals is probably the best way to control green bug and would benefit farmers and the environment (Dean, 1974). In Pakistan, Hamid (1983b) reported locally damaging populations of green bug in the Western hills, Northern hills and Peshawar valley and those of grain aphid in the Western hills, Northern hills, Peshawar and foothills of Punjab (Hashmi *et al*., 1983).

To prevent heavy losses by aphids, several methods of control have been used. These include cultural, physical, mechanical, biological, chemical and host plant resistance. Natural enemies can maintain aphid populations below the economic injury level, however, sometimes the aphids can be extremely injurious if present in large numbers. At this point, chemical control may be the only option. Control by chemicals has however created a number of problems, including killing of the beneficial insects and imparting resistance in pests. Thus to combat the increasing resistance in aphids to pesticides and to reduce its hazardous effects on the environment, adoption of Integrated Pest Management (IPM) strategies are needed (Hatchett *et al*., 1987).

Host plant resistance is an integral part of IPM of the cereal aphids. Partial resistance could well provide adequate control of this pest with only occasional use of pesticides in outbreaks. Host plant resistance is one of the favored control tactics in advanced wheat breeding lines (Souza, 1998).

Keeping in mind the importance of the insect, a project was designed to study and compare the intrinsic rate of natural increase ($r_m = (\log_e M_d)/d$) of green bug on resistant and susceptible wheat cultivars/genotypes, and

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study the antixenosis/non-preference and tolerance components of tested genotypes of wheat.

MATERIALS AND METHODS

Green bugs were collected from a wheat field at New Developmental Farm (NDF - University of Agriculture, Peshawar), and were cultured on susceptible wheat plants for designed experiments, in the screening house of Institute of Biotechnology and Genetics Engineering (IBGE).

All experimental tests were conducted in a screening house on pot reared plants. The potting mixture used in the experiment was purchased from commercial Nurseries in Peshawar.

Preliminary test

Forty three wheat genotypes/varieties were selected for preliminary screening (MPT-V 1, MPT-V 2, MPT-V 3, MPT-V 4, MPT-V 5, MPT-V 6, MPT-V 7, MPT-V 8, MPT-V 9, MPT-V 10, MPT-V 11, MPT-V 12, MPT-V 13, MPT-V 14, MPT-V 15, MPT-V 16, MPT-V 17, MPT-V 18, MPT-V 19, MPT-V 20, MPT-V 21, MPT-V 22, MPT-V 23, MPT-V 24, MPT-V 25, MPT-V 26, MPT-V 27, MPT-V 28, MPT-V 29, MPT-V 30, MPT-V 31, MPT-V 32, MPT-V 33, MPT-V 34, PR-V 98, PR-102, PR-103, PR104, PR-105, Saleem-2000, Pirsabak-2005, Pirsabak-2004 and Pirsabak-2008. These genotypes were sown in a tray (size of the tray was 90 (w) x 210 (l) x12 (h) cm). All the wheat genotypes were sown in rows individually with ten replications of each genotype in screen house. Green bugs were released at an average rate of 10 aphids per seedling at the two leaf stage of growth. Resistance of each genotype was determined on visual damage rating scale. After 15 days, the pest infestation data was recorded. A damage Rating scale of 0-9 (Table I) was used to determine the degree of resistance by using damage rating scale (DRS) from (0---9) where '0' stands for healthy plant and '9' stands for diseased. Genotypes which remained vigorous (DRS = 0-3) were further evaluated for different components of resistance (Webster and Inayatullah, 1988; Akhtar *et al.*, 2011).

Table I.- Damage Rating Scale (DRS)

Resistant			
DR = 0	DR = 1	DR = 2	DR = 3
Moderately resistant			
DR = 4	DR = 5	DR = 6	
Susceptible			
DR = 7	DR = 8	DR = 9	

Antixenosis (Host plant selection)

Single pre-germinated seedlings of each genotype were planted in pot size (40 cm diameter × 7cm height) with equal distance from the center of the pot. Ten pots (replicates) were arranged in a completely randomized design, each pot containing one seedling of each genotype. At two leaf stage of growth, pots with seedlings were infested by releasing 50 green bugs in the center of each pot on a piece of paper. Plants were covered with nylon mesh cages, and after 12, 24 and 48 h of infestation, numbers of aphids on each plant were counted and recorded to determine the degree of Antixenosis among each tested plant (Flinn *et al.*, 2001).

