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[1]Dep. of Field Crops, Faculty of Agriculture, Ege Univ., Bornova, İzmir, Turkey
[2]Directorate of Plant Protection Research Institute, Bornova, İzmir, Turkey
[3]Dep. of Plant Protection, Faculty of Agriculture, Ege Univ., Bornova, İzmir, Turkey
[4]Genomic Services Australia, Darley Circle Bull Creek WA, Australia

Identification of resistance to *Eurygaster integriceps* Put. on some bread wheat genotypes

Fatma Aykut Tonk[1,5], Ekrem Kaya[2], Deniz İştipliler[1], Emre İlker[1], Ferit Turanlı[3], Muzaffer Tosun[1], Erkan Yılmaz[2], Firdevs Ersin[3], Ebru Savran Takak[3], Mehmet Çakır[4]

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Summary

Sunn pest (*Eurygaster integriceps* Put.), is one of the most important pests of wheat in Eastern Europe including Turkey, West and Central Asia. Its damage on leaves, stems, spikes and grains reduce the baking quality of flour made from damaged grains. In this study, some wheat genotypes from Turkey and ICARDA were evaluated for the pest resistance. The genotypes were planted in a randomized block design using hill plots in nylon mesh screening cages in wheat growing season of 2011-2012 and 2012-2013. Sunn pest population was collected from Çanakkale province, where the pest was intensely found in recent years. The plants of each hill plots were infested with one male and one female Sunn pest adults. The results with 12.5% sucking damage showed that the genotypes from ICARDA had higher resistance than the landraces from Turkey to Sunn pest. Especially, the genotypes IC3 and IC4 from ICARDA and TR7 from Turkey with respect to their SED and DSED values were found the most promising genotypes resistant to Sunn pest for future breeding programs.

Abbreviations

ICARDA, International Center for Agricultural Research in the Dry Areas; NSS, Number of Sucking Symptoms; DR, Damage Ratio; TGW, Thousand Grain Weight; SED, Sedimentation; DSED, Delayed Sedimentation; IPM, Integrated Pest Management; ANOVA, Analysis of Variance; DGW, damaged grain weight; UDGW, undamaged grain weight

Keywords

Sunn pest, bread wheat, resistance, sedimentation, delayed sedimentation

Introduction

Sunn pest (*Eurygaster integriceps* Put., Hemiptera; Scutelleridae), is one of the most important pest of wheat in Eastern Europe including Turkey, West and Central Asia. Sunn pest adults overwinter in mountains under the plants such as oak, wild liquorice and echinacea, and they migrate to wheat fields when it gets warm in spring. It can cause damage on leaves, stems, spikes and grains (Lodos, 1961, 1982; El Bouhssini et al., 2007; Trissi et al., 2006; Popov et al., 1996). Typical feeding behavior of Sunn pest is piercing and cutting tissues and injecting digestive enzymes through salivary canal to predigest food. After predigestion, food is ingested through the alimentary channel for further digestion (Cohen, 2000; Boyd et al., 2002). During the predigestion, the insect injects an enzyme complex into the grains, which reduces the baking quality of flour made from damaged grains. The damage of the pest causes break down of gluten rapidly by powerful proteolytic enzymes. If the insects feed on as little as 2 to 5% of the grain, the entire lot may be rendered unacceptable for baking purposes due to the baking quality of the flour being damaged (Lodos, 1982; Hariri et al., 2000).

Sunn pest has been one of the most harmful pests in Turkey since 1927. A serious outbreak occurred during 1987-89 in the Thrace region and thousands of hectares of wheat were damaged. The wheat growing areas in the Central Anatolian region infested by Sunn pest were stated 0.3 million ha and the cost of chemical treatment was about 2 million dollars in 1996 (Anonymous, 1996; Kinaci et al., 1998). In 2014, Sunn pest management was carried out in 6.460.213 ha of wheat production area of Turkey. The cost of this management in 22 provinces of Turkey was 1.7 million dollars (Özkan and Babaroğlu, 2015). It is also estimated that a total of 40 million dollars is spent per year for Sunn pest control in Turkey and neighbors Syria, Iran and Iraq (Javahery, 1995).

