

Oxalic acid as an alienate factor for wheat and barley resistance to cereal leafminer *Syringopais temperatella* (Lederer, 1855) (Lepidoptera: Scythrididae)

F. Al-Zyoud, D. Hassawi & I. Ghabeish

Summary

The cereal leafminer, *Syringopais temperatella* (Lederer, 1855) is a major pest of wheat and barley leading to a sharp decline in their production. Identification of plant cultivars with resistance to *S. temperatella* as a part of integrated pest management is effective, economic, durable and environmentally safe. In this work, percentage of infestation, oxalic acid and moisture content of the leaves were determined to understand the precise correlation for the resistance to *S. temperatella* using thirty four wheat and barley accessions during the 2011/12 and 2012/13 cropping seasons. The results indicated that wheat and barley accessions' effects were statistically significant for infestation percentages and leaf's oxalic acid level. The accessions revealed significant inverse correlation of oxalic acid content with infestation percentage in wheat ($r = -0.445$, $P = 0.026$, $y = -0.10 \times 34.20$), and barley ($r = -0.263$, $P = 0.494$, $y = -0.07 \times 33.77$). There was positive correlation between leaf moisture content and infestation percentage in wheat ($r = 0.190$, $P = 0.362$, $y = 0.09 \times 16.58$), and barley ($r = 0.733$, $P = 0.016$, $y = 0.40 \times 03.53$). In conclusion, oxalic acid exuding from leaf has been shown to contribute to the pest resistance. The accessions, Acsad 1273 and 1614 may be used as resistant donors in the genetically crossing program to evolve leafminer tolerant accessions of barley and wheat, respectively.

KEY WORDS: Lepidoptera, Scythrididae, *Syringopais temperatella*, cereal leafminer, host-plant resistance, oxalic acid, Jordan.

Ácido oxálico como un factor enajenante para la resistencia del trigo y cebada al minador de los cereales *Syringopais temperatella* (Lederer, 1855) (Lepidoptera: Scythrididae)

Resumen

El minador del cereal, *Syringopais temperatella* (Lederer, 1855) es una de las mayores plagas del trigo y de la cebada, causando la rápida disminución de su producción. La identificación de plantas cultivadas con resistencia a *S. temperatella* como una parte integrada para el control de la plaga es efectiva, económica, duradera y con respecto al Medio Ambiente segura. En este trabajo, se determinaron los porcentajes de infestación, ácido oxálico y contenido de humedad de las hojas, para comprender la correlación precisa para la resistencia a *S. temperatella* usando treinta y cuatro adquisiciones de trigo y cebada durante las temporadas de cultivos 2011/12 y el 2012/13. Los resultados indican que los efectos de las adquisiciones de trigo y de cebada eran estadísticamente importantes para el porcentaje de infestación y el nivel de ácido oxálico de las hojas. Las adquisiciones revelan una significativa correlación inversa del contenido de ácido oxálico con el porcentaje de infestación en el trigo ($r = -0.445$, $P = 0.026$, $y = -0.10 \times 34.20$), y la cebada ($r = -0.263$, $P = 0.494$, $y = -0.07 \times 33.77$). Había correlación positiva entre el contenido de humedad de las hojas y los porcentajes de infestación en el trigo ($r = 0.190$, $P = 0.362$, $y = 0.09 \times 16.58$), y cebada ($r = 0.733$, $P = 0.016$, $y = 0.40 \times 03.53$). En conclusión, se ha demostrado que el ácido oxálico que exuda de las hojas contribuye a la resistencia a plagas. Las adquisiciones Acsad 1273 y 1614 pueden ser usadas

como donantes resistentes en un programa de cruce genético para desarrollar la adquisición de minadores resistentes para la cebada y el trigo respectivamente.

PALABRAS CLAVE: Lepidoptera, Scythrididae, *Syringopais temperatella*, minador de cereales, resistencia planta nutricia, ácido oxálico, Jordania.

