Evaluation of Barley Varieties against Russian Wheat Aphid (Diuraphis *noxia* M.) under Greenhouse Condition

Alemu Araya Kidanu

Department of Dryland Crops and Horticultural Sciences, Mekelle University, Mekelle, Ethiopia.

Corresponding author: allexxx.araya@gmail.com

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Abstract: *Russian wheat aphid (RWA) (Diuraphis noxia M.) is the major insect of barley in many areas in the world. It was reported in the Wukro (Atsbi) and Adigrat regions of northern Ethiopia in 1972/73 and western Welo region of northwestern Ethiopia in 1974. RWA causes severe damage to barley in the highlands of Ethiopia. However, only little information is available on the control of this pest in the country. An experiment was conducted in the 2013/2014 off-season at South Gondar Zone (Debretabor). The experiment aimed at evaluating some resistant sources of barley varieties against RWA was conducted in greenhouse conditions of the university site. Five barley varieties (Burton, RWA-1758, 3296-15, Holker and local susceptible) were studied in complete randomized design. The number of aphids per tiller decreased on the resistant varieties as compared to the control; this is probably due to their own inherent resistant character. There were also significant differences($p<0.001$) in mean chlorosis, leaf rolling, RWA population, leaf number per tiller and tiller number per plant among the resistant and the susceptible varieties. Severe plant damage (36.6%) was observed on the local barley variety while the least damage was observed on Burton, followed by RWA-1758. Burton and RWA-1758 were therefore highly resistant and moderately resistant, respectively. The damage to barley lines 3296-15 and Holker was greater than Burton and RWA-1758 and highly lower than the local one. From the result, it was noted that resistant varieties provided much lower damaged plants and population of RWA per tiller and much higher yield components than the susceptible varieties. This indicates that the most effective approach in managing the RWA is the use of resistant variety. Hence it is concluded that the use of host plant resistance is an important avenue for RWA management, and is one of the favored control options for aphids.*

Keywords: Russian wheat aphid, *Diuraphis noxia, barley*, *Hordem vulgare* L. host resistance

1. Introduction

Barley (*Hordeum vulgare* L.) is one of the most important staple food crops grown in the highlands of Ethiopia and believed to have been cultivated in Ethiopia as early as 3000BC (Hailu and Leur, 1996). In the main season (*Meher,* Amharic version), it is the fifth major cereal crop after maize, sorghum, tef and wheat in terms of area coverage and total production (CSA, 2013). In the off-season (*Belg,* Amharic version), barley is the second major cereal crop after maize in terms of area coverage and total production (CSA, 2013). The crop is grown in diverse ecologies with altitudinal range of 1800 to 3400 m (Lakew *et al*., 1993).

Russian wheat aphid (RWA), *Diuraphis noxia* (Mordvilko), is the major insect that reduces yield of barley and has worldwide distribution including the Middle East, U.S.A., South Africa, and Ethiopia (Girma *et al*., 1993). RWA was reported in the Wukro (Atsbi) and Adigrat regions of northern Ethiopia in 1972/73 and western Welo region of northwestern Ethiopia in 1974 (Adugna and Tesema, 1987). In about a year, the insect was recorded from all barley and wheat growing regions of the country (Adugna and Tesemma, 1987).)

Crops damaged by RWA include wheat, *Triticum aestivum* L.; barley, *Hordeum vulgare* L.; oat, *Avena sativa* L.; rye, *Secale cereale* L.; and triticale, *X triticosecale* (wittmack) (Walters *et al*., 1980); but barley and wheat are the most affected by RWA. Alternate hosts for RWA include volunteer wheat and barley such as wild species of *H. vulgare spp spontaneum* (Badr *et al*., 2000) and a number of cool and warm-season grasses on which it survives the dry period in between harvests (Kindler and Springer, 1989). Bayeh and Tadesse (1994) reported that the successive cropping system of barley and wheat in the highlands of Ethiopia enables the pest to migrate from one field to another and survive from one season to the next.

In Ethiopia, the yield of barley is very low in which 0.96 to 1.33 t ha^{-1} is in both in the *meher* and *belg* seasons (Lakew *et al.*, 1993). This is very low compared to the potential maximum yield of 13.3t ha^{-1} reported by other countries (FAO, 1994). The major reason for low yield is that the crop is produced under numerous constraints including RWA.