The government of Turkey has extensively supported the wheat producers to use the chemical control programs since 1928 but this support was terminated in 2008. Currently, chemical control is still continued in certain areas of the Marmara, the Southeast and the Central Anatolian regions. As a result of these chemical applications, the biological control agents that could control the pest were negatively affected. Nowadays, Integrated Pest Management (IPM) programs have been implemented to control this pest especially in the Southeast Anatolia and other regions where the pest is a major problem (Kinaci et al., 1998; El Bouhssini et al., 2007; Koçak and Kılınçer, 2002; Kivan, 2005; Canhilal et al., 2007; Keçeci et al., 2007; El Bouhssini et al., 2009; Gözüaçik et al., 2010).

The resistance studies, which are extremely safe in terms of human health and environment, and constitute an important part of IPM strategies, are carried out against Sunn pest in both Turkey and many other countries of the world. The development of varieties that show moderate to high resistance should be seriously considered because it can reduce chemical applications. Besides, certain studies have suggested that high quality bread wheat cultivars were more resistant to the damaging effects of insect proteinase than low quality cultivars (Every et al., 1997; Najafi Mirak et al., 2008). Duric et al. (2014), reported the reduction in quality traits (protein content, sedimentation value, modified sedimentation value, wet gluten content and energy test) in accordance to the number of infested grains. A number of cultivated wheat and wild relatives have been identified as being sources of resistance to this pest at the vegetative stage at ICARDA (El Bouhssini et al., 2007; El Bouhssini et al., 2009). Some of these resistance accessions were stated to originate from Afghanistan and Tajikistan (El Bouhssini et al., 2009).

Identification of resistant genotypes and using them in wheat production or transferring resistance genes to cultivated genotypes and minimizing the chemical applications are effective and economically important control strategies against Sunn pest. The aims of this study were firstly to determine whether there were any differences between the infested and the non-infested Sunn pest cages in terms of observed wheat traits, and secondly to identify sources of

* Corresponding author

resistance against Sunn pest in bread wheat landraces of Turkey and a set of international wheat lines with known resistance to the pest biotype in Syria from International Center for Agricultural Research in The Dry Areas (ICARDA) under artificial infestation.

Materials and methods

Plant material and field screening

Seven bread wheat landraces from Sunn pest prevalent areas of Turkey and four wheat lines from ICARDA, which were identified as resistant to Sunn pest (El Bouhssini et al., 2007; El Bouhssini et al., 2009), were used for resistance screening (Tab. 1). The pest population was collected from Çanakkale province located at northwest part of Turkey, where the pest intensely caused damage to wheat crop in recent years.

In the first year it should be determined whether Sunn pest causes any notable damage to the bread wheat genotypes in the infested nylon mesh screening cages compared to the control cage. Therefore, four landraces originated from the Central and East Anatolia were obtained from the National Gene Bank in Izmir, Turkey and seven resistant lines from ICARDA were planted in a randomized block design in wheat growing season of 2011-2012. The experiment was conducted with three infested and one control cages. In 2012-2013 wheat growing season, the same genotypes were planted in three infested cages without control cage in order to identify the responses of the wheat genotypes to Sunn pest. In both seasons, the genotypes were grown under natural rainfall conditions. The average monthly rainfall and temperature during 2011-2012 and 2012-2013 growing seasons of wheat are shown in Tab. 2.

