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SCREENING FOR RESISTANCE TO APHID IN BARLEY

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Abstract:-The resistance levels of six barley (*Hordeum vulgare* L.) entries namely B-00080, NRB-37, V-8, Haider-93, B-99036 and JAU-87 of 2003-04 National Uniform Barley Yield Trial (NUBYT) were evaluated against the aphid, *Rhopalosiphum padi* (L.). Evaluation was done by seedling bulk test method for finding out resistant, moderately resistant and susceptible entries. The entry B-99036 was found to be resistant. Further studies were conducted to determine the mechanisms of resistance like antixenosis, antibiosis and tolerance. Results indicated that the entry B-00080 had antibiosis and B-99036 was the tolerant one. So, on the basis of present studies the entry having high level of resistance against *R. padi* (B-99036) should be incorporated in the future breeding programmes.

Key Words: Hordeum vulgare; Resistance; Rhopalosiphum padi; Screening; Evaluation; Pakistan.

INTRODUCTION

Pakistan is one of those countries in the world, which are mainly dependent on agriculture. To meet the food requirements of such a huge population it is imperative to make candid efforts to enhance agricultural productions per unit area (Akhtar et al. 2006). Aphids damaging the wheat crop in Pakistan are *Rhopalosiphum padi* (L.) *Rhopalosiphum madis* F. *Schizaphis graminum* R. and *Sitobion avenae* F. (Akhtar et al., 2010). Aphid population affects the produce adversely (Wratten and Redhead, 1976; Girma et al., 1993) by causing 35-40% yield losses directly (Kiechefer and Gellner, 1992) and 20- 80% yield losses indirectly by transmitting viral and fungal diseases (Marzochi and Nicoli, 1991; Rossing et al., 1994; Trdan and Mileroj, 1999). Aphid (*Rhopalosiphum padi* L.) is a serious pest having a wide host range of at least 60 plant species including wheat, barley, sorghum and corn. Bowling et al., 1998). It sucks sap and injects toxin into the plant that interferes with grain formation (Kannan, 1999). An abundance of aphids adversely affects the nitrogen and protein contents, weight of 1000 grains, number of grains per ear (Ciepiela, 1993) and results in a decrease in carbon assimilation rate, transpiration and total chlorophyll (Ryan et al., 1987) and reduction in plant biomass (Holmes et al., 1991).

The incidence of aphids has been reported to be significantly different on different cultivars of wheat (Hinz and Daeber, 1976; Aheer et al., 1993; Parvez and Ali, 1999; Ahmad and Nasir, 2001) because their pre-reproductive, reproductive and post-reproductive periods and fecundity are significantly affected by crop varieties (Saikia et al., 1998). In IPM, plant resistance to insects refers to the use of resistant crop varieties to suppress insect pest damage. Plant resistance could be used in conjunction with other direct control tactics (Akhtar et al., 1991).

The present work was conducted to screen some barley varieties/ lines for resistance against the aphid (*Rhopalosiphum padi* (L.).

MATERIALS AND METHODS

Studies were conducted in the laboratories of the Insect Pest Management Programme (IPMP), Institute of Plant and Environmental Protection (IPEP), National Agricultural Research Centre (NARC), Islamabad. Barley seeds of 2003-04 National Uniform Barley Yield Trials (NUBYT) were

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collected from Wheat Programme, NARC. The varieties/lines tested were B-00080, NRB-37, V-8, Haider-93, B-99036 and JAU-87. The variety JAU-87 was included as a local check. These were screened by flat test method and then categorized as Resistant (R), moderately resistant (MR) and susceptible (S). These varieties/lines were further tested to find out their mechanisms of resistance i.e., antixenosis (non preference), antibiosis (anti-fecundity) and tolerance.

Survey and Establishment of *Rhopalosiphum padi* (L.) Culture

Survey was conducted at NARC, Islamabad. Wheat fields were thoroughly observed for the collection of bird cherry oat aphid *(R. padi*). The culture of aphids was reared on a susceptible wheat *Triticum aestivum* L. in the laboratory. The experiments were conducted in the laboratory at 27± 2 °C, and 65 ± 5% R.H. with a photoperiod of 16: 8 h(light: dark).

