Evaluating the resistance to sunn pest (Eurygaster integriceps Put) and its relationship with high-molecular-weight glutenin subunit in wheat

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INTRODUCTION

Wheat, *Triticum aestivum* L., and barley, *Hordeum vulgare* L., are very important food crops in the near East, Middle East, and South-Western Asian countries. They are very strategic crops for Iran as well as many other countries. Wheat is grown on approximately 6.41 million ha in Iran. Total production of wheat is 13.44 million t and the yield is 2355 kg/ha in average (FAO, 2003).

The crops are attacked by several species of bugs. Sunn pest, is the most important pest constitutes a major threat to wheat production and, to a lesser extent, barley production. Sunn pests are a complex of true bugs which belong to the genera *Eurygaster* (Scutelleridae), *Aelia*, *Carpocoris* and *Dolycoris* (Pentatomidae). *Eurygaster integriceps* is probably the most important species in Afghanistan, Iran, Iraq, Jordan, Pakistan, Syria, Lebanon, Germany, Spain, Hungary, and Turkey (Moore, 1998).

There are two economically important species of *E. integriceps*, *E. maura* L. and *E. austriaca* Schrk. (Aydemir, 1998; Simsek, 1998). Over 15 million ha can be affected annually and during outbreaks, infestations may result in 100% crop loss. Damage commonly results in yield losses of 20–30% in barley and 50–90% in wheat. This pest also injects chemicals into the grain that destroy the gluten and greatly reduce the baking quality of the flour (Moore, 1998; Hariri et al., 2000).It is now generally recognized that the sole use of pesticides for controlling pests should be avoided as much as possible in favor of a more integrated pest management (IPM) approach, utilizing cultural practices as well as biological control in the first instance with chemical control being used only as a last resort when other measures have failed to keep pest populations below damaging levels (Brain, 1998).

The genetics and biochemistry of high-molecular-weight (HMW) glutenin subunits in wheat (*Triticum aestivum*) are now well understood by virtue of various studies (Payne et *al*. 1987; Shewry *et al*. 1992). HMW subunits are encoded at the Glu-1 loci of the group 1 chromosomes (1A, 1B, 1D), and each locus consists of two genes encoding an x-type and a y-type subunit (shewry *et al*. 1995). Because some genes are silent, wheat cultivars contain three, four or five subunits. Glutenins consist of HMW (high molecular weight)and LMW (low molecular weight) glutenin subunits. Each cultivar contains three to five HMW subunits that can be distinguished by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Payne et al 1987). For synthetic lines and the Iranian wheat cultivars, 15 different alleles were identified, 3 corresponding to the *Glu- A1* locus, 8 to *Glu-B1*, and 4 to *Glu-D1*. Each pattern included 3–5 bands of HMW glutenin subunits. It has been reported that HMW glutenin subunit composition is a useful system for wheat variety identification (Payne *et al*. 1984). HMW is one of the molecular markers that can be used for identification wheat advanced lines/cultivars with resistance to sunn pest.

The research reported in this paper was carried out to evaluate advanced wheat lines/cultivars for resistance to sun pest (*Eurygester integriceps* Put) and to find its relationship with HMW subunits.

MATERIAL AND METHODS

Fifty lines/cultivars of wheat were planted in a randomized block design in three replicated plots at the Research Farm University of Tehran in Karaj, during the autumn season of 2004/2005. The plots were 2 m long with a space of 20 cm between-rows and 10 cm between plants within-row. Aluminum cages (25 by 100 cm) were placed on wheat plants at head initiation. In early seed development stage, six sunn pest (nymph3) were introduced in each cage. The nymphs fed on wheat developing seed for 40 days. After seed maturity the cages were collected and transferred to the lab. After recording the evaluated traits, the spikes of each cage were treshed separately. And the number of damaged seed was counted then the percent damaged seed for each genotype was determined. Seed colour, seed coverage by glume and glumel, plant height, spike density, awn length, peduncle length, number of tiller, number of days to maturity, above-ground biomass, grain yield per plant, number of seed per spike and 1000-seed weight were measured. For measuring the spike density the follow formula was used:

$$D=(10N/L)$$

Where; N: number of spikelet in each spike, L: length of rachis.

