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## Phenotypic diversity of Jordanian wild oat relative *avena sterilis* using multivariate analysis

Nawal Al-Hajaj<sup>1</sup>, Israa Alhasanat<sup>1</sup>, Abd-Alnaser Mousa<sup>1</sup>, Khaled Al-Sham'aa<sup>2</sup> and Salvatore Ceccarelli<sup>3</sup>

<sup>1</sup>National Agricultural Research Center NARC, 639, Baq'a 19381, Jordan

<sup>2</sup>ICARDA, Dalia Building 2nd Floor, Bashir El Kassar Street, Verdun, Beirut, Lebanon 1108-2010

<sup>3</sup>Rete Semi Rurali, via di Casignano 25, 50018 Scandicci (FI), Italy

\*Correspondence: [nawal@narc.gov.jo](mailto:nawal@narc.gov.jo) Accepted: 18 Aug2018 Published online: 31Dec. 2018

The wild ancestral form of Oat, *Avena sterilis* L., collected from the oat diversity center, is a valuable source for gene enrichment in oat crop improvement. This study is aimed at assessing the extent and the pattern of morphological variation in the wild populations of oat with respect to populations' passport data, and to identify the major traits contributing to population's diversity and classifying it in groups. Ten plant and panicle traits were evaluated in forty-eight populations of *A. sterilis* L. collected in Jordan. Three distinct clusters were identified; two grouped according to heading and maturity dates and varied in panicle yield traits, and the third cluster showed earliness in heading and maturity dates. The first three principle components (PC) explained 74.85% of the total variation among populations. 59.27% of the variance was accounted by the first and second PCs and panicle length and spikelet number loaded the largest portion of the variability. The discriminant analysis showed that 58.3% and 14.6% of the 48 populations were attributed correct to their original region and altitude, respectively. Altitude was associated to the diversity in wild oat populations more than collection region. The variation in the population traits reveals plenty of information for better exploitations of collected materials for genetic improvement, and the populations' analysis emphasized the importance of short distance sampling collection to capture most of the variation within the gene pool.

**Keywords:** Diversity, characterization, *Avena sterilis* L., multivariate analysis.

### INTRODUCTION

Oats is an important source for human food and livestock feed (Rasane et al., 2015; Iannucci et al., 2011). It is a secondary crop due to their weedy spread in wheat fields (*Triticum dicoccum* L.) (Vavilov, 1992). *Avena sativa* L. is a cereal crop of Mediterranean origin (Stevens et al., 2004) and the Middle East region is an oat diversity center (Zwer, 2010). Oat tracing goes back to the beginning of Christianity (Warburton, 1910), where it gradually evolved into a separate crop due to its greater cold resistance and adaptation to poor soil comparing with other cereals crop. Wild relative species within the same gene pool

had contributed to crops evolution and cultivars development, as they possess many desirable characteristics. The wild progenitor of cultivated hexaploid oat is *Avena sterilis* L. that represents an important and readily accessible genetic resource in conventional oat breeding, and constitutes a large reservoir of genetic diversity (e.g. see Coffman, 1977; Frey, 1991; Harder et al., 1992).

Many studies reported that *A. sterilis* L. is a potential source for traits such as grain yield, growth rate, harvest index, resistance to diseases such as crown, stem rust, powdery mildew, smuts, root rots, and nematode. In addition, biochemical

quality traits and commercially valuable traits (e.g. see Lawrence and Frey, 1975; Takeda and Frey, 1976; Szejnberg and Wahl, 1976; Loskutov, 2008; Loskutov et al., 2017; Henningsen et al., 2018).

Genetic diversity and crop variability studies based on ecogeographic patterns and plant traits are useful for crop evolution studies (Harlan, 1971; Ren et al., 2013; Abbo et al., 2014), for deciding efficient of germplasm collection (Gepts, 2006; Endresen, 2010; Li et al., 2013), and for parental selection for plant breeding program (Bhatt, 1970; Parra-Quijano et al., 2012 a,b). Morphological characterization is a crucial step for effective germplasm utilization. Phenotypic traits are the most important targets for conventional breeding. Phenotypic variation of crops has been studied vigorously and these studies have been concentrated on the desired traits that would benefit the modern cultivars of annual crops, like barley (Vanhala et al., 2004), tomato (Lippman and Tanksley, 2001) and rice (Brar and Khush, 2002).