Introduction

The Jordanian wheat and barley area harvested covers nearly 193,000 and 865,100 dunums producing 19,800 and 29,300 tons, respectively. Jordan is not self-sufficient in the production of wheat and barley and the self-sufficiency ratio is only 1.8 and 6.1%, respectively. The country imported 1,076,650 and 447,332 tons of wheat and barley, respectively (JORDAN STATISTICAL YEARBOOK, 2011) to cover the national needs for the local population and their livestock. However, the cereal leafminer, *Syringopais temperatella* (Lederer, 1855) is a major insect pest of wheat and barley and causes a serious production loss in many places where these crops are grown in Jordan (AL-ZYOUN, 2012, 2013), Iraq (ICARDA ANNUAL REPORT, 2007), Iran (FARD, 2000; JEMSI *et al.*, 2002; JEMSI & RAJABI, 2003), Cyprus (MELIFRONIDES, 1977), and Turkey (GOZUUACIK *et al.*, 2008). In Jordan, *S. temperatella* larvae emerge from the ground in early February and penetrate the plant leaves, and gnaw mines between the epidermal layers (AL-ZYOUN, 2007) leading to a sharp decline in production.

The current program for the management of *S. temperatella* is restricted to the application of insecticides in many countries of the region (FARD, 2000; VRIEZE, 2002; JEMSI & RAJABI, 2003; AL-ZYOUN, 2013). The development of insecticide resistance in *S. temperatella* populations (GEORGHIOU & LAGUNES-TEJEDA, 1991), coupled with increasing awareness of possible detrimental effects of intensive insecticides use (GERSON & COHEN, 1989), has stimulated interest in the development of integrated pest management (IPM) method of pest control that reduce pesticide inputs and produce more farming systems such as the identification of cultivars/accessions with resistance to pests (RAJPUT *et al.*, 2003), which are effective, economic, durable and environmentally safe (SINGH & WELGAND, 2006). Although considerable efforts have been made to develop pest management strategies over the past years, we are still unable to manage pests in an environmentally adequate (SARWAR, 2013). Concerted efforts to transfer insect resistance into improved cultivars with acceptable yield and quality has not been successful (NASER *et al.*, 2009).

Since wheat and barley growers have to spend much of inputs like applications of agrochemicals to control *S. temperatella*, it was considered viable to search the available germplasm for sources of resistance to such a pest for use in breeding insect resistant cultivars. According to SHAHZAD *et al.* (2005), the resistant chickpea genotypes caused high antibiotic and antixenotic effects for the larvae of *Helicoverpa armigera* (Hübner, [1808]) at vegetative and flowering stages of the plant. It was found that chickpea genotypes resistant to *H. armigera* accumulate more oxalic acid on the leaves than susceptible genotypes (YOSHIDA *et al.*, 1995; ICARDA ANNUAL REPORT, 2005; SARWAR, 2013). Acidic exudate in chickpea plants, in which oxalic acid is a major acidic component (REMBOLD & WEIGNER, 1990; YOSHIDA *et al.*, 1995), has been correlated with reduced plant damage (SRIVASTAVA & SRIVASTAVA, 1989). It is possible that the antibiotic properties of oxalic acid may negate differences due to ovipositional antixenosis and determine the size of the larval population, and therefore pod damage, on a particular genotype (YOSHIDA *et al.*, 1995). Furthermore, oxalic acid accumulation is considered to be one of the mechanisms of *H. armigera* resistance in chickpea (YOSHIDA *et al.*, 1995, 1997). COTTER & EDWARDS (2006) mentioned that oxalic acid exuding from leaf trichomes have been shown to contribute to resistance to pod-feeding caterpillars, *H. armigera* and *H. punctigera* (Wallengren, 1860).

Nevertheless, the continued damage to wheat and barley due to *S. temperatella* is a daunting challenge and requires the use of novel technology. Therefore, there is an urgent need of replacing agrochemicals with safer alternatives and adopting IPM to provide adequate crop protection for sustainable agriculture, and increase wheat and barley production. However, to the best of our

knowledge, oxalic acid in wheat and barley as a chemical-resistant factor to *S. temperatella* has not been identified. In this work, the level of oxalic acid was analyzed to check out the variations among the accessions tested in order to understand the precise correlation to the resistance, and to evaluate the effect of oxalic acid on infestation by *S. temperatella*. It is hoped that this study will help in the promotion of effective plant protection measures, compatible with environment and human health.

