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RAPD analysis and field screening of bread wheat and barley accessions for resistance to cereal leafminer *Syringopais temperatella*

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Abstract

(*Syringopais temperatella* Led.) is a threat to wheat and barley. Resistant varieties are preferable due to environmental and human considerations and for their sustainability. However, no attention has been focused on resistant studies against cereal leafminer worldwide. Concomitantly, this study aimed at screening wheat and barley accessions as sources of *S. temperatella* resistance under semi-arid conditions of Karak-Jordan. It was also designed to evaluate the RAPD markers potential for identifying the accessions based on their resistance. The rank accessions order resulted from least to most according to infestation percentage and presence of larvae on leaves: Acsad 1245 (wheat), Acsad 1273 (wheat), 1614 (barley) and Umkais (wheat). The rank order of the same accessions from most to least according to grain yield and straw biomass: 1614, Acsad 1245, Umkais and Acsad 1273. Data on DNA (RAPD) markers revealed higher polymorphism level among accessions. Two hundred bands were noticed; and 199 were polymorphic. Total number of amplification products/primer ranged from 16 with primer (OPI-08) to 25 with primer (OPA-10), and the PCR size products ranged between 200-3500 bp.

Keywords: Cereal leafminer, varietals identification, RAPD markers, resistant accessions, *Syringopais temperatella*

1. Introduction

Wheat and barley are important crops in semi-arid and arid regions of Jordan (Al-Bakri *et al.*, 2011). Yield gained in these regions is low and variable from year to year due to insufficient rainfall and bad distribution (FAO, 2011). Wheat and barley varieties grown in Jordan differ from one area to another, and their productivity depends on the average amount of rainfall and severity of pest attack. In Jordan, low yield or/and crop failure are common (Einfeldt, 1999), and thus Jordan is not self-sufficient in wheat and barley production, depending on imports to cover the national needs (Al-Ghzawi *et al*., 2019; Jordan Statistical Yearbook, 2018).

Several abiotic (mainly drought, salinity and low soil fertility) and biotic factors (i.e. soil-borne diseases and insect pests) limit wheat and barley production (ICARDA, 2007). Dozens of insects' attack wheat and barley, and many of these pests cause neglected damage, others cause considerable forage and yield reductions across international borders (El-Bouhssini *et al.*, 2009; Ennahli *et al*., 2009). As cultural practices (as control measures) have many negative effects on the biotic and abiotic factors that would suppress the pest numbers, many levels of pests have an outbreak, wreaking huge damage to crops (Harlan, 1992). Nevertheless, many pests are difficult to control with conventional control measures, and because of the low inputs on these two cereal crops in non-developing countries, enough resources are not timely available (Srivastava *et al.*, 1988).

In many West Asian countries, *Syringopais temperatella* Led. (Lepidoptera: Scythrididae) is a major pest that attacks wheat and barley, significantly damaging the crops (Jemsi and Rajabi, 2003; Al-Zyoud, 2013b; Al-Zyoud and Ghabeish, 2015). The pest is endemic to Jordan, being reported since 1960s (Klapperich, 1968). The pest has great significance effect on wheat and barley throughout Jordan, and outbreaks of this pest have been mostly reported in Karak District since 2001 (Al-Zyoud, 2013b). Despite the intensive applications of chemical insecticides against the pest, this did not prevent further crop damage and spread of the pest in Karak, (Al-Zyoud, 2013b). Wheat infestation by *S. temperatella* in Karak District has exceeded 70% in some fields (Al-Zyoud and Ghabeish, 2015). *S. temperatella* populations increased over the years because of frequent drought, not applying the proper crop rotation and unsuitable farmer practices (Al-Zyoud, 2012).

Because of the importance of wheat and barley in Jordan; control tactics of this plague are crucial. Intensive application of chemical insecticides has been used to suppress the pest (Jemsi and Rajabi, 2003; Al-Zyoud, 2013a). Synthetic insecticide usage is neither economically feasible nor ecologically friendly; causing many side effects on humans and the environment (Gerson and Cohen 1989). In addition, the insecticides' use on wheat and barley has typically lagged as low-input crops owing to the cost constraints associated with these two crops (Debach and Rosen, 1991). As the agricultural community became increasingly aware of the negative effects of continuous reliance on chemicals, the idea that control of pests can be based on sound ecological principles reemerged. One of the most important control methods in such low-input crops is the use of resistant varieties. Finding such resistant varieties to agricultural pests are the main goal of wheat and barley breeders nowadays (Smith and Clement, 2012; Razmjou *et al*., 2014).

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Molecular biology methods are used for varietals identification and differentiation among species. Molecular markers could directly detect variations of the DNA sequences among cultivars independent of environmental effects and allow genotypes identification during earlier stages of plant development (Tar'an *et al.*, 2005). Different molecular-marker techniques have been used for accessing the genetic diversity in plants. The random amplified polymorphic DNA (RAPD) technique is used to determine the polymorphism of genomic DNA (Maric *et al.*, 2004; Sapna *et al.*, 2007), and is successfully used for wheat resistant studies (Iqbal *et al.*, 2007; Sapna *et al.*, 2007), and barley (Tinker *et al.*, 1993). The RAPD markers are dominant markers and because of its simplicity and speed, RAPD technique has been used for diversity analysis in many crops (Hernendez *et al.*, 2001; Zenglu and Randall, 2001; Mezghani-Khemakhem *et al.*, 2012).