Multivariate analysis is a useful tool for the characterization and classification of plant genetic resources evaluated for several phenol-morphological and agronomic traits. It also assesses the relative contributions of various traits to the total variability in a crop collection (Kumari et al., 2017; Oliveira et al., 2017; Singh et al., 2017; Öner, 2018; Zanklan et al., 2018), and in various cereals (Assefa et al., 2003; Santos et al., 2012; Sood et al., 2015; Boczkowska et al., 2017; Lodhi et al., 2017; Varthini et al., 2017). In this work we use multivariate analysis to determine the diversity for agro-morphological traits among *A. sterilis* collected from Jordan. However, previous DNA-based markers studies for genetic diversity of *A. sterilis* L. collected from Jordan have shown narrow genetic diversity (Fu et al., 2007), these germplasm still requires detailed knowledge of their genetic and agro-morphological evaluation and characterization (Vincent et al., 2013; Al-Hajaj et al., 2018). The objectives of this study are to identify the extent and pattern of diversity of the germplasm populations (*A. sterilis* L.) with respect to collection regions and altitude, to classify the populations into groups regarding to agro-morphological traits; to assess the traits contributing to the gross diversity of the populations.

## MATERIALS AND METHODS

### Plant materials

Field collection mission was undertaken by the National Agricultural Research Center (NARC) during the cropping season 2013/2014. The collection covered 48 populations in 10 provinces widely distributed in North Western Jordan (Table 1 and Figure 1). Site information (as altitude, longitude, latitude, site description and soil) were also recorded (Table S1- not all data shown). The populations were collected based on their region and altitude (Table 2). From each location, 10 -15 individual plants were randomly selected and seeds from each plant were collected and kept separately.

### Field Experiment

Forty eight populations were used for the characterization of the phenotypic diversity study (Table 1 and Figure 1). The population samples were grown in a pot trial at NARC during the 2014/2015 growing season. Incomplete block design with two replicates was used, each block included 24 pots. Seeds of *Avena sterilis* L. were germinated in the greenhouse in October 2014 and transplanted to pots at NARC station on 1 November 2014. Pots were irrigated as needed. Plants were bagged before flowering to avoid seed loss. Data recorded during the growing season, at harvest, and post-harvest for described the quantitative traits of: days to heading and to maturity, plant height excluding panicles, plant height, number of tillers, panicle length, branches per panicle, number of spikelet, awn length, and glumes length.

### Statistical analysis

Data were analyzed using the restricted maximum likelihood (REML) model using GenStat software (19th Edition). The REML model can handle unbalanced data while accounting for differences in the amount of data available for each population (Bernardo, 2002; van Etten et al., 2008) and generates best linear unbiased predictors (BLUPs), which for the data of each population were used in subsequent analyses.

Before undertaking multivariate analysis, the raw data for each trait within populations were standardized to mean of zero and a variance of one in order to avoid biases created by differences date scales (Sneath and Sokal 1973). PROC PRINCOMP was utilized to group the variables into subsets that are relatively independent from each other and to reduce the dimensionality of the structure.

In addition, PROC DISCRIM was used to examine the validity of the origin-based groupings of the populations, and PROC CLUSTER was used for clustering the 48 populations and the 10 provinces of collection into relatively homogeneous groups based on their similarity with respect to the ten evaluated traits. Values of the cubic clustering criterion (CCC), pseudo-F statistics (PSF) and Hoteling's pseudo-T<sup>2</sup> statistics were considered for defining optimum

number of clusters (Abebe et al., 2010). The measure of dissimilarity was according to the Euclidean distance and the populations were clustered using WARD method. Descriptive analysis (means; standard deviations (SD), coefficient of variations (CV) and ranges) are valuable indicators of diversity. All multivariate analyses were performed by the Statistical Analysis System package v.9 software (SAS, 2007).