Materials and methods

PLANT TEST MATERIAL

Thirty four wheat and barley accessions were tested in this study. Of them, twenty five accessions were wheat and nine were barley. The accessions were obtained from the National Centre of Agriculture Research and Extension/Jordan. They were screened for resistance to *S. temperatella* during the two successive cropping seasons, 2011/12 and 2012/13. The accessions were sown at a uniform depth of 5 cm in December of each year in an experimental field in Karak, Jordan (approximately latitude of 31° 11", longitude of 35° 42", and altitude of 980 m). A rough seedbed was prepared to avoid packing of the cloddy surface due to winter rains, and to facilitate soil aeration and easy seedling emergence. A randomized complete block design with three replications of 34 treatments (wheat and barley accessions) was used. Each replicate (plot) consisted of a row of 2 m long with inter- and intra-row spacing of 2 and 0.5 m, respectively. All the recommended agronomic practices were adopted for raising the crops. No irrigations and fertilizers were applied to the plants. The test material was kept free from any insecticidal spray throughout the cropping seasons to put more pest pressure on the test material during the whole experimental period. The experimental site is characterized by semi-arid conditions with a relatively moderate rainfall (300 mm long-term annual average). The weather in the study area is characteristically warm, and temperature increases gradually during the spring months.

EVALUATION OF *SYRINGOPAIS TEMPERATELLA* INFESTATION

Level of resistance/susceptibility on each of the accessions was assessed under field conditions by recording the percentage of infestation on plants from each test accession at early April. The percentage of infestation has been estimated independently three times within the same day, and the average values were calculated for both cropping seasons.

OXALIC ACID ANALYSIS AND LEAF-MOISTURE CONTENT DETERMINATION

SAMPLING

Duplicated samples of wheat and barley fresh leaves of 3-5 gm were picked up from the 34 accessions grown in the field on the same day as the pest infestation estimation. The first group of samples including samples from all accessions was separately kept in plastic bags and weighted before and after oven drying at 80° C for 3 days. Leaf moisture content was calculated for each accession. Samples of the second group were also separately kept in paper bags and transferred to the laboratory for oxalic acid analysis.

OXALIC ACID ANALYSIS

For oxalic acid analysis, the minimal sample weight that is suitable to determine oxalic acid content is found using 3 different graduated weights; 1.0, 2.5 and 5.0 gm. It was found that 2.5 gm is enough for the acid analysis. The leaf samples were individually homogenized in liquid nitrogen using pestle and mortar. Then the pH was adjusted at 3.0 ± 0.1 with HCl (1M) and the sample volume was

completed to 50 ml using de-ionized distilled water. The samples were boiled for about 15-30 min in a boiling bath. Volume of 2000 l of reagent 1 (R1) was used for blank, standard and sample. The R1 is a liquid buffer (buffer > 20 mmol/L, pH 3.1±0.1, 3-methyl-2-benzothiazolinonehydrazone, MBTH > 0.2 mmol/L, 3-dimethyl-aminobenzoic acid, DMAB > 0.9 mmol/L, activators, stabilizers. For filtration, 100 l of each of sample, blank and standard were added to the buffer and incubated for 5 min at 37 C. Readings of absorbance 1 (A1) were recorded at 590 nm wavelength using a spectrophotometer. After that, 200 l of reagent 2 (R2) was added to the blank, sample and standard, and incubated at 37C until the end of the reaction (about 15 min). The R2 is a lyophilized enzyme (oxalate oxidase from barley > 2 KU/L, peroxidase > 1000 U/L). Readings of absorbance 2 (A2) were recorded at 590 nm and the color (blue quinone) is stable for 60 min (TIETZ & SAUNDERS, 1999). The principle of the method used for the analysis is that oxalate is oxidized to carbon dioxide and hydrogen peroxide by oxalate oxidase. Hydrogen peroxide reacts in the presence of peroxidase with MBTH and DMAB forming a blue quinone compound. The intensity of color is proportional to the concentration of oxalate in the sample at 590 nm. Standard is a liquid oxalic acid 0.5 mmol/L. Enzytec™ oxalic acid kits, code no. E2100 (R-Biopharm AG, Germany), were used for the oxalic acid analysis. It is an end point method of colorimetric assay.

CALCULATIONS

Reading from curve: the equation used is $\ddot{A}A = (A2 - df \times A1) \text{ sample or standard} - (A2 - df \times A1) \text{ RB} = 0.765$. Where RB: reagent blank; df: dilution factor of the optical densities by reagent volume = (sample vol + R1) / (sample vol + R1 + R2) = 0.913. C sample (mg/L) = (C standard (mg/L) / $\ddot{A}A$ standard) $\times \ddot{A}A$. Since the concentration of the standard is fixed at 45 mg/L, then the formula becomes C sample (mg/L) = ($\ddot{A}A$ sample / $\ddot{A}A$ standard) $\times 45$. In ppm = reading from curve \times (dilution factor/sample weight). The ppm = reading from curve \times (50 / 2.6503).