However, no attention has been paid to studies on wheat and barley resistance to the cereal leafminer in Jordan and surrounding countries. Concomitantly, screening many wheat and barley accessions under field and laboratory conditions to investigate sources of resistance for wheat and barley to *S. temperatella* under semi-arid climatic conditions of South Jordan was our main goal. Thus, the outcomes of the current study could develop proper low cost and environmentally friendly integrated pest management (IPM) program to combat the cereal leafminer. Additionally, this research was designed to evaluate the potential of RAPD markers to determine the genetic diversity of accession included in this study.

2. Materials and Methods

2.1. Syringopais temperatella infestation in the field

Twenty-four accessions of bread wheat and three accessions of barley obtained from the Seed Bank of the National Agricultural Research Center (NARC, Baq'a, Jordan) were used in this study. The study was performed in a naturally *S. temperatella* infested field in Jordan, Al-Qasr, Karak area (Latitude of 31°11", Longitude of 35°42", and altitude of 845 m). The experimental site has semi-arid conditions with moderate rainfall; the long-term annual average is of 300 mm. The field soil was sandy clay loam with 1.63% organic matter.

Seeds of the different accessions were sown during the first week of December for the cropping seasons, 2011/2012 and 2012/2013 in a randomized complete block design (RCBD) with three replications. Each accession was sown in three rows (in 3 blocks), in which 30 g of seeds were sown/row of 2-m length with 25 cm spacing among rows. Seeds were directly irrigated after completion of sowing. Neither fertilizers nor insecticides were applied to the barley plants during the experiment. Routine cultural activities especially weeding have been performed every other week during seasons. Bird-net was used to prevent the plant ears from a possible attack by birds near the ripening stage of the plants. The percentage of leafminer's infestation was recorded early April for both cropping seasons. Three researchers have independently estimated the infestation percentage. In addition, the number of *S. temperatella* larvae/plant was counted. Moreover, grains and straw biomasses of all plants in each of the 3 rows (per season) for each accession were collected and weighed, then divided on the number of plants in the concerned raw to find the grain and straw yields per plant.

2.2. Molecular analysis of the accessions

2.2.1. Samples collection and DNA extraction

Fresh leaf samples from each accession were collected from the wheat and barley plants and stored at –80°C for the molecular part. Then, the fresh leaves (3 g) were grounded into fine powder with liquid nitrogen. The powder was mixed with 20 ml of hot CTAB buffer (100 mM Tris-HCl, pH 8.0, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 1% PVP, 0.2% β-mercaptoethanol, 0.1% $NaHSO_3$). Following that, the samples were incubated in a water bath at 65°C for 1 h. After cooling at the room temperature for 10 min, equal volume of chloroform-isoamyl alcohol (24: 1) was added to each sample and gently mixed for 10 min. The samples were centrifuged at 15,000 rpm at 10°C for 15 min. The aqueous phase was transferred into a clean tube and mixed with an equal volume of cold absolute ethanol, and the tubes were kept stand at –20°C for 20 min. The samples were then gently mixed, and the precipitation was recovered with a glass rod. The precipitation was washed with 10 ml of 10 mM ammonium acetate in 76% ethanol, and then air-dried at the room temperature overnight. The samples were resuspended in 400 µl of TE buffer (10 mM Tris-HCl, pH 8, 0.1 mM EDTA). RNA that could interfere with PCR was digested with 2 µl of DNase-free RNase for each sample (Doyle and Doyle, 1987; Maguire *et al*., 1994). The quality of DNA was estimated by calculating the ratio of the absorbance at 260 and 280 nm according to Johnson (1994).

2.2.2. Polymerase chain reaction (PCR)

A total of 10 RAPD primers (Operon Technologies, Inc) were used for PCR amplification. Amplification reactions were carried out in a total volume of 25µl, containing 30-50 ng of genomic DNA, 1.5 mM $MgCl_2$, 0.25 µM10-mer primer, 0.2 mM dNTPs, 1X PCR buffer from 10x buffer [100 mM Tris-HCl (pH 8), 500 mM KCl, 15 mM $MgCl_2$, 0.1% Difco Gelatin], 1 unit AmpliTaq DNA polymerase (Promega, Madison, Wis), and a drop of mineral oil to prevent evaporation. A control PCR mix containing all components, except the genomic DNA, was checked for DNA contamination. Each reaction was repeated at least twice for accuracy. The DNA amplification reactions were set up in a thermal cycler (Gene, UK) according to the following program: 1 min at 94°C for initial strand separation, followed by 40 cycles of 1 min at 94°C, then 2 min at 34°C, and 2 min at 74°C, and a final extension step of 5 min at 74°C.

2.2.3. Agarose gel electrophoresis

Amplified DNA fragments were separated on 1% agarose gel. Ethidium bromide was added to the gel to stain the DNA. The gel was viewed under ultraviolet (UV) light (BIO-RAD, USA), and then was photographed via a Video Polaroid Photograph Camera. A 100 bp-DNA ladder (Sigma Chemical Company, St. Louis) was used to estimate the molecular size of amplification products.