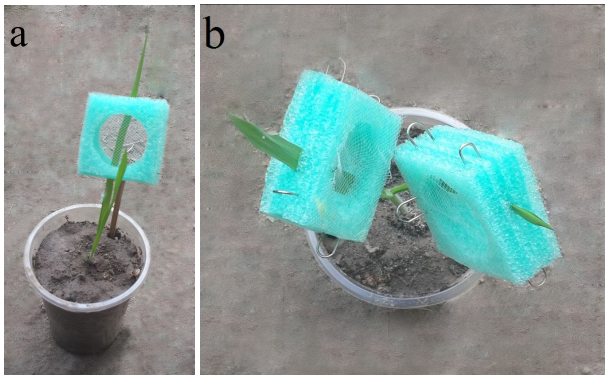

Fig. 1. (a) P1 aphid caging, {b) P1 and F1 (offspring) caged in antibiosis test.

Antibiosis (Antagonistic association between organisms)

Antibiosis was determined from the mean progeny produced by Green bugs on the infested plant of each variety in each replication. A single seed of each variety was planted in separate pots (6cm diameter × 8.5cm height). Ten replications of each variety were arranged in a completely randomized design (CRD). When the plants reached the two leaf stage, the midsection of a first leaf was enclosed in a cage. The cage was made of using two pieces of plastic foam (0.9cm thickness and 1 inch inner diameter) and ventilated with nylon mesh on the outer sides of cage (Fig. 1). A late instar aphid nymph (P1) was released inside a cage and the cage was closed using clips. The P1 aphid on each plant was observed twice a day until reproduction. When the reproductive P1 produced its 1st nymph (F1 aphid), time was recorded, and the mother (P1) of F1 was moved to the second leaf of the same plant and caged as described before. When the F1 aphid produced its 1st offspring, the time in days (d) was recorded, and at that time the number of nymphs produced by P1 (M_d) were counted and recorded. For each plant, M_d and d were calculated, using the method of (Birch 1948) $r_m = 0.738 (\log e Md) / d$, the mean intrinsic

rate of increase (r_m) for each plant was calculated, where M_d is the total number of progeny produced by [P1] the mother of F1, (d) is the time taken by F1 aphid from its birth till the production of its 1st offspring. The value 0.738 is the mean regression slope of m_d/d for four aphid species (Wyatt and White, 1977).

Tolerance

For determining the presence of tolerance factor in a plant, the method of 'Reese *et al.* 1994' was used. The proportional plant dry weight change (DWT) and tolerance index (TI) were calculated for each plant. The DWT was calculated as DWT= [(WC-WT) / WC] X 100, where WC is non-infested/ controlled plant dry weight and WT is infested/treated plant dry weight. From DWT, TI was calculated as TI= DWT/number of aphids produced on the infested plant (Reese *et al*., 1994). The TI was determined to compensate for the confounding effect of differing numbers of green bug on infested plants. The plants/cultivars having TI values significantly lower are considered tolerant as compared to susceptible control.

The pre-germinated seedlings of each genotype were planted individually in pots (6cm diameter × 8.5cm height), replicated 20 times. In each genotype, when the plants were at two leaf stage they were paired on basis of equal plant height and growth. In each pair, one plant was left as the untreated control, while the other plant was infested with 20 green bug aphids. The experiment was setup as a completely randomized block, such that each block has one pair of plants from each genotype. Each pot was covered separately with a nylon mesh cage, and aphids were allowed to feed for 15days until susceptible plants died. Cages were then removed and aphids on each infested plant were collected on a sheet of wax paper, placed in 70% alcohol, and counted. Shoots from infested and non-infested plants were cut at the soil surface and placed in pre-weighed aluminum foil pouches. Attached soil particles were washed from roots and placed in similar pre-weighed aluminum foil pouches. Pouches with the shoots or roots were dried in an oven at 75ºC for 48 h. Tissue weights were determined by subtracting the weight of the foil pouch from the combined pouch and tissue weight.