STATISTICAL ANALYSIS

The statistical analysis was performed using the proc GLM of the statistical package SigmaStat version 16.0 (SPSS, 1997). The data were analyzed using one way ANOVA to detect any differences in the parameters studied (WILKINSON, 1990). When significant differences were detected, means were separated using LSD at 0.05 probability level (ABACUS CONCEPTS, 1991). Correlations' analysis between infestation and leaf's oxalic acid level as well as between infestation and leaf's moisture content of wheat and barley was conducted using Spearman's correlation method (ZAR, 1999).

Results

SYRINGOPAIS TEMPERATELLA INFESTATION AND LEAF'S OXALIC ACID LEVEL

The results indicated that wheat and barley accessions' effects were statistically significant for infestation percentages ($P < 0.05$). The infestation percentages were between 10.6 and 32.3% for wheat (Table 1) and 14.6 and 35.3% for barley (Table 2). The lowest infestation percentage was significantly recorded for the wheat accession, Acsad 1273 with 10.6% and the barley accession, 1614 with 14.6%, whereas the highest infestation percentages were significant for the wheat 1131 (32.3%), and the barley accessions, Wi 2291 (35.3%) and Eyl (34.9%).

In regards to leaf's oxalic acid level (ppm), the results indicated that the wheat accessions, Acsad 1187 (84.4), 1315 (92.0), 1131 (92.4) and 969 (93.5) had significantly the lowest oxalic acid content, while the accessions, 1103 (180.3), Sham (167.8), 885 (167.50) and Tari 885 (163.3) had the highest oxalic acid content ($P < 0.05$) (Table 1). In case of barley, the accessions, Wi 2291 (79.4) and Nabawi (84.3) had significantly the lowest oxalic acid content, while the accessions, Mutah (120.5) and Yarmouk (168.9) were the highest ($P < 0.05$) (Table 2).

Table 1.— Average (\pm SD) leaf's oxalic acid and moisture contents, and *Syringopais temperatella* infestation percentage of wheat accessions in the 2011/12 and 2012/13 cropping seasons.

No.	Accession name	Oxalic acid content (ppm)	Moisture content (%)	Infestation (%)
1	Acsad 1187	84.40 \pm 2.9 a	71.6	27.8 \pm 8.4 fgh
2	1315	92.00 \pm 1.1 b	40.0	24.4 \pm 3.6 defgh
3	1131	92.40 \pm 3.8 b	45.8	32.3 \pm 4.3 h
4	969	93.50 \pm 2.7 b	48.9	29.8 \pm 4.5 gh
5	Safra Maan	104.6 \pm 2.7 c	61.4	19.1 \pm 14.2 abcdefg
6	Petra	107.6 \pm 2.2 c	63.6	26.4 \pm 7.5 efg
7	Acsad 357	114.9 \pm 3.9 d	47.5	12.2 \pm 7.0 ab
8	Horani	116.4 \pm 5.1 d	46.8	17.1 \pm 6.1 abcde
9	981	129.4 \pm 1.6 e	72.7	27.5 \pm 7.1 fgh
10	Amoon	129.4 \pm 6.6 e	62.3	28.3 \pm 1.6 gh
11	Deir Alla	130.7 \pm 3.8 ef	34.9	19.6 \pm 7.1 abcdefg
12	1110	130.8 \pm 1.2 ef	40.3	28.5 \pm 3.4 fgh
13	Acsad 1273	133.5 \pm 2.8 efg	47.5	10.6 \pm 2.6 a
14	Irbid Norseih	135.7 \pm 1.6 fg	44.1	18.9 \pm 1.9 abcdefg
15	Acsad 1275	137.4 \pm 4.4 g	34.4	16.0 \pm 5.0 abcd
16	Umkais	144.9 \pm 2.3 h	60.0	14.9 \pm 4.6 abc
17	Acsad 65	145.9 \pm 1.2 h	56.4	21.5 \pm 13.4 cdefg
18	Acsad 1105	150.4 \pm 2.2 hi	68.6	21.1 \pm 13.2 bcdefg
19	Tari 889	151.9 \pm 2.2 i	68.6	25.6 \pm 8.4 efg
20	Acsad 1245	155.3 \pm 10.1 ij	61.7	13.7 \pm 3.8 abc
21	Horani Nawawi	160.4 \pm 1.7 j	37.0	17.3 \pm 9.1 abcde
22	Tari 885	163.3 \pm 0.8 jk	67.1	22.1 \pm 5.5 cdefg
23	885	167.5 \pm 1.9 k	77.7	17.9 \pm 7.6 abcde
24	Sham	167.8 \pm 1.6 k	59.0	20.3 \pm 8.7 bcdefg
25	1103	180.3 \pm 2.3 l	44.2	18.9 \pm 7.5 abcdefg

[Different small letters within the same column indicate significant differences among the different leaf oxalic acid content and among the different infestation percentages by the pest at $p < 0.05$ (one-factor analysis of variance)].