To determine the electrophoretic mobility of each HMW glutenin subunit by SDSPAGE, we used standards (Chinese Spring, Hirmand, Falat) that included the spectra of subunits expected to find . According to the procedure of Payne *et al.* (1979), gels were made with

7.5% (w/v) acrylamide and 0.2% (w/v) bisacrylamide and contained 1.5 M Tris-HCl, pH 8.8, and 0.27% SDS. The stacking gel contained 0.25 M Tris-HCl, pH 6.8. Wheat flour (10 mg) was suspended in 300 mL 0.25 M Tris-HCl buffer (pH 6.8) containing 2% (w/v) SDS, 10% (v/v) glycerol, and 5% 2-mercaptoethanol and was shaken for 2 h at room temperature. The suspension was heated at 95°C for 3 min. The top portion of the supernatant was collected after centrifugation for 3 min at 12 000 rpm, and a portion (30 µL) of the extract was loaded onto a gel slot. The buffer was 0.025 M Trisglycine, pH 8.3, containing 0.1% (w/v) SDS. Electrophoresis was conducted at 10 mA constant current for 15 h until the tracking dye, bromophenol blue, reached the bottom of the gel. The gels were stained for several hours with Coomassie Blue R in aqueous ethanol and acetic acid. The system for numbering HMW glutenin subunit bands and that for allelic classification at *Glu-A1*, *Glu-B1*, and *Glu-D1* loci, proposed by Payne and Lawrence (1983), were followed.

Due to the un-equal number of sunn pest in each cage, the umber of sunn pest was used as covariate in the analysis of covariance. All statistical analyses were carried out using the SAS and Minitab software (SAS Institute, 1996; Minitab Inc., 2000).

RESULTS AND DISCUSSION

The analysis of variance showed that all traits of genotypes were significantly different at P=0.05. Line18 and Gaspard with 82 and 75.6% of seed damage were the most susceptible genotypes. Line20 line18 with 38.8 and 48.5% seed damage were the most resistant ones. There was no significant correlation between percent damaged seed and morphological traits like seed colour, covering seed by glum and glumel, glume hairiness, plant height, spike density, awn length, number of tiller, number of days to maturity, above-ground biomass, grain yield per plant, number of grain per spike and 1000 seed weight. Rezabaigi *et al*. (1997) found significant positive/negative correlation between percent damaged seed with awn length and seed hardness. Also Ghanadha and Ayene (2003) reported that the damage seed of awnless varieties was higher than varieties having awn. In this study there was significant negative correlation between seed damage and pedunkel length. This result is in apposition to the result of Rezabeigi et al (2000), Rezabeigi (1997), Sosidko and Felko, (1977). As the Line8 was the most susceptible late maturity by increasing the period of seed maturity sunn pest has more time for damaging the wheat seed. According to the percent damaged seed, the varieties and lines were grouped as; Resistant: Lines 20 and 39, Semi-Resistant: Lines 4, 7, 15, 18, 21, 26, 31, Falat, Semi-Susceptible: Lines 1, 2, 9, 12, 14, 19, 23, 24, 28, 29, 30, 32, 34, 36, 37, 38, Bolani, Zardak, Golestan and Ghafghaz, Susceptible: Lines 3, 5, 6, 10, 11, 13, 16, 17, 22, 25, 27, 33, 35, 40, Chamran, Frontana, Sardari and Azadi and Highly-Susceptible were Line 8 and Gaspard.

2^{***}+12 subunit encoded by GLU-D1 was reported among native strain of Pakistanian bread wheats as one of the new alleles with low frequency (Tahir and Lafiandra, 1994). In this study this subunit was observed in 7 of the evaluated lines and cultivars.