Statistical analysis

Data obtained from experiments were analyzed by using STATISTIX 8.1.

RESULTS

Preliminary test

On the basis of preliminary screening, the tested genotypes were grouped into susceptible, moderately susceptible and resistant. The genotypes, MPT-V11, MPT-V24, MPT-V31, MPT-V15, MPT-V4, PR-103, SALEEM-2000, MPT-V32, MPT-V29, PIRSABAK-2008, MPT-V2, MPT-V19, MPT-V20, MPT-V7, MPT-V34, MPT-V23, MPT-V18, MPT-V9 and MPT-V10 fell in the 'susceptible' category. Thirteen genotypes, MPT-V30, MPT-V8, MPT-V27, MPT-V25, PR-98, PR-105, MPT-V14, PIRSABAK-2004, PIRSABAK-2005, MPT-V21, MPT-V16, MPT-V22 and MPT-V12, were 'moderately susceptible', while ten genotypes, MPT-V13, MPT-V3, MPT-V6, PR-104, MPT-V17, MPT-V26, MPT-V5, MPT-V33, MPT-V28 and PR-102, showed a 'resistance' response during preliminary test. The genotypes were further evaluated for detail category of resistant components. Thirty three genotypes were excluded from the experiment and the remaining lot was further tested for resistant category (Table II).

Table II.- Preliminary test for assessment of resistance study.

Susceptible		moderately resistant	Resistant
MPT-V 11	MPT-V 2	MPT-V 30	MPT-V 13
MPT-V 24	MPT-V 19	MPT-V 8	MPT-V 3
MPT-V 31	MPT-V 20	MPT-V 27	MPT-V 6
MPT-V 15	MPT-V 7	MPT-V 25	PR-104
MPT-V 4	MPT-V 34	PR-98	MPT-V 17
PR-103	MPT-V 23	PR-105	MPT-V 26
MPT-V 1	MPT-V 18	MPT-V 14	MPT-V 5
SALEEM-2000	MPT-V 9	PIRSABAK-2004	MPT-V 33
MPT-V 32	MPT-V 10	PIRSABAK-2005	MPT-V 28
PIRSABAK-2008	MPT-V 29	MPT-V 21	PR-102
		MPT-V 16	
		MPT-V 22	
		MPT-V 12	

Table III.- Average population density of green bug on different wheat genotypes at 12, 24 and 48 h post infestation.

	12 h	24 h	48 h
MPT-V 28	3.9	3.8	4.4
MPT-V 5	3.9	4.2	4.1
MPT-V 13	4	3.9	4.3
PR-102	4.2	3.8	4.1
MPT-V 33	4.1	4.3	4.2
PR–104	4.2	4.1	3.9
MPT-V 6	4	4.2	4.5
MPT-V 26	3.8	4	4.2
MPT-V 3	4.2	4.1	4
MPT–V 17	3.9	3.8	4.2

Antixenosis

In free choice test (Antixenosis test), the preferences and non-preference tendency of the green bug were observed. None of the tested lines showed significant differences after 12 h, 24 h, or 48 h post infestation period. Thus no antixenosis was present in the tested lines against green bug (Table III).

Antibiosis

The results of antibiosis experiment are presented in (Table IV). When the green bug was caged on the tested genotypes including resistant and susceptible cultivars, the aphid produced significantly lower number of progeny on genotypes: MPT-V 28, MPT-V 13, PR-102 and MPT-V 33 as compared to the susceptible cultivar 'Khaniwal' but more progeny were produced on cultivar Khaniwal compared to the resistant Tatara-98. Only the genotype 'MPT-V 5' responded similarly to the resistant cultivar Tatara-98, in terms of progeny production. In pre-reproductive time (d) the *S. graminum* took similar time and no statistical differences were found among the tested genotypes compared to the susceptible cultivar 'Khaniwal. While, the genotype 'MPT-V 5' took significantly longer (pre-reproductive time) for progeny production compared to the tested genotypes including the resistant cultivar Tatara-98

Table IV.- Mean values of progeny produced (M_d), pre-reproductive period (d) and rate of natural intrinsic increase (r_m) of green bug on tested genotypes.