Tab. 1: Origin of wheat genotypes used in the study

No.	IC/TR no.	Entry name	Type	Origin
1	IC1	ICBW209272	*T. aestivum*	Afghanistan
2	IC2	IG139883	*T. aestivum*	Afghanistan
3	IC3	IG139814	*T. aestivum*	Afghanistan
4	IC4	IG139753	*T. aestivum*	Afghanistan
5	TR1	TR 46774	*T. aestivum*	Turkey
6	TR2	TR 38895	*T. aestivum*	Turkey
7	TR3	TR 62808	*T. aestivum*	Turkey
8	TR4	TR 63314	*T. aestivum*	Turkey
9	TR5	TR 63410	*T. aestivum*	Turkey
10	TR6	TR 46826	*T. aestivum*	Turkey
11	TR7	TR 56095	*T. aestivum*	Turkey

In both growing seasons, the genotypes were planted using hill plots. Every hill contained 15 seeds and the distance among the hills was 50 cm in each cage. The genotypes were infested with the pest in three cages only for the first year, one cage was allocated for the control having no pest in Field Crops Department of Faculty of Agriculture, Ege University in İzmir, Turkey. The sizes of the mesh screening cages were 2 by 2.2 meters with height of 2.5 meters. Insecticide application (Alphacypermethrin, 100 g/l) was done to clean up the cages from other pests thirty days before infestation of Sunn pest for all cages. The plants of each hill plot were infested with two adults (one male and one female) at the time of the insects' migration to wheat fields in April, and allowed to feed on the plants. The sucking damage ratio was predicted by number of Sunn pest adult/m^2 (Kılıç, 1988; Özkan and Babaroğlu, 2015).

Measured traits

Before harvest, each of the experimental cages were sprayed with Dimethoate (400 g/l) to kill the remaining Sunn pest and the hill plots were separately harvested. One hundred kernels representing each hill plot were randomly selected and checked under magnifying glass; damaged and undamaged kernels were separated and weighted then recorded as damaged grain weight (DGW) and undamaged grain weight (UDGW), respectively. The feeding punctures on damaged kernels for each genotype caused by Sunn pest were counted and recorded as number of sucking symptoms (NSS). Kernels with at least one puncture site were considered as damaged grain. To calculate thousand grain weight (TGW), one hundred kernels were counted four times, weighted and average value was multiplied with ten for each genotype. The damage ratio (DR) was calculated with the following formula:

$$DR\ (\%) = DGW/(DGW+UDGW)*100$$

Gluten quality was analyzed in flour sample for each genotype using the Zeleny sedimentation (SED) (Aacc, 2000) and delayed Zeleny sedimentation (DSED) test (Greenaway et al., 1965). Swelling of the gluten fraction of flour in lactic acid solution affects the rate of sedimentation of a flour suspension in the lactic acid medium. For two parallel analyses, 3.2 × 2 = 6.4 g of weighted flour samples previously milled in a suitable laboratory mill were placed in a graduated cylinder, the reagents were added and mixed thoroughly. After mixing with all reagents, the cylinder was left to stand for exactly 5 min and the volume of sediment was read. Delayed sedimentation values were recorded after two hours. The expected damage ratio was calculated according to Technical Instruction of Agricultural Protection (2008).

Statistical analyses

A paired t-test was used to compare the mean of the infested and the control cages for each trait in the first year of the study. The analysis

Tab. 2: Average monthly rainfall (mm) and temperature (°C) in 2011-12 and 2012-13 growing seasons of wheat in the experimental site

Climate	Years	November	December	January	February	March	April	May	June	Total and average
Rain Distribution (mm)	2011-12	0.0	140.5	127.7	128.2	34.7	105.0	86.6	19.9	642.6
	2012-13	56.9	218.2	252.5	187.0	56.8	30.2	43.7	27.1	872.4
	LYA	109.7	137.9	112.2	99.7	82.9	46.4	25.4	7.5	621.7
Average Temperature (°C)	2011-12	11.1	10.7	6.8	7.6	11.3	17.5	20.5	27.3	14.1
	2012-13	16.4	10.7	9.4	11.2	14.0	17.3	22.7	25.7	15.9
	LYA	13.8	10.5	9.0	9.2	11.8	16.1	21.0	26.0	14.7

LYA: Long year average

of variance (ANOVA) was performed using TOTEMSTAT software (Açıkgöz et al., 2004) for all measured traits with combining the average data of the genotypes obtained from each infested cage in two years. Probability was accepted at the $P \le 0.01$ and $P \le 0.05$ levels. The means were compared using Fisher's least significant differences (LSD) at the $P \le 0.05$ level.