**Table 1. List of forty eight *A. sterilis* populations studied.**

Population No.	Province	Latitude	Longitude	Altitude (M)
1	Albalqa	31.96162	35.71253	251.2
2	Albalqa	31.92123	35.65073	-107.6
3	Jerash	31.75573	35.69110	200.6
4	Madaba	31.76685	35.72522	673.6
5	Madaba	31.61423	35.61100	126.8
6	Madaba	31.60842	35.61022	-316.0
7	Amman	31.86815	35.73933	316.4
8	Madaba	31.84992	35.79958	826.9
9	Jerash	32.26612	35.78337	939.1
10	Jerash	32.28333	35.85022	739.1
11	Ajloun	32.36658	35.70002	609.0
12	Ajloun	32.31398	35.67453	595.9
13	Jerash	32.24855	35.88236	165.8
14	Ajloun	32.32931	35.73324	899.5
15	Madaba	31.55647	35.76382	456.9
16	Madaba	31.59450	35.78203	636.7
17	Irbid	32.41083	35.68415	338.6
18	Irbid	32.55113	35.85490	591.3
19	Irbid	32.42087	35.92113	878.1
20	Irbid	32.51545	35.75325	410.3
21	Jerash	32.21552	35.89370	284.4
22	Jerash	32.27795	35.89083	579.4
23	Irbid	32.46863	35.85373	879.0
24	Jerash	32.27080	35.87107	621.8
25	Jerash	32.34945	35.91651	928.1
26	Jerash	32.27238	35.88018	585.2
27	Irbid	32.46677	35.85012	792.2
28	Irbid	32.61657	35.63833	-96.3
29	Irbid	32.60772	35.63623	-69.5
30	Amman	32.07753	35.90847	872.0
31	Amman	32.05823	35.92667	921.7
32	Alzarqa	32.07573	35.93542	982.7
33	Alzarqa	32.10128	36.32617	181.4
34	Alzarqa	32.14003	36.09042	511.0
35	Madaba	31.77410	35.80217	790.0
36	Madaba	32.69418	35.73162	1477.1
37	Madaba	32.70660	35.85007	417.0
38	Alzarqa	31.75403	36.75536	530.0
39	Alkarak	31.30803	35.78173	883.0
40	Madaba	31.76783	35.81170	785.8
41	Alkarak	31.44662	35.81511	51.5
42	Irbid	32.49547	35.98262	578.0
43	Maan	30.18649	35.76430	1119.0
44	Albalqa	32.18906	35.63973	-198.0
45	Almafraq	32.40008	36.05012	700.0
46	Albalqa	32.08002	35.84270	637.0
47	Madaba	31.76783	35.80117	785.8
48	Jerash	32.28328	35.68348	641.0

**Table 2. Number of the Oat populations tested by regions and altitude classes of oat collection mission (m).**

Region	Altitude classes of oat collection mission (m)					Total
	I (below0)	II (0-300)	III (300-600)	IV (600-900)	V (over 900)	
Irbid	2	–	4	3	–	9
Ajloun	–	–	1	2	–	3
Jaresh	–	3	2	3	2	10
Almafraq	–	–	–	1	–	1
Alzarqa	–	1	2	–	1	4
Amman	–	–	1	1	1	3
Albalqa	2	1	–	1	–	4
Madaba	1	1	2	6	1	11
Alkarak	–	1	–	1	–	2
Ma'an	–	–	–	–	1	1
<b>Total</b>	<b>5</b>	<b>7</b>	<b>12</b>	<b>18</b>	<b>6</b>	<b>48</b>

## RESULTS

Wild oat populations had a relatively wide diversity (CV over 10%) for most of the ten examined characters. Phonological traits such as tillers per plant, branch per panicle and spikelet number had the highest CV (63.3, 31.2 and 42.9%, respectively) (Table 3).

All traits with a CV higher than 10% would be reliable morphological descriptors (Enriquez and Soria, 1967; Dotlacil et al., 2000; Karagöz et al., 2007; Santos et al., 2012). On the other hand, correlation coefficients between morphological traits (Table 4) showed positive correlations, particularly among spike traits (panicle length, number of branch per panicle and number of spikelet). A negative correlation between number of tillers and day of heading and maturity was reported; however, day of heading and number of spikelet have a significant positive correlations. No correlation between plant heights and all other traits was recorded.