2.3. Statistical analysis

To validate the basic assumptions of the data to be statistically analyzed, the normal distribution and the homogeneity of variance were firstly evaluated using the Barlett method (Kohler *et al.*, 2002). After fulfilling the aforementioned two assumptions, analysis of variance was conducted using the Statistical Package Sigma Stat (SPSS) version 16.0 (Proc General Linear Model) (SPSS, 1997). For determining significant differences among means, least significant differences (LSD) test at a probability level of 0.05 was used (Abacus Concepts, 1991). Spearman's correlation analysis was performed to examine pair-wise association among the variables (number of larvae versus grain yield and straw biomass) (Zar, 1999). Data generating from

RAPD analysis were analyzed using the Nei similarity index (Nei and Li 1979). A dendrogram was constructed based on the similarity matrix data, by applying Unweighted Pair Group Method with Arithmetic Averages (UPGMA) cluster analysis using the Numerical Taxonomy System for personal computer (NTSYSpc) program (Exeter, Software, N.Y.).

3. Results

Results indicated that wheat and barley accessions varied in their susceptibility to the cereal leafminer. Averages of infestation level, number of larvae, grain yield and straw biomass showed significant differences among accessions (Tables 1&2). Results revealed that the wheat accessions, Acsad 1273 and 1245, as well as Umkais were the most resistant ones in terms of low percentage of infestation and minimal number of larval attacked plants foliage. The barley accession, 1614 (14.6% infestation) and Tadmur (15.9%) were significantly more resistant than Mutah (21.1%), and both were exhibited a low larval attack. On the contrary, the wheat accessions; 1115, 1069 and 1131 showed susceptibility based on infestation percentage and larval abundance on plant foliage. Moreover, Mutah barley accession showed susceptibility, but with a moderate number of larval attack. The harvested grain of the accessions tested, Acsad 1245 was the best one among the most wheat-resistant accessions; and located in the top 9 accessions from the standpoint of grain yield production. Furthermore, Acsad 1245 showed good straw biomass (1.86 g/plant). The most resistant barley accession, 1614 was one of the top 5 accessions from the standpoint of grain weight and one of the top 4 accessions in terms of straw biomass.

None of the resistant wheat accessions was promising in straw biomass produced. The most susceptible wheat accessions (1115 and 1131) were ranked of the worst 9 accessions in the weight of grains obtained. Results showed that the most susceptible barley accession; Mutah was the top barley accessions in grain yield production. The most susceptible accessions of wheat (Al-Raba) and barley (Mutah) showed the least straw biomass production. As expected, all the susceptible accessions were among the first-third accessions heavily attacked with the highest number of pest larvae.

Results of the Spearman correlation analysis showed significant relationship between the infestation% and the foliage larval number for wheat accessions, and showed non-significant relationship for barley accessions. The relationship was moderately positive for wheat accessions and was weakly positive for barley accessions. Moreover, results showed significant negative relationships between foliage larval infestation and each of the grain yield and the straw biomass for wheat and barley accessions. Spearman coefficient values are higher in wheat accessions than in barley accessions (Table 2).

After an initial screening of several decamer primers available in the laboratory, amplification products of ten primers were selected for further analysis. The genomic DNA of twenty-seven wheat and barley accessions was amplified with these random 10 base arbitrary primers (Table 3). The primers that generated polymorphic amplification fragments were conspicuous and highly reproducible (Figures 1 and 2). The gel of each primer was separately analyzed by scoring the presence/absence of all fragments of PCR in individual lanes, where (+) was allocated for the presence of an amplified fragment, while (-) for its absence (Table 3).

Two hundred DNA fragments were generated by 10 random primers, averaging 20 bands/ primer. Some reactions were duplicated more than once for checking the amplified products' consistency. Among the 200 amplification products recorded, 199 bands (99.5%) were polymorphic, and only one band (0.5%) was monomorphic. The primer (OPA-10) produced the highest number of bands (25), while primer (OPI-08) produced the lowest number (16). The DNA fragments size ranged from 200 bp with primers (OPB-09) to 3500 bp with primer (OPI-08). The primer OPI-08 was the only one that generated fragments above 2000 bp molecular size (2500, 3000, and 3500 bp) with some of wheat accessions. In general, the three barley accessions (1614, Tadmur and Mutah) produced smaller fragments size ranging between 250 to 1500 bp. On the other hand, primers (OPH-15, OPJ-13 and OPK-2) generated more numbers of polymorphic bands with the aforementioned three barley accessions. RAPD data were used to produce dendrogram using cluster tree analysis, NTSYSpc (Figure 3). The 24 wheat accessions were grouped into two main clusters; the first one contained 23 accessions, while the second one contained the accession, Acsad 1129 only. The clustering pattern of the accession indicated that most of the accessions are closely related. This is expected to be caused by the selection of those accessions from a single population.

Table 1. Average (±SD) infestation percentage, grain yield, straw biomass and larvae number of wheat and barley accessions in both the 2011/2012 and 2012/2013 seasons.