Results

Sunn pest population density was calculated as 5 overwintered adults/m^2 in infested cages (2 adults * 11 hill plots = 22 adults for 4.4 m^2). The expected damage ratio was equal to 12.5% sucking damage in each of the infested cages.

The results from the control and the infested cages were found statistically different for all measured traits except DSED in 2011-2012 growing season (Tab. 3). Unexpectedly, some grains with sucking symptoms were observed in the control cage. This damage could be the result of the insects such as stink bugs at low population level remaining after insecticide spraying in the control cage. The highest t value was found for the DR and the cages significantly differed for this trait. The TGW in the control cage were higher than the mean of infested cages and according to t test, the difference between them was statistically significant. Although both of the SED and DSED values were higher in the control cage than the mean of infested cages, the genotypes in the infested and the control cages did not statistically differed for DSED (Tab. 3). All these findings indicated that this population level of Sunn pest significantly damaged the wheat plants and therefore this level of damage through infestation would be suitable to differentiate the wheat genotypes for response to Sunn pest.

The ANOVA showed that the differences between the years were found significantly important for all traits except NSS and DR (Tab. 4). Also, the differences among the genotypes were significant for TGW, SED and DSED. However, genotype × year interaction was significant for only DSED trait (Tab. 4).

Tab. 3: Results of t test for the genotypes in the control and the infested cages in 2011-2012

Traits	Infested cage	Control cage	t value
NSS (%)	55.28	6.00	8.35**
DR (%)	31.14	4.47	9.16**
TGW (g)	50.07	53.28	-2.08*
SED (ml)	11.43	15.72	-3.21**
DSED (ml)	5.56	7.63	-1.34

*, ** : Significant at $P \le 0.05$ and $P \le 0.01$, respectively.
NSS: Number of Sucking Symptoms, DR: Damage Ratio, TGW: Thousand Grain Weight, SED: Sedimentation, DSED: Delayed Sedimentation.

In the infested cages, the values of the genotypes in each year and the mean of two years for the measured traits are separately shown in Fig. 1a-e. The NSS values of the genotypes were higher in 2011-2012 than 2012-2013. In both years, the NSS of IC genotypes were lower than TR genotypes. For the NSS, the genotypes TR6 (23.27%) and IC3 (25.93%) had the lowest values while TR3 (56.23%) had the highest NSS for two years mean (Fig. 1a). In parallel with the NSS, the DR values of the genotypes were found higher in the first year than the second year. The higher DR values of TR genotypes than the IC genotypes could be seen especially from the first year results (Fig. 1b). The lowest DR mean measured was for TR6 (15.60%) and IC3 (16.10%) while the highest DR obtained for TR5 (31.13%) (Fig. 1b). When the TGW of the genotypes was analyzed, the first year values of all genotypes were higher. The TGW of IC genotypes had higher values than TR genotypes. The highest and the lowest mean values were observed for IC2 (48.11 g) and TR5 (30.04 g), respectively (Fig. 1c).

The SED of all genotypes are viewed in Fig. 1d. In contrast to NSS, DR and TGW, the SED values of all genotypes in 2011-2012 were detected higher than 2012-2013. The SED of IC genotypes had higher values than TR genotypes. The highest SED values were measured for IC1 (21.54 ml) and IC3 (23.66 ml) while the lowest value was obtained from TR7 (11.66 ml) (Fig. 1d). Unlike the other traits, the mean square of genotype × year interaction was found statistically significant for the DSED (Tab. 4). Therefore, the genotypes were grouped separately for each year. While, all genotypes were placed in the same group during first year, they were classified in different groups in the second year of the study. In the second year, IC4 (16.00 ml) and TR7 (15.00 ml) had the highest DSED values whereas TR1 (6.50 ml) and TR5 (6.33 ml) had the lowest values (Fig. 1e).