The PCA showed that the first three components that Eigen values greater than 1.0 contributed about 74.58% of the total variation among collected populations for the ten quantitative traits (Table 5). The first PC, accounted for 30.65% of the total variation with the mostly predominant characters being days to

heading and maturity, panicle length and number of spikelet. However, tillers per plant were loaded with negative value. The second principal component contributed 28.62% of the total variation, which was mostly determined by a positive value of tillers per plant, panicle length, branch per panicle, number of spikelet, and awn length and the negative value for heading date and maturity date. The panicle length and number of spikelet explained the largest portion of PC1 and PC2 variability. The third principal component explained 15.31% of the total variation with predominant loadings from awn and glumes lengths.

Several conclusions could be extracted for the collected materials from the principal component analysis matrix. Oat populations in third group were associated with negative value of component 1 (left hand side) due to short panicle and awn, low number of spikelet and branch per panicle, early heading and maturity, and a high number of tillers. On the other hand, the first group was characterized mainly by high yielding traits and late heading and maturity, while the second group was characterized by moderate yielding traits and late heading and maturity dates.

**Table 3. Descriptive statistics of morphological data for wild Oat collection.**

Variable	Mean	StdDev	CV%	Minimum	Maximum
Days to heading	148.5	15.6	22.5	106.4	172.5
Days of maturity	192.2	7.4	13.6	168.8	203.6
Tillers per plant	3.60	0.3	63.3	3.10	4.20
Plant height (cm)	80.6	0.0	20.8	80.6	80.6
Total plant height (cm)	98.7	0.0	19.8	98.7	98.7
Panicle length (cm)	18.0	0.4	28.5	17.4	19.4
Branch per panicle	4.90	0.2	31.2	4.50	5.50
Number of Spikelet	14.7	0.5	42.9	14.0	16.6
Awn Length (cm)	5.50	0.4	9.10	4.50	6.60
Glumes length (cm)	3.20	0.1	10.6	2.90	3.40

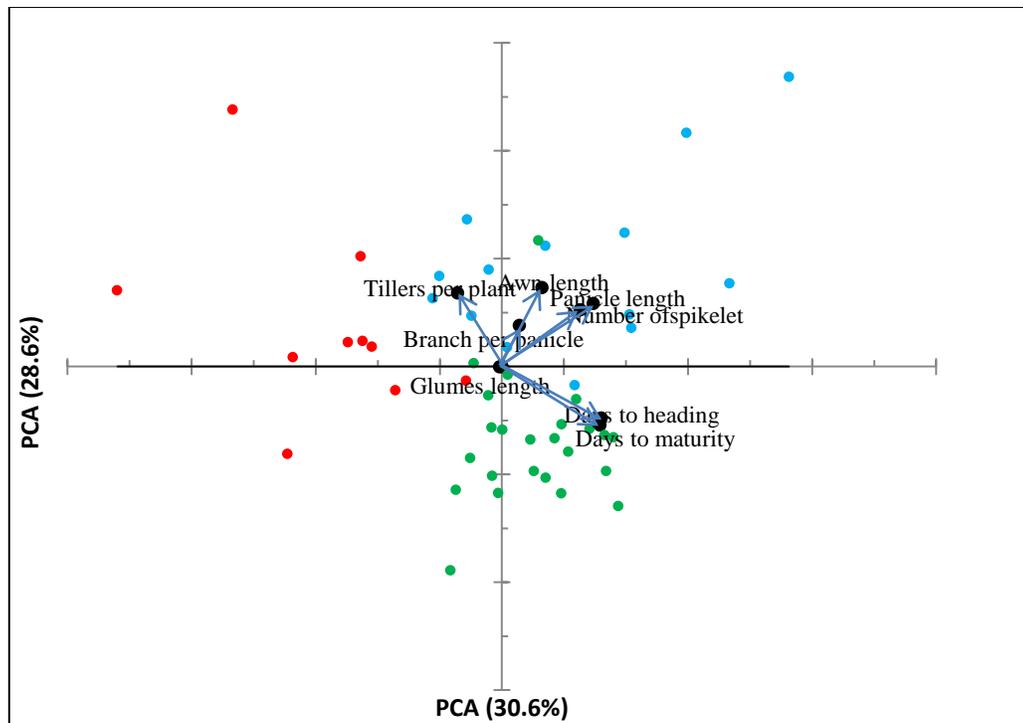
**Table 4. Matrix correlation between the ten examined quantitative traits.**