No	Crop	Accession	Infestation (%)	Grain yield (g/plant)	Straw biomass (g/plant)	No. of larvae per plant
1	Wheat	Acsad 1273	10.6±2.69 a	0.21±0.18 abc	1.56±0.98 abcd	2.57±0.85 a
2	Wheat	Acsad 1245	13.7±4.41 ab	0.45±0.29 abcdef	1.86±0.94 bcde	2.51±1.52 a
3	Barley	1614	14.6±7.76 ab	0.53±0.35 cdef	2.08±0.89 cde	2.61±1.42 a
4	Wheat	Umkais	14.9±4.61 ab	0.32±0.27 abcdef	1.67±0.73 abcde	2.86±2.39 ab
5	Wheat	Acsad 1129	15.7±10.2 abc	0.13±0.07 a	1.85±0.44 bcde	3.21±1.56 abc
6	Barley	Tadmur	15.9±6.36 abc	0.49±0.21 bcdef	1.39±0.15 abc	2.36±1.24 a
7	Wheat	Acsad 1275	16.0±5.07 abc	0.16±0.10 ab	1.56±0.37 abcd	2.65±1.18 a
8	Wheat	Horani	17.1±6.19 abc	0.21±0.20 abc	1.96±0.29 bcde	2.34±1.31 a
9	Wheat	Horani Nawawi	17.3±9.16 abc	0.45±0.35 abcdef	2.09±0.40 cde	3.25±3.11 abc
10	Wheat	885	17.9±7.65 abc	0.63±0.30 ef	1.84±0.70 bcde	4.15±2.89 abc
11	Wheat	Al-Raba	18.5±5.53 abcd	0.39±0.16 abcdef	0.97±0.55 a	2.22±0.79 a
12	Wheat	Safra Maan	19.1±14.3 abcde	0.20±0.18 abc	2.35±0.90 de	2.51±1.82 a
13	Wheat	Sham 4	20.2±8.72 bcde	0.32±0.23 abcdef	1.65±0.86 abcde	2.85±1.04 ab
14	Wheat	Sham 1	20.3±9.22 bcde	0.37±0.26 abcdef	1.68±0.81 abcde	2.89±1.65 ab
15	Wheat	Amra	20.5±13.8 bcde	0.23±0.21 abcd	1.93±0.22 bcde	5.45±3.43 bc
16	Barley	Muta'h	21.1±9.82 bcdef	0.55±0.50 def	1.36±0.39 abc	3.89±2.05 abc
17	Wheat	Acsad 65	21.5±13.4 bcdef	0.32±0.22 abcdef	1.38±0.36 abc	3.11±2.53 abc
18	Wheat	Tari 885	22.1±5.52 bcdef	0.62±0.30 ef	1.83±0.30 bcde	2.84±1.31 ab
19	Wheat	1315	24.4±3.66 cdefg	0.32±0.31 abcdef	2.06±0.95 cde	3.57±2.63 abc
20	Wheat	Petra	26.4±7.75 cdefgh	0.26±0.22 abcd	1.97±0.99 bcde	5.67±5.04 c
21	Wheat	899	27.2±6.73 defgh	0.38±0.37 abcdef	1.40±0.80 abcd	4.33±2.94 abc
22	Wheat	981	27.5±10.7 defgh	0.65±0.31 f	1.57±0.21 abcd	3.07±1.65 abc
23	Wheat	Acsad 1187	27.8±8.41 efgh	0.41±0.15 abcdef	2.13±0.67 de	3.03±1.77 abc
24	Wheat	969	29.8±4.50 fgh	0.46±0.09 abcdef	1.97±0.73 bcde	3.88±1.36 abc
25	Wheat	1131	32.3±4.36 gh	0.30±0.26 abcde	2.05±0.47 cde	4.49±4.39 abc
26	Wheat	1069	33.0±6.94 gh	0.44±0.35 abcdef	2.03±0.75 cde	4.12±1.98 abc
27	Wheat	1115	33.6±6.21 h	0.24±0.11 abcd	1.24±0.23 ab	3.58±2.48 abc
LSD value			9.10	0.34	0.75	2.80

*Means with different letters in the same column are significantly different at 0.05

Table 2: Correlation analysis of larval population size of the leafminer *Syringopais temperatella* versus grain yield and straw biomass of the infested barley and wheat accessions during the 2011/2012 and 2012/2013 seasons.

Correlated variables	**Spearman coefficient (r)**	**Significances**
Barley:		
Infestation% vs. larval number	0.254	NS
Larvae vs. straw biomass	-0.781**	000
Larvae vs. grain yield	-0.721**	000
Wheat:		
Infestation% vs. larval number	0.440 **	000
Larvae vs. straw biomass	-0.589**	000
Larvae vs. grain yield	-0.593**	000

**Correlation is significant at the 0.01 level. NS: Not significant.

Table 3: Number of different size and monomorphic markers, and percentage of monomorphic and polymorphic markers generated by each of the ten primers with the 27 wheat and barley accessions.

Primer number	Primer sequence (5`-3`)	No. of different size markers	No. of monomorphic markers	Monomorphic markers (%)*	Polymorphic markers (%)**
OPA-10	GTGATCGCAG	25	0	0%	100%
OPB-09	TGGGGGACTC	17	1	6%	94%
OPC-10	TGTCTGGGTG	18	0	0%	100%
OPD-12	CACCGTATCC	20	0	0%	100%
OPG-02	GGCACTGAGG	18	0	0%	100%
OPH-15	AATGGCGCAG	22	0	0%	100%
OPI-08	TTTGCCCGGT	16	0	0%	100%
OPJ-13	CCACACTACC	22	0	0%	100%
OPK-02	GTCTCCGCAA	20	0	0%	100%
OPO-02	ACGTAGCGTC	21	0	0%	100%
Total	-	199	1	0.5%	99.5%

*Calculated by dividing the number of common markers among accessions on the total number of markers produced by each primer.