The obtained results revealed that the NSS, DR and TGW values of the genotypes in 2011-2012 were higher than those of 2012-2013. Therefore, the SED and DSED values of the genotypes in 2011-2012 were lower compare to 2012-2013 (Fig. 1a-e).

The mean TGW, SED and DSED of IC genotypes were identified more than TR genotypes. In contrast, the NSS and DR of TR genotypes were higher than IC genotypes. The mean of NSS value were detected as 32.59% for IC genotypes and 42.65% for TR genotypes. The DR means were 20.64% and 25.15% for IC and TR genotypes, respectively. The mean of SED value were found 19.60 ml for IC and 14.37 ml for TR genotypes. The mean of DSED values obtained from the IC and TR genotypes were 8.92 ml and 7.42 ml, respectively (Fig. 1f).

Discussion

A number of studies on resistance screening against Sunn pest were conducted on vegetative stage of wheat genotypes. Seven accessions originated from Afghanistan and Tajikistan with this kind of re-

Tab. 4: Analysis of variance for measured traits

Source of variation	Mean of Squares					
	df	NSS (%)	DR (%)	TGW (g)	SED (ml)	DSED (ml)
Year	1	21318.8	4718.00	7785.8**	1849.5**	420.3**
Genotype	10	860.9	1361.74	287.1**	107.6**	21.1**
Genotype × year	10	825.63	170.90	4.5	17.2	13.1*
Error	54	582.3	103.54	18.2	14.2	5.0

*, ** : Significant at $P \le 0.05$ and $P \le 0.01$, respectively.
NSS: Number of Sucking Symptoms, DR: Damage Ratio, TGW: Thousand Grain Weight, SED: Sedimentation, DSED: Delayed Sedimentation.