Variable	Hd	Md	TillerNo.	PncIng	PncBrch	SpikNo	AwnIng
Md	0.90**						
TillerNo.	-0.43*	-0.56**					
PncIng	0.21	0.15	0.13				
PncBrch	-0.13	-0.12	0.24	0.34*			
SpikNo	0.28*	0.24	0.12	0.72**	0.69**		
AwnIng	-0.03	-0.06	0.14	0.26	0.18	0.14	
GlumLng	-0.06	0.01	-0.05	0.04	-0.11	-0.03	0.23

where: \* , \* \*: significant at  $P = 0.05$ ,  $P = 0.0001$  level, respectively, and other non significant at  $P > 0.05$ . Hd, Days to heading; Md, Days to maturity; Tiller No., tillers per plant; PncIng, Panicle length; PncBrch, Branch per panicle; SpikNo, number of spikelet; AwnIng, Awn Length; GlumLng, Glumes length.

**Table 5. The Eigen values, proportion of variation, and cumulative variations across the axis of the first ten principal components**

Eigenvectors			
Traits	PC1	PC2	PC3
Days to heading	0.51	-0.31	-0.02
Days of maturity	0.50	-0.36	0.02
Tillers per plant	-0.23	0.45	-0.07
Plant height (cm)	0.00	0.00	0.00
Total plant height (cm)	0.00	0.00	0.00
Panicle length (cm)	0.40	0.35	0.09
Branch per panicle	0.21	0.48	-0.20
Number of Spikelet	0.47	0.39	-0.12
Awn Length (cm)	0.09	0.25	0.59
Glumes length (cm)	-0.01	0.00	0.76
Eigen value	2.45	2.29	1.22
Variation (%)	30.65	28.62	15.31
Cumulative Variation (%)	30.65	59.27	74.58



**Chart1. Two dimensions of PCA analysis showing a relation among 48 populations of wild Oat *A. sterilis* collected in Jordan.**

Data from quantitative characters were analyzed, legend for populations are 'green' for cluster one, 'blue' for cluster two, 'red' for cluster three.

Traits recorded on the wild oat populations were used to perform cluster analysis to examine the populations aggregation patterns (Figure2). The 48 populations were grouped in three main clusters (Figure1; Table 6).

About 50% of the populations in the first cluster and the remaining populations were distributed within the second and the third clusters. About 20% of the populations collected from Madaba and Jerash provinces grouped in first cluster. Thirty five percent of the total populations grouped in first cluster were from Madaba and Jerash. The first and second clusters demonstrated late heading and maturity and good spike characteristics. Cluster I and II represent 79% of the populations. However, the third cluster was from seven provinces with almost uniform distribution within. These populations showed early heading and maturity and high number of tillers per plant. The late heading and maturing populations in the first and second clusters originated from altitudes class III (32%) and class IV (29%). Seventy one percent of early heading and maturing populations that were grouped in the third cluster from locations at altitudes above 600m and 50% of those populations originated

from altitude class IV (600-900m). Madaba contributed with highest percentage (33%) of populations from altitude class IV. Furthermore, the third cluster was the only cluster without populations from altitude class I.

Discriminant analysis using the regions of collection of the populations as a grouping variable revealed that almost half of the populations (20) out of 48 (41.7%) were classified in their respective regions.

When we used the altitude of the collection site as the grouping variable, 41 populations out of 48 (85.4%) were classified in their respective altitude. The percentage of populations correctly classified varies with the collection region and altitude (Table 7). Alkrak, Almafraq, and Ma'an populations were completely subjected in their respective collection regions, while the rest population relatively subjected in their respective collection regions. Most of the populations from the different altitudes class were scattered over the range of altitude class. None of the populations from altitude class I (below 0 sea level) were grouped in same altitude class and 77.7% of populations were grouped under this altitude from altitudes class III (300-600 m) and

class VI (600-900 m), also 54.5% of populations were grouped under collection altitude class II (0-

300 m) from collection altitude class VI (600-900 m).