**Calculated by subtraction the % of monomorphic markers from 100%.

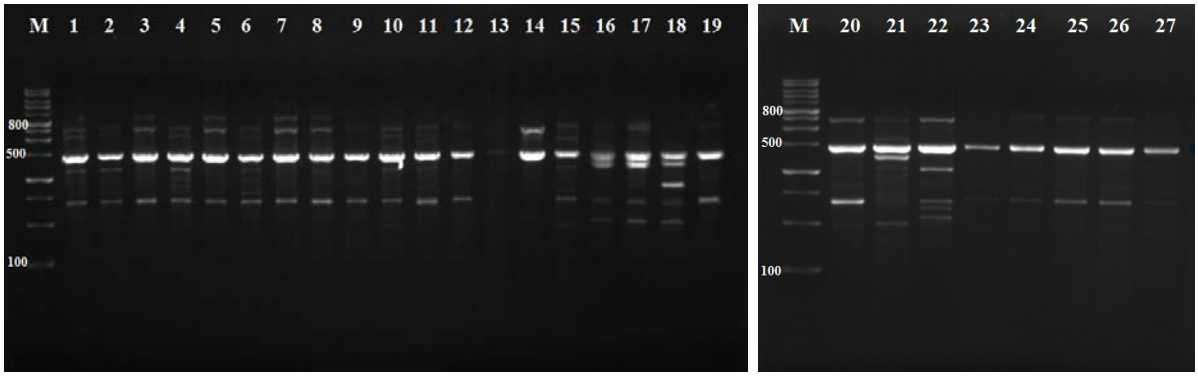

Figure 1: RAPD PCR products profile of wheat and barley accessions with primer OPI-08. M = Marker, 1 = wheat 981, 2 = wheat 1131, 3 = wheat Horani Nawawi, 4 = wheat Al-Raba, 5 = wheat Safra Maan, 6 = wheat 1069, 7 = wheat Sham 4, 8 = Acsad 1187, 9 = wheat Umkais, 10 = wheat 885, 11 = wheat Tari 885, 12 = wheat Horani, 13 = wheat Petra, 14 = wheat Amra, 15 = wheat 1115, 16 = barley Mutah, 17 = wheat Acsad 1129, 18 = barley Tadmur, 19 = wheat Sham 1, 20 = Acsad 1275, 21 = barley 1614, 22 = wheat 1315, 23 = wheat 969, 24 = wheat Acsad 65, 25 = wheat Acsad 1273, 26 = wheat Acsad 1245, 27 = wheat 899.

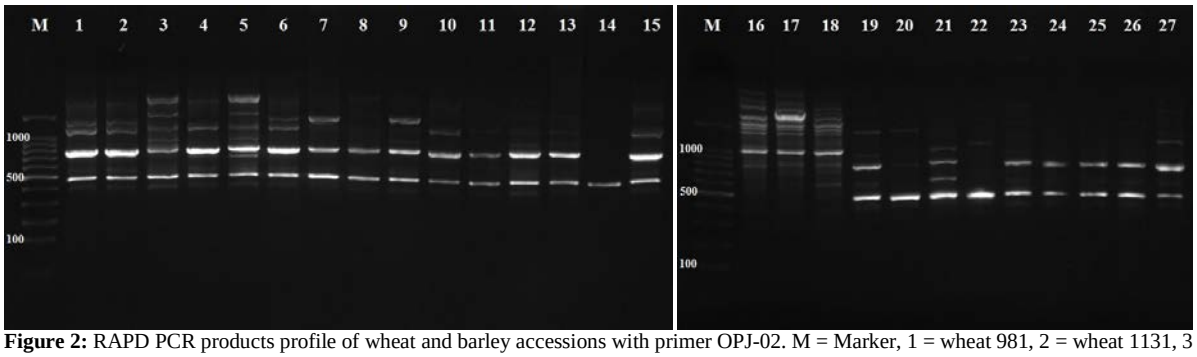

Figure 2: RAPD PCR products profile of wheat and barley accessions with primer OPJ-02. M = Marker, 1 = wheat 981, 2 = wheat 1131, 3 = wheat Horani Nawawi, 4 = wheat Al-Raba, 5 = wheat Safra Maan, 6 = wheat 1069, 7 = wheat Sham 4, 8 = Acsad 1187, 9 = wheat Umkais, 10 = wheat 885, 11 = wheat Tari 885, 12 = wheat Horani, 13 = wheat Petra, 14 = wheat Amra, 15 = wheat 1115, 16 = barley Mutah, 17 = wheat Acsad 1129, 18 = barley Tadmur, 19 = wheat Sham 1, 20 = Acsad 1275, 21 = barley 1614, 22 = wheat 1315, 23 = wheat 969, 24 = wheat Acsad 65, 25 = wheat Acsad 1273, 26 = wheat Acsad 1245, 27 = wheat 899.