Evaluation through Seedling Bulk Test (Flat Test)

One row of each test entry was sown in the soil in a metal tray, measuring 51 cm x 35 cm x 9cm. There were eight rows in total in one tray and one variety/ line was sown in each row. The rows were made with a wooden mold. There were 20 seedlings of an entry in a row. When the seedlings were about 5 cm high, *R. padi* were released @ 10 aphids per seedling on them. The lodging started after about 10-15 days. The damage to each entry was visually recorded on 0 to 9 damage rating scale (Webster and Inayatullah, 1984). The entries were classified as highly resistant with DR (damage rating) 0-3, moderately resistant with DR, 4 -6 and susceptible with DR, 7-9.

Determination of Components of Resistance

Antixenosis Test

For Antixenosis test of 2003-04 (NUBYT) years against *Rhopalosiphum padi*, six entries were planted randomly in a circular pattern, about 3 cm from the edge of 30 cm diameter plastic pot. There were ten replications for each entry. There was one seedling of each entry in the pot. When the seedlings were about 5 - 8 cm tall, 60 adult apterous *R. padi* were released in the center of the pot (Webster and Inayatullah, 1988). A round cage made of transparent acetate sheet, 30 cm in height was placed inside of the plastic pot around the seedlings infested with *R. padi.* The tops of cages were covered with muslin cloth. There were two ventilation holes (5cm dia) on the sides of cage covered with muslin cloth. After 24h all the plants were observed and *R. padi* on each plant were counted. The second reading was taken after 48 h.

Antibiosis Test

For antibiosis test of NUBYT--2003-04 against *R. padi*, there were ten replications for each entry while the total entries were six. Two seeds of each entry were sown in 7cm diameter pots which were thinned out to single seedling in each pot for experimental purpose. These individual plants were infested with one aphid per seedling and placed in transparent acetate sheet round cages (6cm x 30cm). The top and the side ventilation holes were covered with muslin cloth (Inayatullah et al., 1993). The plants and *R. padi* were observed daily. When reproduction started the adult and nymphs were removed, leaving only one nymph per seedling. This one nymph was allowed to grow on the test plant until it matured and began to reproduce. The nymphs laid were counted and removed daily until the adult stopped reproduction.

Tolerance Test

For tolerance test of NUBYT--2003-04 against *R. padi*, there were ten replications for each entry while the total entries were six. Individual seedlings were grown in the pots of same size as described in antibiosis test. When seedlings attained the height of 5 cm they were infested with laboratory reared apterous *R. padi* @ 10 per seedling (Inayatullah et al., 1993). The plants were observed daily. Nymphs of *R. padi* produced were counted and removed daily leaving 10 apterous adults. After 12 days the infested plants were visually rated for damage on damage rating scale through visual observation from 0 (no damage) to 9 (dead or dying plant).

Statistical Analysis

The data obtained were analyzed statistically using analysis of variance (ANOVA) (Steel and Torrie, 1980) and the mean values were compared by using Duncan's multiple range test (DMRT) at 5 % level of significance (Duncan, 1955).

RESULTS AND DISCUSSION

Seedling Bulk/Flat Test

Data revealed that out of six NUBYT 2003-2004, the entry B-99036 was resistant whereas B-00080, NRB-37 ,V-8, Haider-93, JAU-87 were moderately resistant (Table 1). Among moderately resistant entries, Haider-93 and JAU-87 (local check) were comparatively more resistant with DR 4.5 followed by B-00080, NRB-37, V-8, with DR 5. Thus, there was more variability in moderately resistant lines. Results were similar to those of Schliephake and Geissler (1995), who reported that a total of 203 accessions of barley and wheat from the gene bank Gatersleben were tested for resistance to *Sitobion avenae, Rhopalosiphum padi* and *Metopolophium dirhodum* under defined conditions. Five of 152 accessions tested showed resistance to *R.padi* 16 of 125 to *S. avenae* and 12 of 109 to *M. dirhodum*. Nahid et al. (1991) reported that Green bug, *Schizaphis graminum (*Rondani), is an important aphid species attacking barley. Eighty one barley lines were tested in the laboratory to explore the new sources of resistance. They advocated that use of resistant varieties is an effective tool of management against this pest.