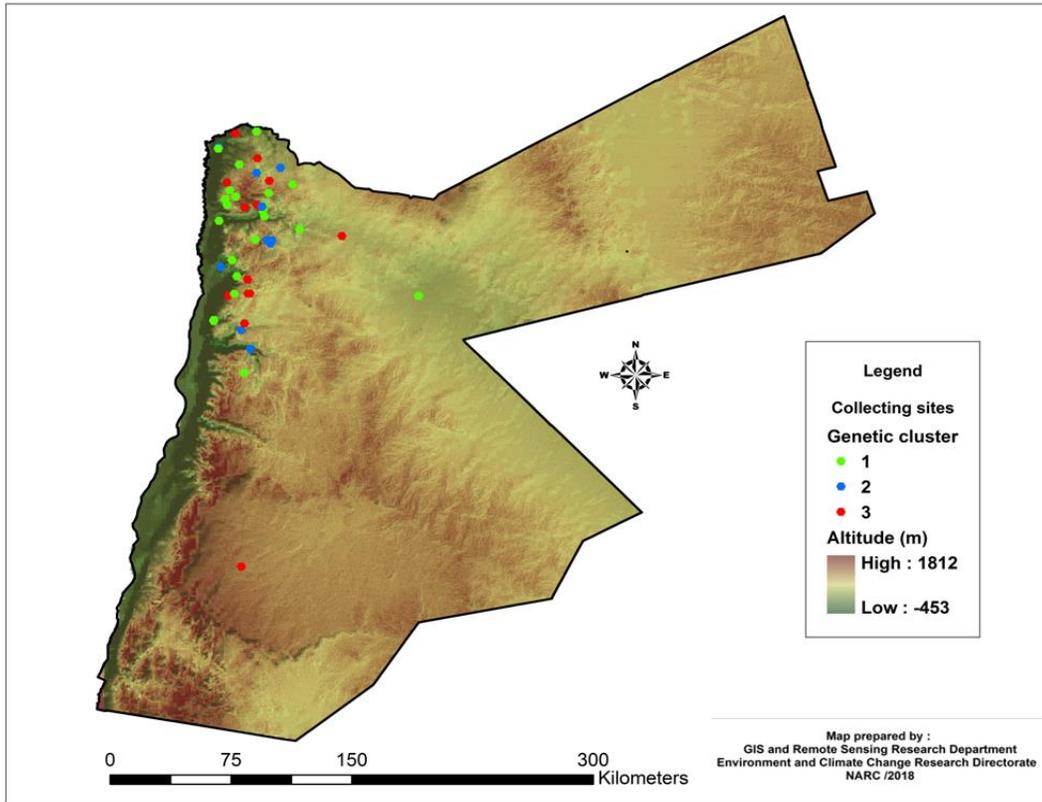


Figure 1. Distribution of *A. sterilis* collection locations in North-Western Jordan.