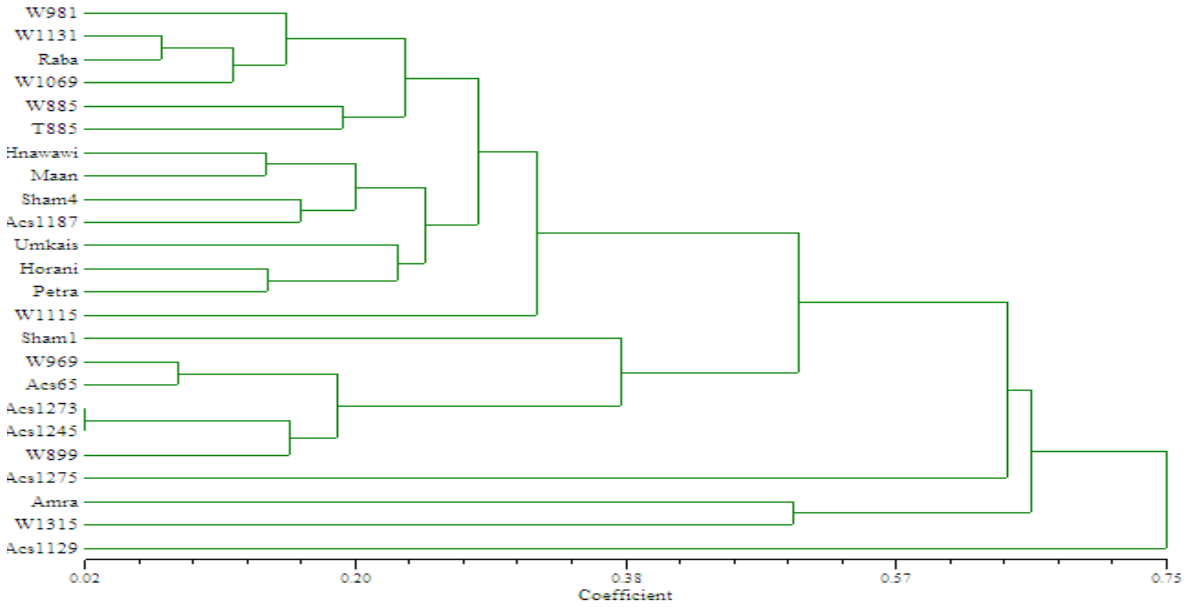

Figure 3: Dendrogram illustrating genetic relationships among 24 wheat accessions generated from RAPD data.

4. Discussion

Breeding of wheat and barley resistant varieties is of vital importance for cereal growers, since cereal crops characterizing by relatively low financial returns, and thus, costly control measures is considered undesirable. Resistant varieties usage against agricultural pests of such crops does not entail extra cost, and the only cost is the price of the seeds, and thus the use of these varieties is a desirable practice for their effectiveness, safe to environment (Singh and Weigand, 2006), and durable as well. Smith and Clement (2012) mentioned that arthropod-resistant for rice and sorghum cultivars and, to a lesser extent, wheat and raspberry cultivars are major elements of IPM programs throughout the world. Hence, this study aspires to constitute the first step for the development of resistant varieties to *S. temperatella*.

The current results indicated that some accessions, especially Acsad1273 (as it has higher resistance than 1245) (wheat) and 1614 (barley) are promising, based on the field resistance score, and could be useful as genetic source materials for further studies in breeding programs for producing *S. temperatella*-resistant cultivars. The aforementioned accessions indicated low infestation level, high grain yield and straw biomass as well as few numbers of larval populations attacking their foliage, and this is confirmed by the results of the correlation analysis; Spearman coefficients are relatively high for barley (0.78 for straw and 0.72 for grain yield vs. larvae number), and of moderate values for wheat (0.58 for straw and 0.59 for grain yield). Furthermore, results of the correlation analysis explained the low grain yield and straw biomass in the susceptible accessions which is due to the larger larval population size of the leafminer attacked their foliage and vice versa for the resistant accessions. Nevertheless, accession resistance to *S. temperatella* might be due to plant physical barriers, or variations in plant chemical composition (Al-Zyoud et al., 2009; 2015). Moreover, the accession's genetic make-up might also stand behind *S. temperatella* resistance. Although, no accession tested was completely immune to *S. temperatella*, some accessions indicated a type of resistance that is promising to the cereal leafminer and could be used in a future breeding program. Al-Zyoud *et al.* (2009), however, found susceptibility variations in 12 cultivars of wheat and barley to the pest, in which Acsad 65 (wheat) and Athroh (barley) had pest resistance more than the rest of the tested cultivars. In addition, in Iraq, it was reported that Sham 6 (wheat) is more resistant to the pest, whereas Tell-After 3, Karunyia and Om Rabee showed less resistance to *S. temperatella* (ICARDA, 2007).

Ten RAPD primers were selected and used in this study. The number of reproducible polymorphic fragments for wheat accessions with primers ranged between one band such as in the wheat accessions, Petra and Amra with primer OPI-08 and OPJ-13, respectively to twelve bands for the wheat accessions, Acsad 65 with primer OPA-10 and Al-Raba with primer OPO-02. For barley accessions, the number of bands ranged from three for 1614 and Tadmur with primers OPG-02 and OPO-02, respectively to twelve bands for Mutah with primer OPH-15 and Tadmur with primer OPJ-13. The fragment size ranged between 200 bp for primer OPB-09 with the accessions; wheat Tari 885, wheat Amra and barley 1614 to 3500 bp for primer OPI-08 with the wheat accessions; Horani Nawawi, Safra Maan, Sham 4, and Acsad 1187.

The PCR amplification products allowed us to investigate the genetic relationship of the accessions. The total number of bands scored for the ten primers was 200. The relatively large number of polymorphic bands (199) obtained with these primers is consistent with the earlier findings of Maric *et al.* (2004) and Sapna *et al.* (2007), who reported that both wheat and barley are highly polymorphic plant species. The results of this study revealed that the diversity of wheat and barley, which is displayed by the 27 accessions could be attributable to evolutionary forces like selection, mutation, migration and genetic drift that act continuously and result in continuous changes in allelic frequency in a population as well as genetic diversity.

All amplification patterns obtained with the ten primers were clear, but the intensity was not the same after being illustrated under UV. It is suggested that intensity of the band might reflect differences in the copy number of the amplified sequence among the different accessions (Yang and Quiros, 1993).

