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INHERITANCE OF RESISTANCE TO RUSSIAN WHEAT APHID (*Diuraphis noxia* Kurdjumov) IN BREAD WHEAT (*Triticum aestivum* L.)

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ABSTRACT

Diuraphis noxia **(Kurdjumov) (Hemiptera; Aphididae), Russian wheat aphid (RWA) causes death at heavily infested bread wheat (*Triticum aestivum* L.) plants. The pest is living in rolling leaves so its chemical control is difficult. The most effective and economical means of controlling Russian wheat aphid is through the use of resistant cultivars. Two resistant lines, 15 (FL302//BUC/PVN/3/RSK/CA8055//CHAM6) and 27 (BJN C 79/F96PYN3-1828) were crossed with susceptible cultivar Basribey in 2010 and the F_1 plants were selfed to produce the F_2 progeny to determine the inheritance mode after exposing to pest in 2011. Chi-square test showed that the segregation ratio fits a one gene segregation ratio of 3:1 resistant:susceptible phenotyping segregation ratio at the P≤0.75-0.50 and P≤0.50-0.25 level of probability in combinations respectively. In both combinations, chi-square test results indicated that a single dominant gene with minor genes governed resistance to RWA. Differential set from USDA (United State Department of Agriculture) with different *Dn* resistance genes used to find out the single dominant gene carrying the resistance. Variance analyses showed that the differences between reactions of resistant genes were important for chlorosis and leaf rolling. The lines carried *Dn7* resistant gene exhibited high level of resistance to the pest while moderate resistance conferred by the plants containing *Dn6* gene. Reactions of other *Dn* genes were close to susceptible control cultivars.**

Key words: Resistance, Inheritance, resistant cultivars, *Dn7* gene, Russian wheat aphid

INTRODUCTION

As a wheat pest Russian wheat aphid (RWA), is one of the most important and widely distributed insect species in the world. Pest causes leaf rolling and streaking, head trapping and even death heavily infested plants. Russian wheat aphid after the first record as a pest in Crimea in 1901, is one of the most devastating pest of wheat. After occurring in South Africa in 1978 it is a limiting factor in wheat production. It is also known as the key pest of wheat in the USA and in the other significant grain producing countries of the world. It was found that the pest caused the economic yield losses over one billion dollar in the USA, including not only losses in the wheat yield but also insecticide usage (Stoetzel, 1987; Elmali, 1998; Morrison and Peairs, 1998; Haley et al., 2004).

In Turkey, *D. noxia* was first recorded in Bitlis province in 1959. A few years later, it was recorded in Isparta, Ankara and wider areas of Central Anatolia, and in some areas of Adiyaman and Malatya provinces (Tuatay and Remaudiere, 1964). An epidemic occurred in 1962 and caused 25-60% crop losses in Konya province (Duran and Koyuncu, 1974; Altinayar, 1981). A survey was carried out to determine the current status of the *D. noxia* in major wheat-producing areas of Turkey in May 2010. In the survey conducted in the important wheat growing areas in Turkey (Izmir, Manisa, Usak, Kutahya, Eskisehir, Aksehir, Ankara, Konya, Aksaray, Nevsehir, Yozgat and Erzurum provinces) were checked in view of distribution, damage and population level of the pest. The survey showed that almost all areas were infested with the pest (Turanli et al., 2012).