In spite of the increasing importance of RWA on barley production in Ethiopia, only few works have been done in the area of varietal host resistance. Host plant resistance to insect pests of crop plants is generally seen as an effective, environmentally responsible, economically and socially acceptable method of pest control which plays an integral role in sustainable agricultural systems (Wiseman, 1999).

Host plant resistance is an important avenue of pest management, and it is one of the favored control tactics for the cereal aphids (Robinson, 1992). The use of host plant resistance in Ethiopian situation is often limited to avoidance of susceptible barley varieties and the subsequent shift to early maturing varieties by farmers. The only barley variety so far identified by Holetta Agricultural Research Center as resistant to RWA was a barley line 3296-15.

Yield losses due to RWA are severe with individual plant losses as high as 90% possible (Du Toit and Walters, 1984). Robinson (1992) recorded crop losses of 68% in Ethiopia and 35-60% in South Africa for wheat. This insect generally causes yield losses of 41-79 % in barley and up to 86% in wheat in Ethiopia (Miller and Adugna, 1988).This severe grain and biomass yield reduction is associated with these symptoms. Typical white, yellow and purple to reddish purple longitudinal streaks occur on the leaves of plants infested with RWA. The aphids are found mainly on the adaxial surface of the newest growth, in the axils of leaves or within rolled leaves. Heavy infestations in young plants cause the tillers to become prostrate, while heavy infestations in later growth stages cause the ears to become trapped in the rolled flag leaf (Walters *et al.*, 1980). RWA infestation leads to a drastic reduction in chlorophyll content (Kruger and Hewitt, 1984) and reduced photosynthetic ability (Fouche *et al.*, 1984)which, when combined with the characteristic leaf rolling that occurs, causes a considerable loss of effective leaf area of susceptible plants (Walters *et al.*, 1980).

In an attempt to better understand host plant resistance to RWA and their use as management measures in the form of resistant cultivars, this study is highly significant to investigate mechanisms (i.e. antixenosis, antibiosis and tolerance) of resistance to RWA and the influence of resistance on population development of RWA in the field. This may assist breeders in future efforts to better understand and therefore, successfully exploit genetic resistance to this damaging pest. In addition, quantifying the yield loss due to RWA damage, in commercially available resistant cultivars will illustrate the practical application of this resistance under field conditions. It is therefore necessary to evaluate host plant resistance efficiently against RWA on barley. Thus, this study was initiated with the objective of evaluating barley varieties against Russian wheat aphid populations under green house conditions.

2. Materials and Methods

2.1. Description of the study area

The pot experiment was carried out in South Gondar Zone at Debretabor University's site. Debretabor is located at the latitude of 11o 51' N and longitude of 38o 00' E. The elevation is 2500 m above sea level. The area is situated in woina dega agro-ecological zone of the region, which is characterized by low and erratic rainfall. Annual rainfall ranges from 1500 to 2000 mm while the average maximum and minimum temperature is 22.1 and 9.5 0C, respectively. The soil type is mainly clay loam. The major crops grown in the area are barley, wheat, potato, bean, millet, and lentil.

2.2. Experimental design and treatments

The study was conducted in a greenhouse as pot experiment in controlled conditions. Two Russian wheat aphid resistant barley varieties from the United States (Burton and RWA-1758) (Bregitzer *et al.*, 2005) (MARC), one tolerant barley variety (3296-

15) from HARC, one improved malty barley variety (Holker), improved standard check from AARC and a susceptible local check (kinkina) from North Western Ethiopia (Debretabor) were included in the experiment. The experimental design was complete randomized design (CRD) with three replications. There were a total of 15 treatments.