Genetic diversity is referred to the diversity present within different genotypes of same species. RAPD analysis indicated that wheat Acsad 1273 and Acsad 1245 are closely related to each other, as they showed similar pattern with the different RAPD

primers, and they are far related to wheat Acsad 1129; this also proved in the different RAPD patterns of this accession compared to the other two mentioned above. These three accessions showed lowest infestation%. However, RAPD patterns in this study revealed the genetic diversity of the whole genome of the accessions and not similarity or diversity of single gene polymorphisms for resistance to leafminer. RAPD primers are random and not specific; to acquire a molecular marker closely linked to leafminer resistant gene, a saturated genetic linkage map is required using other molecular markers techniques such as Amplified Fragment Length Polymorphism (AFLP), Single Nucleotide Polymorphisms (SNPs), Simple Sequence Repeats (SSRs), and Restriction fragment length polymorphism (RFLP), …etc., which is beyond the scope of this study.

The existence of genetic diversity among wheat and barley accessions used in this study may serve as the source of desirable alleles and may assist plant breeders in breeding for leafminer resistant and new insect-pests. Additionally, the presence of genetic diversity among these accessions may permit breeders to select superior genotypes to be used as parents in hybridization programs.

5. Conclusions

None of the accessions checked in the current study have been immune to *S. temperatella*; however, some accessions were found having a promising degree of resistance to the pest infestation, which could be exploited in future breeding programs. Depending on pest infestation level and number of larvae on plants, the rank order of accessions from least to most was as follows: Acsad 1245 (wheat), Acsad 1273 (wheat), 1614 (barley) and Umkais (wheat). The rank order of the same accessions from most to least from the standpoint of grain yield and straw biomass produced was 1614, Acsad 1245, Umkais and Acsad 1273. The plant materials used in this study are well adapted to our region; thus, they could be directly used as genetic resources without any further adapting.

Genetic diversity revealed by RAPD analysis and dendrogram results for wheat accessions in relation to field resistance to leafminer indicated that accession Acsad 1129 is far related to two other accessions, Acsad 1273 and Acsad 1245; also, the three accessions showed least Infestation%. For future leafminer breeding programs and for fast and efficient release of leafminer resistant variety, accession Acsad 1129 can be crossed with Acsad 1273 as first choice and Acsad 1129 with Acsad 1245 as second choice for this purpose. RAPD analysis can help plant breeders in deciding on selecting parents in breeding programs, which otherwise is not possible to perform based solely on field resistance.

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References

Abacus Concepts. 1991. **SuperAnova User's Manual**, Version 1.11, Abacus Concepts, Berkeley, CA.

Al-Bakri J, Suleiman A, Abdulla F and Ayad J. 2011. Potential impact of climate change on rainfed agriculture of a semi-arid basin in Jordan. *Phys Chem Earth Parts A/B/C*. **36(5):** 125-134.

Al-Ghzawi A, Al-Ajlouni Z, Al Sane K, Bsoul Y, Musallam I, Bani Khalaf Y, Al-Hajaj N, Al Tawaha A, Aldwairi Y and Al-Saqqar H. 2019. Yield stability and adaptation of four spring barley (*Hordeum vulgare* L.) cultivars under rainfed conditions. *Res Crops*. **20(1):** 10-18.

Al-Zyoud F. 2012. Effect of field history on the cereal leafminer *Syringopais temperatella* Led. (Lep., Scythrididae) and its preference to different wheat and barley cultivars. *Pak J Biol Sci*. **15:** 177-185.

Al-Zyoud F. 2013a. Efficacy of insecticides' applications against the cereal leafminer *Syringopais temperatella* Led. (Lep., Scythrididae) on barley under field conditions in Karak-Jordan. *Dirasat Agric Sci.* **39:** 65-74.

Al-Zyoud F. 2013b. Towards integrated pest management of the cereal leafminer *Syringopais temperatella* Led. (Lep., Scythrididae): Status, current and future control options. *Am-Eur J Agric Environ Sci*. **13:** 1582-1594.

Al-Zyoud F and Ghabeish I. 2015. Significance of the larval population size of the cereal leafminer *Syringopais temperatella* Led. (Lep., Scythrididae) and the diapausing depth in the soil on the yield variables of wheat and barley. *Jordan J Agric Sci.* **11:** 725-734.

Al-Zyoud F, Hassawi D and Ghabeish I. 2015. Oxalic acid as an alienate factor for wheat and barley resistance to cereal leafminer *Syringopais temperatella* (Lederer, 1855) (Lepidoptera: Scythrididae). *SHILAP Revta lipid.* **43:** 113-123.

Al-Zyoud F, Salameh N, Ghabeish I and Saleh A. 2009. Susceptibility of different varieties of wheat and barley to cereal leafminer *Syringopais temperatella* Led. (Lep., Scythrididae) under laboratory conditions. *J Food Agri Environ.* **7:** 235-238.

Debach P and Rosen D. 1991. **Biological control by natural enemies**. Cambridge University Press, Cambridge, UK.

Doyle JJ and Doyle JL. 1987. A rapid isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bul.* **19:** 11-15.

Einfeldt CHP. 1999. Effects of heterozygosity and heterogeneity on yield and yield stability of barley in the dry areas of north Syria. Ph.D. Dissertation, University of Hohenheim, Stuttgart, Germany.