Cultivar	Total progeny production (M_d)	Pre-reproduction time (d)	Rate of intrinsic increase (r_m)
MPT-V28	40.3 B	6.9 C	0.172 A
MPT-V5	31.1 C	8.5 A	0.130 C
MPT-V13	40 B	6.87 C	0.172 A
PR-102	39.3 B	6.93 C	0.170 A
MPT-V33	40.1 B	6.92 C	0.171 A
Khaniwal	44 A	7.13 C	0.170 A
Tatara-98	29.6 C	7.45 B	0.146 B
LSD (0.05)	3.3560	0.2694	7.605

Tolerance

In term of percent plant dry weight change (DWT); all genotypes showed independent responses, except genotype MPT-V28 and MPT-V5 (Table V). The accession 'MPT -V13' showed significantly higher percent dry weight change (DWT) for all the parameters; shoots (57.2), roots (51.7) and plant total (108.9). While genotype 'MPT -V33' had shown significantly minimum percent dry weight change (DWT) for shoots (28.8), roots (22.7) and plant total (51.5). The genotypes 'MPT-V28' and MPT-V5, also showed statistical significance compared to the other tested genotypes while between them there was no significant difference. The genotype PR-102 had also showed statistical difference from all the tested genotypes in term of proportional plant dry weight change (DWT).

Table V.- Means of percent dry weight change (DWT) for shoots, roots and total plant of different wheat genotypes to green bug.

Cultivar	Shoot	Roots	Plant total
MPT -V28	48 b	44.4 b	92.4 b
MPT -V5	48.25 b	45.32 b	93.57 b
MPT -V13	57.2 a	51.7 a	108.9 a
PR-102	40.7 c	35.3 c	76 c
MPT -V33	28.8 d	22.7 d	51.5 d
LSD (0.05)	2.8239	3.0644	4.7336

The results of tolerance index (TI) values are presented in (Table VI). The tolerance index value of 'PR-102' (plant total=0.533) was significantly greater than all other tested genotypes. The genotype 'MPT -V33' (0.248) had a significantly lower tolerance index compared to the tested genotypes and genotype MPT-V5 showed moderate response in term of tolerance index. The genotypes MPT-V28 and MPT-V13 showed no statistical difference between each other but they were significantly different from other tested genotypes.

Table VI.- Means of tolerance index (TI) for shoots, roots and total plant of different wheat genotypes to green bug.

Cultivar	Shoot	Roots	Plant total
MPT–V 28	0.183 c	0.170 c	0.353 c
MPT-V 5	0.232 b	0.218 b	0.450 b
MPT-V 13	0.200 c	0.181 c	0.382 c
PR-102	0.285 a	0.247 a	0.533 a
MPT-V 33	0.138 d	0.109 d	0.248 d
LSD (0.05)	0.0270	0.0254	0.0496

DISCUSSION

The plant resistance mechanism has a strong influence on insect population/infestation, most specifically phloem feeding insects (Gallum, 1977; Saxenal and Barrion, 1985; Puterka *et al*., 1992; Porter *et al*., 1977). That's why the knowledge and role of resistance categories is very important. Many accessions of barley, wheat and triticale have been identified having

antixenosis, antibiosis, tolerance or a combination (Du Toit, 1987, 1989; Formusoh *et al*., 1992, Smith *et al*., 1992). The focal problem related with both antixenosis and antibiosis resistance mechanism is the selection pressure on the insect, which finally results in the development of a new resistance breaking biotype which may have the potential to feed on those plants which had previously shown resistance to the pest. In the case of tolerance resistance mechanisms, no selection pressure is exerted on the pest.