RELATIONSHIP BETWEEN INFESTATION AND LEAF'S OXALIC ACID AND MOISTURE CONTENTS

The correlation between leaf oxalic acid level of wheat and barley and the infestation percentage by *S. temperatella* is characterized by a decreasing function (Figure 1A, B); while the oxalic acid content increased in the leaves; the infestation percentage was decreased. The highest oxalic acid content found in the wheat accession, 1103 (180.3 ppm) showing relatively a low infestation (18.9%). On the contrary, the lowest oxalic acid content of 84.4 ppm showed an infestation of 27.8% in Acsad 1187 (Table 1). Wheat accessions revealed a significant inverse correlation of oxalic acid content with leafminer-infestation percentage ($r = -0.445$, $P = 0.026$) (Table 3). Negative correlation was also found for barley but without significance ($r = -0.263$, $P = 0.494$). The lowest barley accession, Wi 2291, in leaf oxalic acid (79.4) showed the highest percentage of pest infestation (35.3%) (Table 2).

The regression equations derived from the linear relationship between oxalic acid and infestation were $y = -0.10 \times 34.20$ for wheat and $y = -0.07 \times 33.77$ for barley (Table 3). Leaf's moisture content varied from 46.7 to 70.3% in the barley accessions (Table 2), corresponding to infestation percentages of 20.4 and 35.3%, respectively. Leaf moisture content showed an increasing function with the pest infestation percentage for both crops (Figure 2A, B). This positive function is stronger in barley than in wheat. The r value in barley was significant ($r = 0.733$, $P = 0.016$), while it was not significant for wheat ($r = 0.190$, $P = 0.362$) (Table 3). The highest leaf moisture content (70.3%) of the barley