El-Bouhssini M, Street K, Joubi A, Ibrahim Z and Rihawi F. 2009. Sources of wheat resistance to Sunn pest *Eurygaster integriceps* Put in Syria. *Evol.* **56:** 1065-1067.

Ennahli S, El-Bouhssini M, Grando S, Anathakrishnan R, Niide T, Starkus L, Starkey S and Smith CM. 2009. Comparison of categories of resistance in wheat and barley genotypes against biotype 2 of the Russian wheat aphid *Diuraphis noxia* Kurdjumov. *Arthrop Pl Interact.* **3:** 45-53.

FAO. 2011. **Production Yearbooks**.

Gerson U and Cohen E. 1989. Resurgence of spider mites (Acari: Tetranychidae) induced by synthetic pyrethroids. *Exp Appl Acarol*. **6:** 29-46.

Harlan JR. 1992. **Crops and man**. Second ed., American Society of Agronomy, Madison, Wisconsin, USA.

Hernendez P, Rosa R, Rallo L and Dorado G. 2001. Development of SCAR markers in olive (*Olea europaea*) by direct sequencing of RAPD products: Application in olive germplasm evaluation and mapping. *Theor App Genet.* **103:** 788-791.

ICARDA. 2007. Iraq-ICARDA-Australia Project: Better crop germplasm and management for improved production of wheat, barley and pulse and forage legumes in Iraq. 2nd Technical Report, ICARDA, Aleppo, Syria.

Iqbal A, Khan AS, Khan FS, Ahmad A and Khan AA. 2007. Study of genetic divergence among wheat genotypes through random amplified polymorphic DNA. *Genet Mol Res.* **6:** 476-481.

Jemsi G and Radjabi G. 2003. Study on harvesting agronomic measures and effect of chemical application in controlling the cereal leafminer *Syringopais temperatella* Led. (Lep., Elachistidae) in Khuzestan province. *App Entomol Phytopath*. **70:** 45-61.

Johnson JL. 1994. Similarity analysis of DNAs. In: Methods for general and molecular bacteriology, (Gerhardt P, Murray RGE, Wood WA and Krieg NR., Eds). American Society for Microbiology, Washington, D. C., USA.

Jordan Statistical Yearbook. 2018. Department of Statistics/**Annual Agriculture Surveys**. http://www.dos.gov.jo /dos_home_a/main/ cd_yb2011/pdf/agri.pdf

Klapperich J. 1968. Entomologisch und wirtschaftlich bedeutsame Schadinsekten in der jordanischen Landwirtschaft. *J Pest Sci*. **41**: 164-168.

Kohler W, Schachtel W and Voleske P. 2002. **Biostatistik**. Springer-Verlag, Berlin.

Maguire TL, Collins G, and Sedgley M. 1994. A modified CTAB DNA extraction procedure for plants belonging to family *Proteaceae. Plant Molecular Biology Reporter*. **12:** 106-109.

Maric S, Bolaric S, Martincic J, Pejic I and Kozumplik V. 2004. Genetic diversity of hexaploid wheat cultivars estimated by RAPD markers, morphological traits and coefficients of parentage. *Pl Breed.* **123:** 366-369.

Mezghani-Khemakhem M, Bouktila D, Kharrat I, Makni M and Makni H. 2012. Genetic variability of green citrus aphid populations from Tunisia assessed by RAPD markers and mitochondrial DNA sequences. *Entomol Sci.* **15:** 171-179.

Nei M. and Li WH. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc Natl Acad Soc*. **76:** 5269-5273.

Razmjou J, Hemati SA and Naseri B. 2014. Comparative performance of the cotton bollworm *Helicoverpa armigera* Hubner (Lep., Noctuidae) on various host plants. *J Pest Sci.* **87:** 29-37.

Sapna G, Pushpa K, Rekha M, Jain S and Jain RK. 2007. Assessment of genetic diversity among some Indian wheat cultivars using random amplified polymorphic DNA (RAPD) markers. *Ind J Biotech*. **6:** 18-23.

Singh KB and Weigand S. 2006. Registration of three leafminer-resistant chickpea germplasm lines: ILC 3800, ILC 5901 and ILC 7738. *Crop Sci.* **36:** 472-472.

Smith CM and Clement SL. 2012. Molecular bases of plant resistance to arthropods. *Ann Rev Entomol.* **57**: 309-328.

SPSS. 1997. **Sigmastat Statistical Software Users Manual**. Statistical Product and Service Solutions Inc., Chicago, United States.

Srivastava JP, Miller RH and van Leur. JAG. 1988. **Biotic stress in dry land cereal production: The ICARDA perspective**. In: Unger PW, Jordan WR and Sneed TV.

Tar'an B, Zhang C, Warkentin T, Tullu A and Vandenberg A. 2005. A genetic diversity among varieties and wild species accessions of pea (*Pisum sativum* L.) based on molecular markers, and morphological and physiological characters. *Genome*. **48:** 257-272.

Tinker NA, Fortin MG and Mather DE. 1993. Random amplified polymorphic DNA and pedigree relationship in spring barley. *Theor App Genet*. **85:** 976-984.

Zenglu L and Randall LN. 2001. Genetic diversity among soybean accessions from three countries measured by RAPDs. *Crop Sci.*, **41:** 1337-1347.

Yang X and Quiros C. 1993. Identification and classification of celery cultivars with RAPD markers. *Theor Appl Genet.* **86:** 205-212.

Zar J. 1999. **Bio-Statistical Analysis**. Prentice Hall, Upper Saddle River, NJ, pp 663.