Table1. Damage rating (DR) of NUBYT (2003-04) against bird cherry oat aphid through screening by flat test

NUBYT 2003-04	Damage rating	Remarks
B-00080	5.0	MR
NRB-37	5.0	MR
V-8	5.5	MR
Haider-93	4.5	MR
B-99036	3.0	R
JAU-87	4.5	MR

R = Resistant, MR= Moderately resistant

Components of Resistance

Antixenosis Test

In the first experiment of Antixenosis, preference of bird cherry oat aphid was recorded through visual observation on six varieties of NUBYT--2003-04. Results showed that entries had shown significant differences in preference level of *R. padi* (Table 2). The maximum number of adults of *R. padi* were found on B-00080 followed by NRB-37 V-8 Haider-93 with mean population of 6.4, B-99036 and JAU-87 (local check). Among these entries, most preferred were B-00080 and NRB-37. The variety JAU-87 was least preferred while V-8, Haider-93 and B-99036 were moderately preferred. Lamb and Mackay (1995) reported that seedlings exhibits antibiotic and antixenosis resistance to the aphids *Metopolophium dirhodum* and *R. padi* because the seedling contain hydroxamic acid or gramine. Messina et al. (2002) examined the effects of prior infestation by the Russian wheat aphid, *Diuraphis noxia* (Mordvilko) and the bird cherry oat aphid *(R. padi)* on the subsequent feeding preference and performance of each species. Aphid colonies fed and reproduced on wheat seedlings for five days and were then removed. The estimated aphid population growth and feeding preference on control and previously infested plants was noted. *D. noxia* adults preferred to feed on leaves from control plants. *R. padi* adults showed antixenosis (no preference). Both aphids preferred leaves from control plants to those from *R. padi* infested plants. The plant quality was lowered by prior *R. padi* infestation but not by *D.noxia* infestation. Results of antixenosis test indicated that line V00BT004 was least preferred (LP) after 24h

Table 2. Antixenosis in NUBYT (2003-04) against *R. padi*

Cultivar	Adults(nos.)	Resistance level
B-00080	10.4a	HP
NRB-37	9.4ab	MP
V-8	7.8ab	MP
Haider-93	6.4ab	MP
B-99036	5.8ab	MP
JAU-87	4.2b	LP

HP = Highly prefered; MP = Moderately prefered; LP= Least prefered
Values followed by same letter(s) do not differ significantly at p= 0.05.

release of aphids with mean preference rating of 2.6 (Akhtar et al., 2007).

Antibiosis Test

There was great variation in resistance and susceptibility of 6 lines. Therefore average fecundity of nymphs laid was taken to check the resistance and susceptibility of the different lines.

Results showed that comparatively higher mean numbers of nymphs were found on NRB-37, followed by Haider-93 , B-00080 , JAU-87 and V-8 in the antibiosis studies (Table 3). The resistant entry was B-99036. All these tests indicated that three lines were comparatively more resistant than others. These lines may be further investigated for their source of resistance (Inayatullah et al., 1993).

Tolerance Test

The mean DR to wheat cultivars after 10 days of infestation in the tolerance test was lowest on PARI-73 followed by Sandal and Punjab-85 (Table 4) in descending order, with the means not significantly different (P > 0.05) (Inayatullah et al., 1993).

Table 3. Antibiosis in NUBYT (2003-04) against*R. padi*

Cultivars	Nymphs (nos.)	Resistance Level
B-00080	1.75b	MF
NRB-37	2.27a	HF
V-8	1.55b	HF
Haider-93	2.15a	HF
B-99036	1.08b	MF
JAU-87	1.17b	MF

HF= Highly fecund; MF=moderately fecund
Means followed by same letter(s) do not differ significantly at P= 0.05.