Several effective management approaches have been proposed to reduce economic losses from *D. noxia*. The pest is living in rolling leaves so its chemical control is difficult. Although cultural and biological controls may have some effectiveness, the most effective and economical means of control growing resistant cultivars with one or more resistance genes. Resistant varieties could be the source of fundamental breeding studies. For almost two decades, there has been a worldwide effort by wheat breeders to identify and use new source of genetic resistance to the *D. noxia* (Du Toit, 1987, 1989). The first sources of resistance to Russian wheat aphid were identified from wheat that originated from countries where the pest is endemic, namely the former Soviet Union, the Balkans, Iran, Turkey and the rest of the Middle East (Harvey and Martin, 1990; Do Toit, 1992). In the United States, winter wheat resistance to the Russian wheat aphid was first identified in 1987 in the PI 372129 genotype (Quick et al., 1991). Inheritance of this type of resistance was governed by a single dominant gene, *Dn4* (Nkongolo et al., 1991a; Saidi and Quick, 1996). The first North American Russian wheat aphid-resistant hard red winter wheat cultivar, 'Halt', contained *Dn4*, was released to growers by the Colorado Agricultural Experiment Station in 1994 (Quick et al., 1996). Since the release of Halt, other cultivars containing *Dn4* have been released and are currently available to growers (Quick et al., 2001a, b, c, d; Haley et al., 2004). Roughly 25% of Colorado wheat acreage is planted with this Russian wheat aphid resistant variety. Many researchers have categorized resistance to Russian wheat aphid-resistant in wheat and have found varying degrees of antibiosis, antixenosis, and tolerance. Hawley et al. (2003) and Miller et al. (2003) reported that wheat containing *Dn4* exhibited antixenosis, antibiosis, and tolerance. Tolerance is the ability of the plant to grow when infested with aphids. Antixenosis refers to non-preference of a host by aphids and is measured by the number of adult aphids per plant (Kogan and Ortman, 1978). Antibiosis is measured by a significantly reduced fecundity of aphids (i.e. the number of nymphs per aphid) grown on plant and can occur together with tolerance or antixenosis. The incorporation of different types of resistance would provide plants with wider range of responses to the aphid.

In the previous studies of resistance, sources of resistance genes have been identified in wheat germplasm accessions including *Dn1* in the PI 137739, *Dn2* in the PI 262660 (Du Toit, 1989), *Dn4* in the PI 372129 (Nkongolo et al., 1991b), *Dn6* in the PI 243781 (Saidi and Quick, 1996), *Dn8 and Dn9* in the PI 294994, and *Dnx* in the PI 220127 (Liu et al., 2001). The PI 292994 was first hypothesized to have a single dominant gene called *Dn5* (Marais and Du Toit, 1993), but later was found to contain more than one gene in some genotypes. Three types of inheritance were observed in the PI292994: a single dominant gene, two dominant independent genes, or one dominant and one recessive gene carrying resistance to the RWA (Zhang et al., 1998).

The identification of a new, *Dn4*-virulent Russian wheat aphid biotype in the southeastern Colorado necessitated a rapid usage of new resistance sources to reduce economic losses from infestations with the new biotype. Information on the effectiveness of the *Dn7* gene or other resistance sources under field conditions would be helpful to evaluate the value of the resistance in breeding wheat cultivars with Russian wheat aphid-resistance (Collins et al., 2005).

Dn7 is a rye gene located on chromosome 1RS that carries resistance to the Russian wheat aphid. This gene was previously transferred from rye to a wheat background via a 1 RS/1BL translocation. The resistant line "94M370" was crossed with a susceptible wheat cultivar which also contains a pair of 1 RS/1BL translocation chromosomes. The F_2 progeny from this cross segregated for resistance in a ratio of 3 resistant 1 susceptible indicating a single dominant gene. The *Dn7* gene confers antixenosis and provides a higher level of resistance than that provided by the *Dn4* (Garret et al., 2003)

The aim of this study was to determine the inheritance mode by using the F_2 segregating generations and identify the gene which carries the resistance to the pest. Thus this study will be one of the first breeding attempt to determine the mode of inheritance to this pest and important problem of wheat production.

MATERIALS AND METHODS

Genetic Materials

Seeds of resistant lines 15 (FL302//BUC/PVN/3/RSK/CA8055//CHAM6) and 27 (BJN C 79/F96PYN3-1828) were obtained from the ICARDA (International Center for Agricultural Research in The Dry Areas) and the CIMMYT (International Maize and Wheat Improvement Centre). Seeds of susceptible cultivar (Basribey) were obtained from the Aegean Agricultural Research Institute. The lines number 15 and 27 were crossed with Basribey in Department of Field Crops, Faculty of Agriculture of the Ege (Aegean) University. The F_1 plants were selfed to produce the F_2 progeny in 2010. The plants of the F_2 generations were infested with the Russian wheat aphid and the injury evaluations were done on individual plants in 2011.

The differential set which have different resistant genes to the RWA, was obtained from the ICARDA (Originated from USDA, United States Department of Agriculture) and was presented in Table 1.