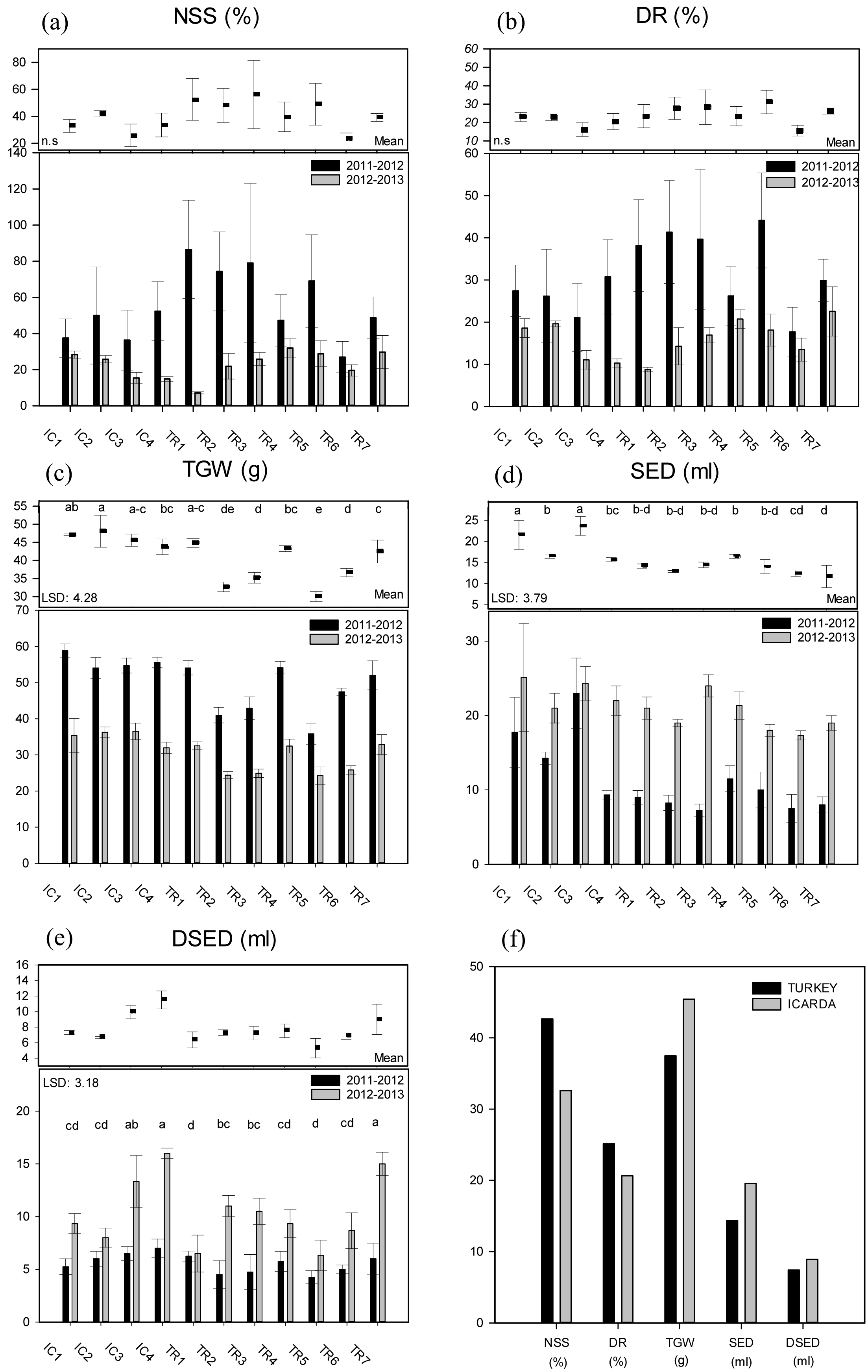

Fig. 1: Effects of Sunn pest on the bread wheat genotypes in 2011-2012 and 2012-2013 growing seasons: (a) NSS, Number of Sucking Symptoms, (b) DR, Damage Ratio, (c) TGW, Thousand Grain Weight, (d) SED, Sedimentation, (e) DSED, Delayed Sedimentation, (f) the mean values of two years for IC and TR genotypes.

sistance reported by El Bouhssini et al. (2009). However, Trissi et al. (2006) emphasized that feeding by Sunn pest at vegetative stage does not affect the bread-making quality of the grain in wheat. Therefore, economic damage of Sunn pest actually occurs in generative stage of wheat plant. Grain damage of Sunn pest at generative stage in wheat was investigated in a few studies in Turkey and other countries (Kinaci et al., 1998; Dizlek and İslamoğlu, 2010; Duric et al., 2014).

In accordance with previous studies, at generative stage of wheat five Sunn pest per m^2 infestation level resulted in significant differences for all traits except DSED between the infested and the control cages. Trissi et al. (2006) stated that protein, sedimentation and damaged kernel values for the infested plot at six Sunn pest individuals per m^2 density were significantly lower than those of the control wheat plot. The reason of the absence of significance for DSED is that in contrast to the TR genotypes, the DSED values of the IC genotypes did not varied between the infested and the control cages. For the SED and DSED traits, the variation between the infested and the control cages can be attributed to the effect of proteolytic enzyme secreted by Sunn pest, which caused significant reduction in gluten level in damaged grains (Lodos, 1982; Hariri et al., 2000).