The entry B-00080 was tolerant. The remaining entries were moderately tolerant with their mean damage rating ranging from (4.4-4.7) followed by JUA-87(4.4), Haider-93 (5.5), NRB-37 (4.6) and B-99036(4.4). The least tolerant line was V-8 (Table 4). Tollo and Richter (1997) also studied the susceptibility of two winter barley cultivars to infestation by *R. padi*. Aphid growth and reproduction were greater on the cultivar Asorbia than on Grete. In spite of a higher number of aphids on Asorbia, changes in growth showed a similar pattern 21 days after infestation in both cultivars. With similar losses in shoot mass, the mass of roots in variety Grete decreased to a greater extent after 28 days of increased feeding activity by aphids. There was no difference in total mass between the cultivars. Asorbia was classed as more tolerant to infestation by *R. padi* than Grete.

It is obvious that out of three components of resistance, entry B-99036 was resistant in seedling bulk test, JAU-87 was least preferred, B-99036 was least fecund and B-00080 was tolerant one. These cultivars may be further investigated for their source of resistance. This information will be useful to incorporate into the breeding programme. Results of antixenosis test showed that least preferred (highly resistant) cultivar was JAU-87(local check) with

Table 4. Tolerance in NUBYT (2003-04) against *R. padi*

Cultivar	Damage rating	Nature of resistance
B-00080	4.0a	Tolerant
NRB-37	4.6a	Moderately tolerant
V-8	5.1a	Least Ttlerant
Haider-93	4.5a	Moderately tolerant
B-99036	4.7a	Moderately tolerant
JAU-87 (L-Check)	4.4a	Moderately tolerant

Means followed by same letter(s) do not differ significantly at P= 0.05.

mean number of 4.2 aphids / plant. Highly preferred (least resistant) cultivar was B-00080 with mean number of 10.4 aphids / plant.

In antibiosis tests, aphids were least fecund (highly resistant) entry was B-99036 producing 1.08 nymphs during the life cycle of female. Highly fecund (least resistant) entries were NRB-37 and Haider-93 produced during the life cycle of a female.

In tolerance test there were non-significant differences in the damage rating (DR) of cultivars. Least preferred (highly resistant) entry was B-00080. Highly preferred/ least resistant entry was V-8 Schliephake and Geissler (1995) reported that a total of 203 accessions of barley and wheat from the gene bank Gatersleben were tested for resistance to *Sitobion avenae*, *Rhopalosiphum padi* and *M. dirhodum* under defined conditions. Five of 152 accessions tested showed resistance to *R. padi,* 16 of 125 to *S. avenae* and 12 of 109 to *M. dirhodum*. Most accessions with resistance to *M. dirhodum* could be found in the barley collections, while in *S. avenae* and *R. padi* wheat species showed resistance. Only *Triticum boeticum*, *Hordeum jubatum* and *H. bogdanil* were resistant to more than one aphid species. Beloshapkin (1998) worked on the food specialization of cereal aphids in relation to the development of resistant varieties of cereal crops. Studies on the antibiosis of wheat species against *Sitobion avenae* F. and *Rhopalosiphum padi* L. demonstrated a relationship between the antibiosis of different species of wheat and their genomes and degree of hygrophily. Plants of the line of *Triticum boeoticum* L. are recommended for use as donors of resistance genes for interspecific hybridization ($F = 2.334$, $P = 0.0732$).

Understanding of these laboratory results towards the inheritance of aphid resistance in these entries would help breeders to develop an effective strategy for utilization of these cultivars in their breeding programmes. The results indicate that resistant entries against *R. padi* would be good addition for enhancing economic yield of barley crop by incorporation of safe, cheap and integral part of IPM (host plant resistance). Discovery of the cultivars conferring resistance would be of an immense value.

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