Table 1. The list of differential set from the United State Department of Agriculture (USDA)

USDA Small Grain	Plant/Variety/ID No	Origin	Resistant Gene
	Yama	USA	*Dn0*
PI 262660 TR05ID	Turtsikum	Azerbaijan, Naxcivan	*Dn2*
PI 372129	Yamar (Colorado)	Turkmenistan	*Dn4*
PI 294994	CO950043 (Colorado)	Bulgaria	*Dn5*
PI 47545 TR05ID	CI 6501	Iran	*Dn6*
RWA-MATRIX	CI 6501	Iran	*Dn6*
Tukey 77 (Rye)	94M370 (South Africa)	Turkey	*Dn7*
Gamatoos R	94M370 (South Africa)	Turkey	*Dn7*

Insect Culture

The insect materials were three different populations (İzmir, Kütahya and Ankara) of *Diuraphis noxia*. The aphid populations which had been taken as alive from wheat fields and reared as purely in the Department of Plant Protection, Faculty of Agriculture, Ege University were used in the studies. Aphid cultures were established and maintained on barley plants in the greenhouse with 20$\pm$1 ^{O}C temperature, 60$\pm$5% humidity and 16:8 photoperiods.

RWA Resistance Phenotyping

The F_2 individual plants were infested with Izmir population to test their reaction to RWA. Test plants were also reared in viols under same conditions. One wheat seed was sown in the mixture of soil and peat (1/1 ratio) in viol from each combinations or line. Irrigation was done every two days.

Tests with the differential set were done by hill plots with four replications. Each hill plots consisted of 5 plants. When the plants reached to 2-4 leaf stage, they were infested with 5 individuals of the pest per plants. The RWA damage was rated at 28 days after infestation. In 2011, leaf chlorosis and leaf rolling were evaluated based on 1-6 and 1-3 scales, respectively (Ennahli et al., 2009) (Table 2). According to leaf chlorosis scale, plants with 1, 2 and 3 values were accepted as resistant and with 4, 5 and 6 values as susceptible. For the leaf rolling scale 1 and 2 values were referred to resistant and 3 referred to susceptible. For this purpose, differential set including different *Dn* resistant genes were exposed to three different RWA populations.

Statistical Analysis

A chi-square test analysis was performed on the F_2 segregation data for the RWA resistance accepting a single dominant–gene model (Toker et al., 2012). All the data obtained from differential set were analyzed by the ANOVA. The average value of three different populations were used in the analyses variance of using TARIST (Acikgoz et al., 2004) and the differences between the means were compared by the LSD multiple range test according to Steel et al. (1997).

Table 2. Descriptors and rating scales used for evaluation of the F_2 population for resistance to Russian wheat aphid (Ennahli et al., 2009).

Scale	Description
Scale for leaf chlorosis (1-6)	
1	no chlorosis
2	<1/3 of leaf area chlorotic
3	1/3- 2/3 leaf area chlorotic
4	>2/3 of leaf area chlorotic
5	necrosis in at least one leaf
6	plant death
Scale for leafrolling (1-3)	
1	no rolling
2	trapping or curling in one or more leaves.
3	rolling in one or more leaves

RESULTS AND DISCUSSION

The Chi-square values of the plants which is belongs to F_2 generations of two crosses [Basribey (♀) x 15 (♂) and Basribey (♀) x 27 (♂)] were shown in Table 3. The 199 F_2 individual plants from the cross between Basribey (Susceptible) and line number 15 (Resistant) were segregated for RWA resistance. In the F_2, 144 plants were found as resistant, while 55 plants were identified as susceptible. Chi-square test showed that this segregation ratio fits to 3 (Resistant) :1 (Susceptible) ratio. The 200 F_2 individuals from the cross between Basribey (Susceptible) and line number 27 (Resistant), segregated for resistance to RWA, were shown in Table 3. Out of 200 individuals in the F_2, 159 plants were scored as resistant, whereas 41 plants were scored as susceptible. Chi-square test results of this combination were also similar with the previous segregation the ratio 3:1 resistant:susceptible.