2.3. Experimental procedures

Five seeds from each entry were placed to a depth of 2.5 cm in a plastic pot filled with a medium composed of 2:1 silt/sand mixture. The height and diameter of the plastic pot used were 25 and 20 cm, respectively. Six kilo grams of soil were used for each pot. Pots were placed on the table with 50 cm height above the ground. The space between pots was 20 cms. Then, emergence seedlings were thinned to three plants per pot. Plants were infested with 5 RWA adults at Zadok's 3- leaf stage (Zadok *et al*., 1974). The RWAs were placed on each plant after 14 days with a soft brush. Each pot was received equal number of insects. Infested plants were immediately covered with mosquito nets (perforated net to allow ventilation) until the plant reaches flowering stage. Pots were covered with a fine net cloth for easy entry of air and to prevent the movement of the aphids from one pot to another. The area covered to avoid the movement of the aphids from one pot to another was 4 m x 2 m (8 m^2). The height and width of the area covered with fine net cloth were 1 m x 2m (2 m^2). The RWA populations (colonies) that served as a source of infestation were obtained from nearby barley fields that were planted one month before the start of the experiment. A local barley variety was planted on the field with plot size of 2 m^2 to harbor these aphids. The aphids were taken from the leaves of the tillers by dusting them over paper using soft brush and then infesting the pots with 5 RWAs properly. Care was also taken by carefully selecting the RWAs to avoid parasitism. Plants were examined for aphid populations and plant damage 14 days after being infested. Each plant was evaluated for the following data (Zadok *et al*., 1974).

2.4. Data collection

Chlorosis was recorded visually from the leaf of tillers after seedling emergence to flowering stage with 14 days intervals using 0-9 scoring scale (Webster *et al*., 1987), where 0: Immune, 1: plants appear healthy, may have small isolated chlorotic spots, 2: isolated chlorotic spots prominent, 3: chlorosis ≤ 15% of the total leaf area, chlorotic spots coalesced, 4: chlorosis > 15% but ≤ 25% of the total leaf area chlorotic lesions coalesced, streaky appearance, 5: chlorosis > 25% but ≤ 40% of the total leaf area, well defined streak, 6: chlorosis > 40% but ≤ 55% of the total leaf area, 7: chlorosis > 55% but ≤ 70% of the total leaf area, 8: chlorosis > 70% but < 85% of the total leaf area, and 9: plant death or beyond recovery.

Leaf rolling was recorded visually from the leaf of tillers after seedling emergence to flowering stage with 14 days intervals on a rating scale of 1-3 (Webster *et al*., 1987) where 1: No leaf rolling, 2: One or more leaves conduplicately folded, and 3: One or more leaves convolutedly folded.

RWA population count per tiller was taken from the leaves of the tillers by dusting the aphids over paper using soft brush and then counting them individually every two weeks interval after infestation.

Plant height was recorded as the length of the plant in cm from the base of the main stem to the tip of the panicle excluding the awns at late flowering stage.

Number of tillers per plant was recorded at the average number of total tillers per plant without panicle excluding the main shoot.

Number of leaves per tiller was recorded at the average number of total leaves per tiller.

2.5. Data analysis

The collected data were analyzed using the GenStat 12^{th} Edition statistical software (VSN international Ltd, 2009). The count data were subjected to square root transformation. Analysis of variance procedure was employed. Fisher's tests were also used to separate the means whenever found significant at 1% probability label.

3. Results and Discussion

3.1. Evaluation of Barley varieties' resistance to RWA population under greenhouse conditions

Analysis of variance for plant damage (chlorosis and leaf rolling), RWA population count, number of leaves per tiller, number of tillers per plant and plant height as influenced by host plant resistance is presented in the respective Tables. Significant differences (p <0.001) were observed among all the varieties for all variables except for the plant height which is not significant at p >0.01 level.

3.1.1. ***Chlorosis***

The mean Chlorosis score of the tested barley varieties is presented in Table 1. Significant differences in leaf chlorosis (p< 0.001) were observed among the tested barley varieties as compared with local check. Larger chlorotic streaks and higher chlorotic scores (7.00) were observed on the local susceptible variety, resulting from the depression of cytokinin synthesis or loss of chlorophyll molecule by the sucking action of RWA population. Wiese (1987) reported that Russian wheat aphid and certain other species inject toxic saliva that causes localized discoloration of host tissue of susceptible varieties. The least chlorotic score was observed on Burton (1.46) followed by RWA-1758 (2.10) (Table 1). As the result indicated, the two tested barley varieties Burton and RWA-1758 are resistant to the RWA populations on a rating scale of 0-9 (Webster *et al*., 1987). In case of cereal plant resistance to aphids, success has been achieved with the RWA that causes easily detectable plant damage, and selections can be based on reduced chlorotic symptoms (Berzonsky *et al*., 2003).