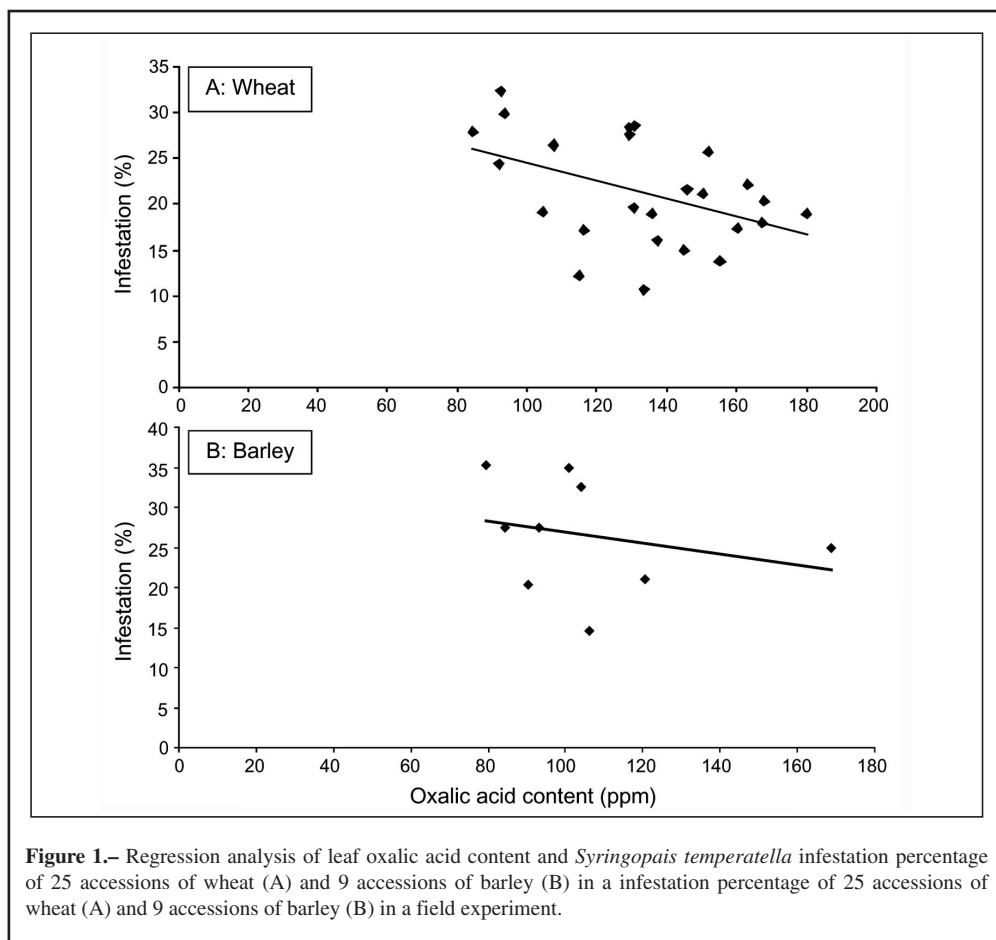


Table 2.– Average (\pm SD) leaf's oxalic acid and moisture contents and *Syringopais temperatella* infestation percentage of barley accessions in the 2011/12 and 2012/13 cropping seasons.

No.	Accession name	Oxalic acid content (ppm)	Moisture content (%)	Infestation (%)
1	Wi 2291	79.40 \pm 3.1 a	70.3	35.3 \pm 10.8 c
2	Nabawi	84.30 \pm 2.3 ab	47.3	27.4 \pm 10.0 bc
3	Anta	90.20 \pm 1.7 bc	46.7	20.4 \pm 0.7 ab
4	Wi 2269	93.10 \pm 3.3 c	66.1	27.5 \pm 2.5 bc
5	Eyl	100.8 \pm 7.7 d	56.9	34.9 \pm 2.3 c
6	Stepter	104.2 \pm 3.3 d	56.6	32.5 \pm 6.6 bc
7	1614	106.1 \pm 4.6 d	51.2	14.6 \pm 7.7 a
8	Mutah	120.5 \pm 2.8 e	51.0	21.1 \pm 9.8 ab
9	Yarmouk	168.9 \pm 1.2 e	68.0	25.0 \pm 9.0 abc

[Different small letters within the same column indicate significant differences among the different leaf oxalic acid content and among the different infestation percentages by the pest at $p < 0.05$ (one-factor analysis of variance)].

accession, Wi 2291 showed the highest percentage of leafminer infestation (35.3%) (Table 2). The regression equations derived from the linear relationship between leaf moisture and infestation were $y = 0.09 \times 16.58$ for wheat and $y = 0.40 \times 03.53$ for barley (Table 3). However, the infestation in some of the wheat and barley accessions was high despite the low levels of oxalic acid in the leaves. This observation indicates that, in addition to oxalic acid, other factors may also determine *S. temperatella* susceptibility in wheat and barley.