Simple correlation coefficients of HMW subunits and percent of damaged seed are presented in Table 1. Results revealed that 7+8 and 2+12 alleles have significant positive correlation with percent damaged seed. In addition 7+9 and 12 alleles have significant negative correlation with percent of damaged seed.

Table 1- A matrix of simple correlation coefficients (r) for HMW subunits and percent damaged seed

Variables	Null	1	2^{*}	6+8	7	7+8	7+9	13+16	14+15	17+18	2+12	2^{***}+12	5+10	12
1	-0.42^{**}													
2^{*}	-0.72^{**}	-0.33^{*}												
6+8	0.01	0.19	-0.15											
7	0.14	-0.06	0.19	-0.03										
7+8	-0.05	-0.06	0.09	-0.18	-0.13									
7+9	0.26	0.03	-0.30^{*}	-0.11	-0.08	0.47^{**}								
13+16	-0.32^{*}	-0.15	0.44^{**}	-0.07	-0.05	-0.30^{*}	-0.18							
14+15	0.15	-0.06	-0.11	-0.03	-0.02	-0.13	0.08	-0.05						
17+18	0.02	0.11	-0.10	-0.09	-0.06	-0.39^{**}	-0.23	-0.15	-0.06					
2+12	-0.24	0.46^{**}	-0.10	0.10	-0.09	0.17	-0.22	-0.06	-0.09	0.09				
2^{***}+12	0.31^{*}	-0.18	-0.18	-0.08	0.06	0.46^{**}	-0.21	-0.13	-0.06	-0.18	-0.25			
5+10	-0.12	-0.24	0.30^{**}	-0.01	0.14	0.44^{**}	0.41^{**}	0.19	-0.15	-0.02	-0.65^{**}	-0.42^{**}		
12	0.15	-0.06	-0.11	-0.03	-0.02	0.16	-0.08	0.05	-0.02	-0.06	-0.09	-0.06	-0.15	
Percent damaged seed	-0.24	0.05	-.20	0.25	-0.01	0.43^{**}	-0.63^{**}	0.02	-0.10	0.09	0.33^{**}	0.10	-0.25	-0.32^{*}

* , **: means significant at 5%, 1% level of probability

Table 2 shows the data representing cumulative R2 as well as the Probability or the accepted limiting four alleles in percent damaged seed prediction. These alleles are: the 7+9 (39.2%), 12 (13.4%), 7+8 (5%) and 6+8 (4.5%). According to the results, 62.1% of the total variation in percent damaged seed could be attributed to these aforementioned four alleles. The other alleles were not included in the analysis due to their low relative contributions.

Table 2- Regression analysis of the accepted alleles that can be used to predict percent damaged seed

Variables	Coefficient of regression (*B*)	Cumulative R^2	Standard error (SE)	T	Sig.
Intercept	61.27		1.35	45.44	0.00**
7+9	-9.43	0.392	2.07	-4.55	0.00**
12	-22.81	0.526	5.35	-4.27	0.00**
7+8	4.99	0.576	1.77	2.83	0.01*
6+8	9.79	0.621	3.93	2.49	0.02*

* , **: means significant at 5% , 1% level of probability

Rezabeigi (1997) by evaluation of sunn pest collected from Varamin reported Ghafghaz variety as resistant and Zardak as susceptible cultivars. In this study Falat was identified as semi resistant cultivar which was agreed with results of Ghannadha and Ayeeneh, (2003). Also Sardari and Zardak were distinguished as susceptible and semi susceptible cultivars, respectively. Golestan and Ghafghaz were evaluated as semi susceptible. These results previously were reported by Rezabeigi *et al.* (2000). According to the results of this study we can conclude that some cultivars like Falat has maintained their resistance while the resistance of others such as Golestan and Ghafghaz has been altered in response to changing insect biotype in various years and sites. In general variation in the time of insect infestation can influence the resistance or susceptibility of cultivars.

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