The chlorosis score for the barley line 3296-15 and Holker (3.76) was in fact higher compared to Burton and RWA-1758, but it was lower than that of the susceptible local variety. According to Bayeh *et al*. (2008), the barley line 3296-15 had a lower leaf chlorosis score of 4.33 to the Shewa RWA populations. In this study, however low leaf chlorosis score was recorded with the Gondar RWA population indicating a probable genetic variation between RWA populations of Gondar and Shewa. Least chlorosis is often associated with a resistant reaction as several workers (Botha *et al*., 2005) have not reported significant changes in leaf color (chlorosis) and a reduction in photosynthetic activity for resistant cereal hosts.

3.1.2. ***Leaf rolling***

The mean of leaf rolling of the tested barley varieties is also presented in Table 1. The leaf rolling caused by RWA was significantly influenced (P<0.001) by the degree of resistance. Leaf rolling was high on the local susceptible variety, whereas Burton had the lowest leaf rolling score, which was significantly different from the other varieties, though the reaction of RWA-1758 was relatively closer to Burton. Similarly, barley line 3296-15 had a leaf rolling score of 1.70 which was again significantly different from the rest of the varieties. The reaction of Burton was considered as flat leaf as stated by Burd *et al*. (1993). So, Burton is highly resistant from all the tested varieties regarding leaf rolling to the RWA populations. The leaf rolling value of the RWA-1758 was (1.33) and that reaction was in sooth highly lower compared to the local variety (2.43) and it was rated as less than fully folded leaves on a rating scale of 1- 3 (Webster *et al*., 1987).

Barley line 3296-15 had a tolerant leaf rolling reaction of 3.17 on a 0-9 scale at Holetta (Bayeh *et al*., 2008). The reduction of leaf rolling score of 1.70 (moderate resistance reaction) on a 1-3 scale of Webester *et al*. (1987) could also be another indication of genetic variation between RWA population of Shewa and Gondar. Holker variety in fact was higher (2.10) than Burton and RWA-1758 but it can be considered as moderate susceptible as compared to the local variety which scored 2.43 according to the rolling scale. Feeding damage by RWA to plant leaves results in yellow or red chlorotic streaks with a convoluted rolling of the leaf for susceptible plants. Khan *et al*. (2011) confirmed that rolling of the leaves reduces photosynthetic area and protects aphids from contact insecticides and natural enemies. The heads that developed on tillers that had severe leaf rolling were trapped and did not extrude from the flag leaf sheath and this was particularly true for the susceptible variety (Table 1). On such heads, there was no seed development at all. Susceptible barley lines become stunted under heavy aphid attacks and prepanicle infestations can result in curling of the flag leaves and panicle deformations (Jones *et al*., 1989; Kindler and Hammon, 1996). In contrast to the reactions of the susceptible variety, the resistant varieties Burton and RWA-1758 barley lines were essentially asymptomatic. RWA feeds on host plants in dense colonies within tightly curled leaves, which result in rolling up of fully expanded leaves and by preventing the normal unrolling of newly emerging leaves (Hewitt *et al*., 1984).

Thus, the observations of far larger RWA populations on the susceptible variety relative to the resistant varieties were expected. Leaves are rolled as a result of the stress created by the sucking action of the aphids and it is quite natural for leaves not to roll when grown host plant resistance and that could be one of the possible reasons for reduction in degree of rolling of leaves. This is in line with the findings that host plant resistance plays important roles in controlling pests and protecting of natural enemies in an agroecosystem (Francis *et al*., 2001; Messina and Sorenson, 2001), and the effect on application of insect resistance plant varieties in reducing pest damage is considered to be conspicuous (Painter, 1958).

3.1.3. ***RWA population per tiller***

The population density of RWA was significantly influenced ($P < 0.001$) by the tested barley varieties. The mean RWA population of the tested barley varieties is presented in Table 1. The highest population of RWA per tiller was recorded on the local variety (26.80) and the lowest populations of RWA were recorded on Burton (7.36) followed by RWA-1758 (10.50) (Table 1). Brewer *et al*. (1999) also reported that the abundance of *Diuraphis noxia* on resistant barley lines was lower than that on more susceptible lines. The number of aphids per tiller was lowest on the resistant varieties as compared to the control; this is probably due to their own inherent resistant character. Leszczynski *et al.* (1995) reported that resistant varieties have higher concentrations of allelochemicals which restrain aphid development on plants, reduced fecundity and inherent rate of increase.