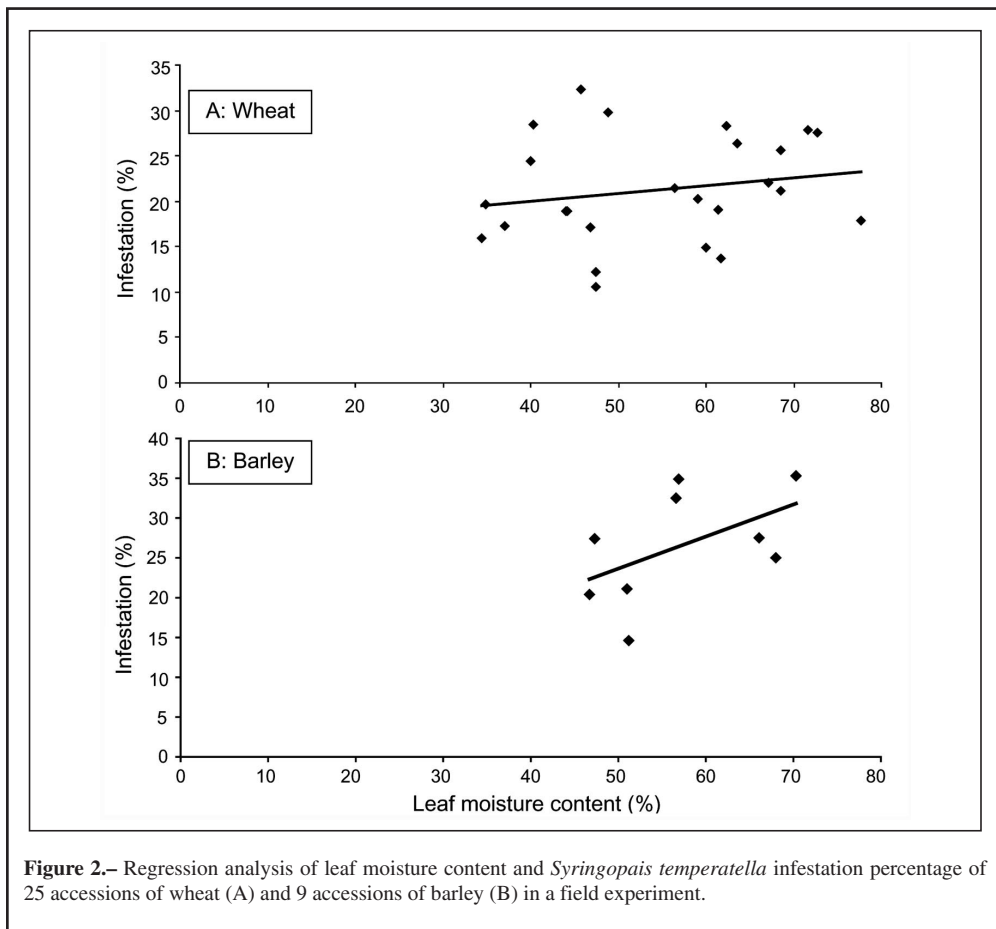


Figure 2.– Regression analysis of leaf moisture content and *Syringopais temperatella* infestation percentage of 25 accessions of wheat (A) and 9 accessions of barley (B) in a field experiment.

Table 3.– Correlation analysis of oxalic acid and moisture contents of wheat and barley leaves and the infestation percentage as resistance factors against *Syringopais temperatella* during the 2011/2012 and 2012/2013 cropping seasons.

Correlated variables	Crop	R value	Sig.	Model	Notes
Infestation vs. oxalic acid content	Wheat	-0.445*	0.026*	$y = -0.10x + 34.20$	Fig. 1A
	Barley	-0.263	0.494	$y = -0.07x + 33.77$	Fig. 1B
Infestation vs. moisture content	Wheat	+0.190	0.362	$y = 0.09x + 16.58$	Fig. 2A
	Barley	+0.733*	0.016*	$y = 0.40x + 03.53$	Fig. 2B

*Correlation is significant at the 0.05 probability level.

Discussion

Abiotic stresses such as drought, heat, cold and nutrient deficiency and biotic stresses such as pests, diseases and weeds prevent realization of the potentially high yield capacity of wheat and barley (WELTZIEN & FISCHBECK, 1990; ICARDA ANNUAL REPORT, 2007; AL-ZYOUD, 2012). In the Mediterranean area, *S. temperatella* causes reduction of wheat and barley that depends on infestation level, cultivar, and the environment; yield loss rates can reach 70% (AL-ZYOUD, 2012). The major reasons for the reluctance of farmers to use insecticides on these crops appeared to be high cost of protection and development of pest resistance against certain chemicals (RAJPUT *et al.*, 2003; SARWAR, 2013). The present study was undertaken to evaluate the performance of different wheat and barley accessions against *S. temperatella* taking into account the role of oxalic acid level in the leaves as a suggested chemical-resistant factor.

Oxalic acid occurs naturally in quite a large number of plants (BRADBURY & HOLLOWAY, 1988). Oxalates are present in plants as soluble salts (potassium and sodium oxalate), oxalic acid, or insoluble calcium oxalate. They are contained both within cells, and as sharp crystals between the cells. Leaves usually contain more oxalates than leaf-stalks (BRADBURY & HOLLOWAY, 1988); therefore, leafminer infestation on leaves was considered in this study. Fortunately, wheat and barley are crops in which man does not eat the leaves, whereas the high leaf content of oxalic acid in leafy crops like spinach is considered undesirable as this causes many health problems (AUSTRALIAN FOOD & GROCERY COUNCIL, 2008). Knowledge of the extent of susceptibility/resistance of crop cultivars and pest status on that crop is a fundamental component of IPM programs for many crops. Such information can help to detect and monitor pest infestation, cultivar selection and crop breeding. Growing a resistant cultivar is an ideal component of IPM strategy and use of less susceptible cultivars may offer one of the suitable components of eco-friendly management approach (AL-ZYOUD, 2008; AL-ZYOUD *et al.*, 2011).