In Turkey, 2% Sunn pest sucking damage ratio was determined as maximum limit in wheat commodity (Özkan and Babaroğlu, 2015). According to Kiliç (1988), the economic threshold for Sunn pest control is 10 nymphs/m^2 and 0.8 overwintered adults/m^2 (Özkan and Babaroğlu, 2015). In this study determining the resistant genotypes, the sucking damage ratio (12.5%) obtained was higher than the economic threshold level because of high population density (5 Sunn pest adults/m^2). Similar to our results, Kahraman et al. (2011) reported the highest rate of sucking damage as 11.7% in greenhouse and the lowest as 2.3% in the field. Due to a very high pest pressure, the means of NSS and DR values were found very high in both years (Fig. 1a). The genotypes from ICARDA, which were reported as resistant against Sunn pest at vegetative stage (El Bouhssini et al., 2007; El Bouhssini et al., 2009), appeared to be more resistant to Sunn pest compared to the TR genotypes at generative stage of wheat (Fig. 1f). Especially the genotypes IC3 and IC4 with noticeably lower mean values for NSS and DR draw the attention and showed higher mean values for SED and DSED. However, amongst TR genotypes, the genotype TR6 did not show higher values for SED and DSED although NSS and DR values were lower like in IC genotypes (Fig. 1a-e). The reason of these differences could be due to a lack of gluten quality or the number of gluten allele in these two genotypes. Fatehi et al. (2008) investigated the correlation of HMW subunits and the percent of damaged seed by Sunn pest and reported that 7+8 and 2+12 alleles have significant positive correlation while 7+9 and 12 alleles have significant negative correlation with the percent damaged seed. In the present study, the SED values of the genotypes were generally higher in less damaged kernels, which is consistent with the results of previous studies (Karababa and Ozan, 1998; Dizlek and İslamoğlu, 2010). Similarly, Canhilal et al. (2005) revealed a strong negative relation between sedimentation values and percentage of damaged kernels.

The NSS, DR and TGW values of the genotypes in the year 2011-2012 were higher compared to year 2012-2013. These differences can be explained with the average amount of monthly rainfall in April and May of two years (Tab. 2). The April and May received the average rainfall of 105.0 and 86.6 ml, respectively in 2012, and 30.2 and 43.7 ml, respectively in 2013 in experimental area. The higher rainfall of these two months in 2012 may extended the milk stage due to the longer grain filling period. As a result, the TGW values of all genotypes were higher in this year (Fig. 1c). The extending milk stage caused more sucking damage on grain by the 2-5th stage nymphs and new generation of adults of Sunn pest on wheat (Özkan and Babaroğlu, 2015). More sucking damage contributed in increasing the number of damaged kernel (NSS) and therefore the percentage of damaged kernel weight in the total grain weight (DR) rose during the first year. However, the reduction in the amount of gluten in the damaged grains, because of proteolytic enzyme of Sunn pest, caused the decrease in the SED and DSED values of the genotypes.

In case of NSS and as a result DR, the values for some genotypes e.g. TR1 differed significantly during two years. It must be because of the differences between the numbers of damaged grain by Sunn pest in two years. The kernels with at least one puncture site were considered as damaged grain. Therefore, the pest damaged more kernels of the genotypes in the first year compared to the second year because of the longer flowering and grain filling period. In contrast, the pest damaged fewer kernels but with more puncture sites during second year due to shorter flowering and grain filling period. Besides, the damage by Sunn pest in wheat kernel is determined by the SED and DSED test (Dizlek and Islamoglu, 2015). Also it has been emphasized that the higher the difference between the values of SED and DSED, the higher the damage caused by Sunn pest will be (Elgun et al. 1998; Ozkaya and Ozkaya, 2005; Dizlek and Islamoglu, 2015).

In conclusion, the extension of the flowering and grain filling period can fairly increase the damage of Sunn pest at generative stage in wheat. Therefore, the resistance of wheat genotypes to Sunn pest may vary among years. The IC genotypes, known resistant to Sunn pest in the vegetative period, also exhibited resistance in the generative period of wheat. In general, these genotypes were identified to be more resistant than the TR genotypes originated from Sunn pest prevalent areas of Turkey. The genotypes IC3 and IC4 from ICARDA and TR7 with noticeable values for DSED can be a promising source of germplasm for resistance to Sunn pest in next breeding programs. The resistance sources identified in the study provides opportunities towards establishing a base to a much broader study of understanding genetic and physiological bases of resistance against Sunn pest in wheat. Furthermore, the genotypes identified here can be used by plant breeders for the introgression of resistance genes into susceptible wheat cultivars.

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Address of the corresponding author:
E-mail: fatma.aykut@ege.edu.tr