Indeed the population density of RWA for the barley line 3296-15 was greater than Burton and RWA-1758, but it was less than that of the susceptible local variety. The aphids were raised on susceptible barley under greenhouse conditions (Starks and Burton, 1977). The RWA population of the local variety was 26.8 and the population number of the barley line 3296-15 was 22.95. This shows that the barley line 3296-15 was by far lower aphid populations as compared to the local variety, but it was higher than Burton (7.36) and RWA-1758 (10.50). The incidence of aphids has been reported to be significantly different on different cultivars of wheat (Aheer *et al*., 1993; 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).

The population of the aphids on Holker was recorded very low (12.15) as compared to barley line 3296-15 (22.95), but the chlorotic and rolling capacity were very high. This is probably due to the lack of inherent resistance/loss of resistant gene behind the variety. Similarly, host plant resistance is one of the most vital factors which can handle aphid infestation well below the economic threshold level. Host plant resistance also lessens the chances of biotype development (Lowe, 1987; Riazuddin *et al.,* 2004).

Similar results were reported by Michel *et al*. (1994) where they found differences in RWA densities among barley lines and significantly more numbers of RWA per plant were recorded on susceptible varieties. In general, significantly lower numbers of RWA per tiller were recorded on the resistant varieties (Table 2). Akhtar and Hashmi (1992) confirmed that adequate aphid resistance against aphid pests could be achieved by implementing resistant varieties.

The findings demonstrate that resistant varieties affect the population of RWA and they may be used in integrated pest management systems. As stated by Smith (1989), the use of a resistant variety alone should not be expected to control pests under all conditions or in all locations where the crop may be grown. Instead, resistant varieties should be used in combination with other pest suppression measures including treatments of the susceptible varieties to reduce RWA damage drastically. When screening for resistance to pests and diseases for the purpose of selecting resistant plant genotypes, the common procedure is to grow different genotypes in greenhouses or climate chambers within a restricted area, in order to compare plants under similar environmental conditions (Smith, 1989).

Table 1. Reaction of barley varieties to RWA population, leaf chlorosis and leaf rolling under greenhouse experiment

Varieties	Plant damage parameters		
	Leaf rolling	Chlorosis	RWA population/tiller

Burton (V_1)	1.10[a]	1.46[a]	7.36[a]
RWA-1758 (V_2)	1.33[a]	2.10[b]	10.50[b]
3296-15 (V_3)	1.70[b]	3.76[c]	22.95[c]
Holker (v_4)	2.10[c]	3.76[c]	12.15[d]
Kinkina (local) (V_5)	2.43[c]	7.00[d]	26.80[e]
Mean	1.73	3.82	15.95
CV (%)	12.55	8.44	5.52

Means in columns followed by the same letter are not significantly different at p<0.001

3.1.4. *Number of leaves per tiller*

The mean of the leaves per tiller of the tested barley varieties is presented in Table 2. Significant differences (p <0.001) were detected in the number of leaves per tiller in the tested barley varieties. A significant difference was observed between the varieties (Burton and RWA-1758), the barley line (3296-15) and the local ones. But non- significant differences were recorded between the resistant varieties Burton and RWA-1758, and between Holker and the local barley variety. The highest number of leaves per tiller was recorded on the RWA-1758 (2.86) followed by Burton (2.83). The densities of trichomes on the leaf surface of some cultivars deter feeding and sometimes oviposition. Leaf trichome density and position may act as a physical obstacle to aphid feeding. According to Oberholster (2002, 2003) the high trichome density on the leaf veins could prevent the aphid from finding a suitable feeding site.