The leaf oxalic acid level is playing an opposite roles in the cereal leafminer infestation. The leaf oxalic acid may be considered as a resistant factor against the pest attack. It may categorize within the non-preference mechanism of resistance in this study. However, none of the wheat and barley accessions tested in this work was completely immune to *S. temperatella* due to the leaf oxalic acid. The accessions revealed significant inverse correlation of oxalic acid level with infestation in wheat and barley. However, the infestation in some of the wheat and barley accessions was low despite the low levels of oxalic acid in the leaves. This observation indicates that, in addition to oxalic acid, other factors may also determine *S. temperatella* susceptibility in wheat and barley such as average rainfall (MADANAT *et al.*, 2012), plant cultivar (AL-ZYOUD *et al.*, 2009), and starting of leaves germination whether earlier or later among the different accessions tested, which reflects the duration of exposure to the pest infestation. Our results are coincident with ICARDA researchers who found that leaf oxalic acid content was significantly higher in the least preferred cultivars by the chickpea leafminer, *Liriomyza cicerina* (Rondani) than in the most preferred ones (ICARDA ANNUAL REPORT, 2005). Such resistant correlation with leaf oxalic acid was also found in other phytophagous insects. The inhibition of *H. armigera* larval growth by oxalic acid in the trichome exudate appears to be a component of host-plant resistance in chickpea; since there was a significant negative correlation ($r = -0.614$) between the oxalic acid level and larval density (YOSHIDA *et al.*, 1997). Similarly, YOSHIDA *et al.* (1995) and SARWAR (2013) reported that genotypes resistant to *H. armigera* accumulated more oxalic acid on the leaves than susceptible genotypes. Furthermore, oxalic acid from trichomes on the surface of chickpea plants (REMBOLD & WEIGNER, 1990) has been correlated with reduced pod damage (SRIVASTAVA & SRIVASTAVA, 1989; REMBOLD *et al.*, 1990; WIGHTMAN *et al.*, 1995; SHARMA *et al.*, 2006). The mechanism of resistance to *H. armigera* was not caused by antifeedant effects but was more likely attributable to antibiosis (YOSHIDA *et al.*, 1995; SHARMA *et al.*, 2006).

In the present study, the infestation correlated positively in both cereal crops with leaf moisture content (increasing function); normally, the mining larvae prefer the more succulent plant tissues. This finding is in full agreement with WEI *et al.* (2000) where host feeding selection (number of feeding

punctures) of the pea leafminer, *Liriomyza huidobrensis* (Blanchard) was positively correlated with the percentage of leaf moisture content. Moreover, one of the most conspicuous correlation between leaf moisture and larval growth aspects is that found in *Bombyx mori* L., in which significantly positive correlation between leaf moisture content and different larval parameters was obtained (RAHMATHULLA *et al.*, 2006). In addition, the preference might be due to physical factors (i.e. hairiness, hardness and thickness) of the leaves (COTTER & EDWARDS, 2006), as well as to differences in chemical composition of the leaves such as oxalic acid level in plant leaves, as indicated in this work.

In conclusion, oxalic acid exuding from leaf has been shown, to a certain extent, to contribute to pest resistance. In spite of the high variations among wheat and barley accessions in the present study, results support that the infestation percentage and oxalic acid level could be used as a selection criterion of a resistant accession as an integral part of management program against *S. temperatella*. It also provides useful information on the mechanism of resistance to *S. temperatella*. On the basis of percent plant infestation, the accessions 1614 and Acsad 1245 may be used as resistant donors in the genetically crossing program to evolve leafminer resistant accessions of barley and wheat, respectively. Therefore, these accessions may be used in hybridization program to evolve high *S. temperatella* resistant cultivars of wheat and barley that may be used as one of the components of IPM. The results of our study have practical implications for wheat and barley germplasm enhancement programs.

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F. A. A.–Z.
 Department of Plant Protection and IPM
 Faculty of Agriculture
 Mu'tah University
 Karak, 61710
 JORDANIA / JORDAN
 E-mail: firaszud@yahoo.com

D. S. H.
 Department of Biology
 College of Science
 Al-Anbar University
 Al-Anbar
 IRAQ / IRAQ
 E-mail: dhassawi@yahoo.com

*I. H. G.
 Department of Plant Production and Protection
 Faculty of Agricultural Technology
 Al-Balqa Applied University
 Al-Salt 19117
 JORDANIA / JORDAN
 E-mail: balappuniv@yahoo.com

*Autor para la correspondencia / *Corresponding author*

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