The present experiment was designed to investigate resistance components in the selected genotypes which can be used for effective management of green bug. In antixenosis test, none of the tested genotypes showed significant antixenosis resistance. This correlates to the work done by Khan *et al*. (2010) investigating wheat breeding lines for resistance components and finding none of the tested lines having antixenosis resistances. Contrary to these results, the antibiosis experiment genotype 'MPT-V 5' demonstrated strong resistance in terms of total progeny production and pre-reproduction period compared to the tested and susceptible cultivar. Moreover, the genotype 'MPT-V 5' was statistically similar to the standard resistant cultivar Tatara-98. Furthermore, during the second experiment (antibiosis) the rate of natural intrinsic increase (r_m) of the genotype 'MPT-V 5' sustained the antibiotic characteristics with significantly lower intrinsic rate (0.130) compared to the other tested genotypes. These results are also similar to that of Goldasten *et al*. (2012) which studied the biology of green bug on four wheat varieties and proved that the intrinsic rate of increase was lower on Zagros variety 0.222±0.00 day^{-1} and higher on Pastor variety 0.276±0.002. In our case, one of major factors *i.e*. allelo-chemicals, physical and morphological barriers may be responsible for resistance of MPT-V 5 to green bug.

The standard mass seedling screening techniques to evaluate small grains for resistance to green bug was developed by Wood (1961), which identifies several wheat accessions having tolerance to green bug. Based on tolerance resistance results during the third experiment, minimum proportional plant dry weight changes (DWT) were observed in the genotype 'MPT-V 33' (51.5 %) and maximum changes in genotype 'MPT-V 13'(108.9). These changes were found in shoot DWT '57.2%', '57.7%' in root and '108.9%' in total plant. However, minimum proportional plant DWT were observed on MPT-V 33. Contrary to our results, Voothuluru *et al*. (2006) focused on the importance of independent root and shoot dry weight measurements rather than whole plant measurements, while, in our case the two parameters have been taken separately but added mathematically for total plant dry weight change.

In the case of tolerance index (TI), minimum values for roots, shoots and plant total were observed for 'MPT-V 33, which indicated that genotype 'MPT-V 33' is relatively more tolerant among the tested genotypes. The tolerance character present in 'MPT-V 33' may be due to activation of the biomass accumulating metabolic genes such as those involved in cell repair or photosynthesis regulation in response to the aphid feeding (Smith and Boyko, 2007; Smith *et al*., 2010).

Most of the authors in Pakistan have evaluated wheat genotypes against different aphids based on, field population, visual count methods or aphid density per tiller/plants, however, no study has ever been conducted in details regarding different components of resistance. Therefore, this study was developed to investigate newly developed detailed procedures and characterize genotypes with different categories of resistance on the basis of established methods for host plant resistance. During the current genotypic evaluation, the tolerance category of resistance was calculated on the basis of proportional plant dry weight change and tolerance index value. Thus 'MPT -V33' is recommended as the new standard tolerant genotype for future line testing while, the genotype 'MPT-V 5' proved antibiosis resistance to the green bug. These results provided base line data for future host plant resistant programs in our country and both these genotypes, can be used as standard samples for any level of research against the green bug in lab/field experiments.

It is summarized that during the current evaluation of the ten genotypes showing some vigor to green bug in preliminary test. During antixenosis, no genotype carried significant resistance, however within the antibiosis experiment; genotype 'MPT-V 5' exhibited significantly high antibiosis resistance to the green bug *Schizaphus graminum* among the tested genotypes, while the genotype 'MPT V-33' showed strong tolerance characteristics.

CONCLUSIONS

Thus it is concluded that none of the tested genotypes showed significant antixenosis resistance, however, during the tolerance experiment genotype 'MPT-V 33' was relatively tolerant compared to all the tested genotypes. The genotype 'MPT-V 33' can be used as a base line for tolerance in future screening programs. While, genotype 'MPT-V 5' proved significantly resistant compared to tested genotypes and even strong resistance than the standard cultivar (Tatara-98). The genotype 'MPT-V 5' is categorized as antibiosis resistance and needs further detailed investigation at the molecular level to manage the green bug.

Statement of conflict of interest

Authors have declared no conflict of interest.

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