The number of leaves per tiller on barley line 3296-15 and Holker compared to the number of leaves on the local variety was higher (Table 2), whereas the least number of leaves per tiller (2.56) was recorded on the local variety on which the highest populations of aphids were recorded. An abundance of aphids adversely affects the nitrogen and protein contents (Ciepiela, 1993) and results in a reduction of total chlorophyll (Ryan *et al*., 1987) and reduction in plant biomass (Holmes *et al*., 1991). RWA had a greater impact on infesting leaf number of the susceptible barley variety. According to Burd *et al*. (1993), RWA feeding typically reduces leaf number in susceptible cereals.

3.1.5. *Number of tillers per plant*

Analysis of variance for the number of tillers per plant revealed significant differences (p<0.001) between the tested barley varieties. The mean of tillers per plant of the tested barley varieties are presented in Table 2. The variety Burton had the largest (3.47) number of tillers per plant followed by RWA-1758 (3.19). This result agrees with the findings of Mornhinweg (1994) who reported that resistant varieties had less percentage of tillers damaged by RWA than the susceptible varieties. The rest varieties had almost equal number of tillers per plant that is why no significant differences were observed between them. Anonymous (1995, 2013) also reported that aphids can affect the development in the early stages of the crops; long lasting infestation can reduce tillering.

3.1.6. *Plant height*

The mean plant height for the tested barley varieties is presented in Table 2. There was no significant difference (p>0.001) among the barley varieties in plant height. Burton and RWA-1758 had almost the same plant height (Table 2). From the result RWA-1758 was the longest (63.57) followed by Burton (58.80) where as the local variety was the shortest (42.40) one. Similarly, in Kenya Kiplagat (2005) reported extensive chlorosis and leaf rolling due to RWA retarded plant development and delayed ear emergence.

The barley line 3296-15 had relatively longer (57.50) plant height as compared with the local susceptible variety. A field study of Russian wheat aphid RWA on yield and yield components of field grown susceptible and resistant spring barley showed highly resistant lines, increased yield components and grain yield (average grain yield increase 5%) under aphids feeding pressure and susceptible cultivars had a large reduction in yield components and grain yield (average reduction 56%) (Mornhinweg *et al*., 2006). On the other hand, the local variety had the shortest one which was noT significantly different from the other barley varieties (Table 2). Burd *et al*. (1993) determined that plant stunting as best predicted the quantitative damage response to RWA infestations in oats, wheat, and triticale and that susceptible germplasm was stunted. While testing 557 wheat lines Li *et al*. (1998) found that there were significant differences in resistance among yield and other yield related parameters (height of plants, number of spikes/plant, number of spikelets/spike, length of spike/plant, and 1000 grain weight).

Table 2. Barley varieties of the number of leaves per tiller, number of tillers per plant, and height of the plant under greenhouse conditions

Varieties	Yield component parameters		
	Number of leaves/tiller	Number of tillers /plant	Plant height
Burton (V_1)	2.83[a]	3.47[a]	58.80[a]
RWA-1758 (V_2)	2.86[a]	3.19[a]	63.57[a]
3296-15 (V_3)	2.73[b]	2.70[b]	57.50[a]
Holker (v_4)	2.63[c]	2.830[b]	53.20[a]
Localvariety (V_5)	2.56[c]	2.50[b]	42.40[a]
Mean	2.72	2.94	55.09
CV (%)	2.62	6.68	17.81

Means in columns followed by the same letter are not significantly different at p<0.001

4. Conclusion and Recommendation

There were significant differences in mean chlorosis, leaf rolling, RWA population per tiller, leaf number per tiller and tiller number per plant among the resistant and the susceptible varieties. Severe plant damage was observed on the local susceptible barley variety. The least damage was observed on Burton variety followed by RWA-1758. From the result, the tested Burton and RWA-1758 barley varieties are resistant on a rating scale of 1-3 leaf rolling and 0-9 leaf chlorosis. The barley line 3296-15 was moderate resistant. From the result, host plant resistance was more effective in the control of RWA compared with the control and use of resistant varieties. It substantially reduced plant damage by RWA. This indicates that the most effective approach in managing the RWA is the use of resistant variety. This study conclusively demonstrated that population abundance of RWA was influenced by using host plant resistance, and the use of host plant resistance did not result in higher aphid infestation, instead their reduction.

Future research should concentrate on the interaction of host plant resistance and natural enemies as it is extremely important to provide the Ethiopian barley producer with an effective and inexpensive RWA control strategy.

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