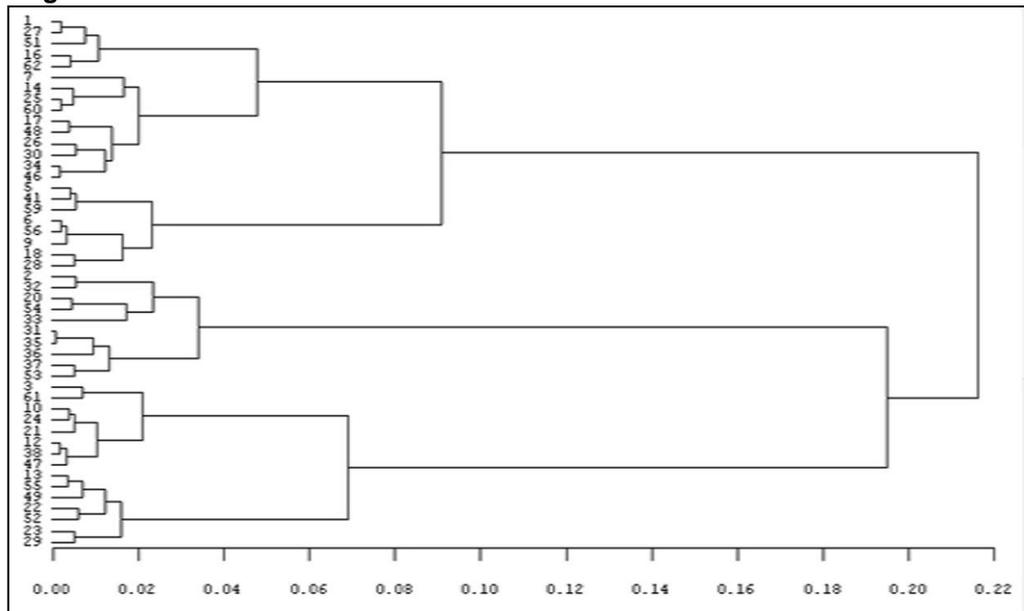


Figure 2. Dendrogram showing the patterns of relationships of 48 wild Oat *A. sterilis* valued for ten traits.

Table 6. Distribution of 48 wild oat populations over three clusters by 10 provinces and five altitude classes.

Regions	Cluster Number			Total
	I	II	III	
Irbid	3	3	3	9
Ajloun	3	–	–	3
Jarash	5	1	4	10
Almafraq	1	–	–	1
Alzarqa	2	1	1	4
Amman	1	2	–	3
Albalqa	3	1	–	4
Madaba	5	1	5	11
Alkarak	1	1	–	2
Ma'an	0	–	1	1
<b>Total</b>	<b>24</b>	<b>10</b>	<b>14</b>	<b>48</b>
Altitude classes				
I	3	2	–	5
II	4	1	2	7
III	7	3	2	12
IV	9	2	7	18
V	1	2	3	6
<b>Total</b>	<b>24</b>	<b>10</b>	<b>14</b>	<b>48</b>

Table 7. Summary of discriminant analysis for wild oat populations

No. of regions	Provinces	Number of populations correctly classified under the provinces/ altitude of Oat collection										%	
		1	2	3	4	5	6	7	8	9	10		
1	Irbid	9	4	1	1	0	0	1	1	0	1	0	44.4
2	Ajloun	3	0	2	0	0	0	0	1	0	0	0	66.7
3	Jarash	10	0	1	6	0	1	0	0	1	1	0	60.0
4	Almafraq	1	0	0	0	1	0	0	0	0	0	0	100
5	Alzarqa	4	0	0	0	1	2	1	0	0	0	0	50.0
6	Amman	3	0	0	0	0	0	2	0	1	0	0	66.7
7	Albalqa	4	1	0	0	0	0	0	2	1	0	0	50.0
8	Madaba	11	2	0	2	1	0	0	0	6	0	0	54.5
9	Alkarak	2	0	0	0	0	0	0	0	0	2	0	100
10	Ma'an	1	0	0	0	0	0	0	0	0	0	1	100
		Altitude classes		I	II	III	VI	V					%
1	I	5	0	1	2	1	1						0.00
2	II	7	1	1	1	2	2						14.3
3	III	12	4	3	1	3	1						8.30
5	VI	18	3	6	2	4	3						22.2
6	V	6	1	0	1	3	1						16.7

## DISCUSSION

The objective of this study was to assess the morphological diversity among 48 *A. sterilis* L. populations collected from various regions and altitudes based on ten quantitative traits. Two traits related to plant height did not contribute to the diversity among the studied populations. The dominance of the trait is determined by the interaction of several exo- and endogenous factors. Moreover, the polygenic nature of this trait was confirmed by earlier studies (Siripoonwiwat et al., 1996; Boczkowska et al., 2016). Morphological variation was strongly affected by the environment (Paczos-Grze et al., 2007; Okoń et al., 2016), hence, the degree of trait variations differed with the collection regions and their altitudes. The presence of morphological diversity within oat populations resulted in using the natural populations as a source for oat breeding to improve phenological characteristics: panicle length, number of branch and number of spikelet per panicle. These traits are important components for grain yield. The results represented in this study show that precipitation and temperature influenced heading and maturing date as well as grain yield or seed production. The population groups have shown interesting combinations of flowering date and yield, thus presence population groups adapted to drought and temperature stress successfully (Ceccarelli, 2014; Dwivedi et al., 2016).