Beside the distribution of the plants in the F_2 generation in two combinations according to leaf chlorosis scale showed that resistant and susceptible plants were not divided only in two groups (Figure 1). Results of the chi-square test and distribution of the plants in the F_2 generation indicated that a single dominant gene possibly with minor genes controls the resistance to the RWA (Table 3 and Figure 1). These results also implied that resistance in the parents (15 and 27) was also governed by a single dominant gene.

Table 3. Number of the F_2 plants, chi-square value and segregation ratio from two crosses

F_2 combinatons	Number of resistant plants	Number of susceptible plants	Total plants	Chi-square value	P value
Basribey X 15	144	55	199	0.735	0.75-0.50
Basribey X 27	159	41	200	2.16	0.50-0.25

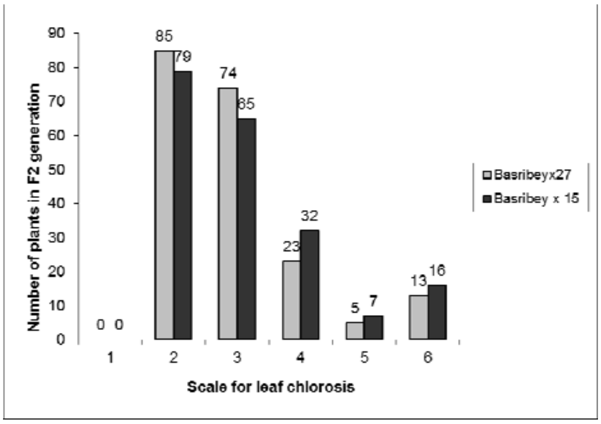

Figure 1. The classification of plants in the F_2 generation for leaf chlorosis scale.

According to Smith et al. (2004), 11 RWA resistance genes have been reported in wheat, rye and goat grass (*Aegilops* sp.). The locations of some of these genes on the chromosome are known, and the *Dn1*, *Dn2*, *Dn5*, *Dn6*, *Dn8* and *Dnx* genes were located on chromosome arm 7DS (Liu et al., 2005). Also, the *Dn7* gene is derived from rye, located on the chromosome arm 1RS, and it carries resistance to different RWA populations. In another study, Peng et al. (2007), the ratio of [H (Heterozygotes Dominance) + B (Homozygotes Dominance)] to A (Homozygotes Recessive) by using the molecular markers fits the expected ratio of 3:1 a single dominant gene model, indicating that the RWA resistance in the line 2414-11 is governed by a single dominant gene. Our findings studies were parallel with previous studies (Peng et al., 2007; Valdez et al., 2012).

In second part of this study, the resistant gene responsible for resistant to RWA was determined by using the differential set. Based on leaf chlorosis and leaf rolling reactions of all *Dn* genes to three RWA populations were similar. Chlorosis and leaf rolling scale values were shown separately in Figure 2 and 3 to demonstrate the reactions to each resistant gene.

The analyses of variance indicated that the differences between reactions of resistant genes were statistically significant for chlorosis and leaf rolling. The lines carried *Dn7* resistance gene exhibited high level of resistance to the pest, while moderate resistance conferred by the plants containing *Dn6* gene. Reactions of other *Dn* genes were found to be close to susceptible control cultivars (Figure 2 ve 3). In breeding studies against to RWA in the USA *Dn4* used successfully. In 2003, a new biotype of the RWA which is virulent to *Dn4* were identify in Canada (Haley et al., 2004). It was understood that *Dn7* were resistant to the new virulent biotype of RWA and it is appear that this gene could be used in resistant breeding programs against to this pest. In this study, the lines carrying the *Dn4* gene were found susceptible to the RWA populations in Turkey. These findings seemed to be parallel to the studies in the USA indicating usage of the *Dn7* resistance genes in a breeding program for resistance.

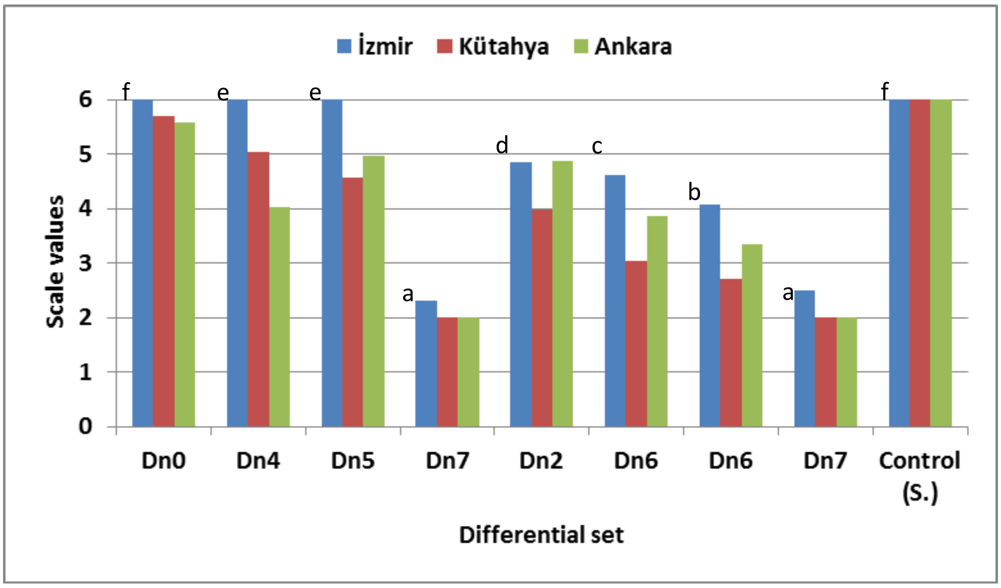

Figure 2. Scale values for chlorosis of three different populations of RWA and their groups based on variance analyses. LSD P≤ 0.05: 0.382.

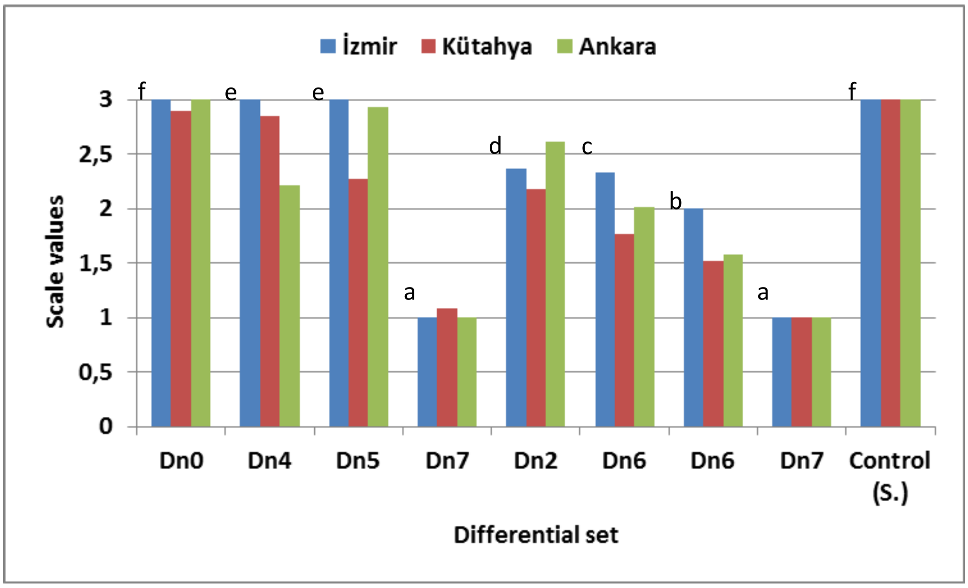

Figure 3. Scale values for leaf rolling of three different populations of RWA and their groups based on variance analyses. LSD P≤ 0.05: 0.157.

CONCLUSION

The usage of host plant resistance at the low cost and environmentally safe is an ideal method to control the Russian wheat aphid. The genetic bases of the resistance to Russian wheat aphid would be crucial step in the future of resistance breeding studies. Continuous efforts are necessary to identify and introduce additional resistance genes into the commercial wheat cultivars in Turkey. The results of this study would be beneficial to the researchers in conducting resistance breeding programs to the RWA. Continuous efforts are necessary to identify and introduce additional resistance genes into commercially acceptable cultivars in Turkey.

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