Generally, the principal component analysis explained the entire variation in terms of few PCs. This, in turn, revealed the involvement of a number of traits in contributing toward the overall observed diversity. In the same context, the contribution of several traits revealed the overall variation and diversity within populations observed in other studies (Assefa et al., 2003; Abebe et al., 2010). The number of spikelet and panicle length constituted to one of the important traits for the variation and the diversity that will contribute in oat breeding program (Coffman, 1977; Frey, 1991; Harder et al., 1992). However, this type of variation indicates the potential of wild oat to become more weedy (Miller et al., 1982; Dai et al., 2012).

Grouping populations according to similar morphological traits that adapted to different environments provides better information for selecting parents in breeding program (Abebe et al., 2010; Boczkowska et al., 2016). Clustering of populations revealed that the variability at the phenotypic level has not grouped according to distinct region, where populations collected from

the same or adjacent regions subjected to different grouping clusters. Same results were reported in wild oat collected from Jordan (Al-Hajaj et al., 2018) in the contrary, Thormann et al., (2017) reported that wild barley collected from Jordan were grouped according to collection sites. The result from this study is in agreement with previous studies that were reported that oat has relatively weak population structure (Hamblin et al., 2010; Newell et al., 2011; Winkler et al., 2016).

The populations clustered together as high or relatively high yielding traits are associated with late heading and maturity date, and the population clustered as low yielding traits characterized with early heading and maturity traits and high number of tillers. These results demonstrate that oat population groups are influenced by adaptation to theseasonal changes in precipitation and temperature (Górny, 2005). This finding is also in agreement with data collected from Irbid, Jaresh, Alzarqa and Madaba.

Discriminant analysis illustrated the detection of regions and altitude diversity patterns in the collected germplasm, through the populations' misclassification to their respective collection region and altitude. This result agreed with the hypothesis that the higher the diversity of the group, the higher the probability of misclassification and vice versa (Holcomb et al., 1977; Pecetti and Damania, 1996; Abebe et al., 2010; Reddy and Gomashe, 2017). A high diversity between populations was revealed in this study and the populations examined for altitude were more diversified than those examined for geographic region. This could be related to finding that outcrossing in *A. sterilis* rate is higher than 10.7% within the natural populations (Shorter et al., 1978) and thus increases the rate of gene flow between agro-ecologies.

This emphasizes that the plant diversity is explained by another factors rather than geographic origin, like plant crossing type, agro-ecology conditions and their interactions.

The results of the present study clarified how the altitude and ecological factors were related to the diversity, and how it influenced the diversity which existed in the collected material. The present results confirmed the results of *A. sterilis* genetic structure collected from Jordan (Al-Hajaj et al., 2018) with regard to region and altitude variation pattern. The significant agromorphological variation between the populations for the studied traits offers ample opportunities for the crop improvement. The collected material

showed high degree of variation a long short distances. This suggests the importance of sampling over short distance of ex-situ conservation expedition to capture most of the variation as possible within of the gene pool and this result agrees with Karagöz et al., (2007).

## CONCLUSION

Forty-eight populations of *A. sterilis* L. were evaluated for ten plant and panicle traits to assess the extent and pattern of morphological diversity and association of traits in Jordanian wild oat in relation to regions of origin and altitudes. The analysis of morphological diversity showed the importance of natural populations as a source of diversity for oat breeding to improve phenological characteristics: panicle length, number of branch and number of spikelet per panicle; and confirm that oat has relatively weak population structure. The populations were grouped in three distinct clusters as high or relatively high yielding traits are associated with late heading and maturity date, and the population clustered as low yielding traits characterized by early heading and maturity traits and high number of tillers. Discriminant analysis revealed that the populations examined for altitude were more diversified than those examined for geographic region. The collected material showed high degree of variation a long short distances and the importance of sampling over short distance of ex-situ conservation expedition to capture most of the variation as possible within of the gene pool.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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## AUTHOR CONTRIBUTIONS

NA designed the experiment. NA, IA and AM collected the germplasm in Jordan. IA and AM maintained the experiment and collected the

project data. NA and KA conducted the phenotypic diversity analysis. NA and SC wrote the paper. All authors contributed to and approved the paper final revision.

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