

ISSN 1682-296X (Print)

ISSN 1682-2978 (Online)



Bio Technology



ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Assessment of Phenotypic Diversity among Jordanian Barely Landraces (*Hordeum vulgare* L.)

¹Aida Al-Nashash, ¹Hussein Migdadi, ²Mohamad A. Shatnawi,
³Hani Saoub and ⁴Sameer Masoud

¹National Center for Agricultural Research and Technology Transfer
²Department of Biotechnology, Faculty of Agriculture and Technology,
Al-Balqa Applied University, Al-Salt 19117, Jordan
³University of Jordan, Jordan
⁴University of Mut'ah, Jordan

Abstract: A field study was carried to assess phenotypic variation for 32 barely landraces in Jordan collected from two diverse environments. Three long-term checks: Harmal, Zanbaka and Arta were also used in this study. Triple lattice design with three replications was used. Half of the collected barely landraces were six-row type that dominated in Ajlun area (favorable environments) and the remaining landraces were two-row type that dominated in Muwaqqar area (dry environment). This reflects the expected landrace adaptability to the stressful climatic conditions. The phenotypic variation exhibited by the landraces for 13 quantitative traits indicated that these landraces are heterogeneous populations to various degrees for most desirable agronomic traits, except for growth habit and early growth vigor that were monomorphic. Considering all traits, the average diversity index (H') for the collected landraces was 0.71 ± 0.05 . Similarity indices using Euclidean distances ranged from 0.99 to 0.60 with an average of 0.81. Wide range of similarity confirmed the high level of phenotypic polymorphism. Collected landraces were completely separated into two main clusters according to the row types and the collection sites. The first canonical variable, on average, explained 89.1% of the total variation and shows that spike weight, grain yield/plant and grains/spikelets are the major discriminating coefficient among clusters. Jordanian barely landraces have higher level of diversity in desirable traits that can be exploited in breeding programs.

Key words: Barley land races, phenotypic diversity, shannon's information statistic, canonical analysis

INTRODUCTION

Barley landraces are genetically heterogeneous populations comprising inbreeding lines and hybrid segregates generated by a low level of random out crossing in each generation (Nevo, 1992). Moreover, having evolved across thousands of years in a multitude of environments and local farming systems, these landraces have developed abundant patterns of variation and would represent a largely untapped reservoir of useful genes for adaptation to biotic and abiotic stresses (Brush, 1995). Although Jordan is one of the countries comprise barley's center of diversity, the land area under landraces is declining and almost of all accessions maintained in ex situ collections are advanced cultivars or breeders' lines.

Landrace populations of barley from Jordan proved to be a rich source of genetic variation for a number of agronomic characters. An important feature, detected in

these landrace populations is inter- and intra- regional diversity. This diversity was noticed more in the six-row group rather than the two-row group of landraces. Obviously, geographic and environmental factors caused these populations to differ from each other. Therefore, Jaradat *et al.* (1996) concluded that the drier regions of Jordan harbor higher levels of variability for most morphological and yield-related characters. Ceccarelli *et al.* (1987) estimated the diversity in barley landraces originating from Jordan found that some lines were better than the improved cultivated check for some agronomically desirable traits, which prove their importance and potential for breeding.

Jaradat *et al.* (2004a) assessed the genetic diversity in the Batini Barley Landrace from Oman for spike and seed characters and also for response to salinity stress found that total genetic variation and genetic differentiation estimates for qualitative traits were 25% lower than for quantitative traits strong, nonrandom trait

associations among four seed phenotypic markers showed a hierarchical pattern, indicating an adaptive response to environmental conditions and human selection and they concluded that the long history of *in situ* conservation of this landrace in a multitude of subsistence farming systems, undoubtedly, contributed to the high variability and it contains diversity for salt tolerance that remains to be exploited (Jaradat *et al.*, 2004b).

Although farmers may have several socio-economic incentives to replace landraces with modern introduced varieties (Kebebew *et al.*, 2001), this landrace is still being cultivated by subsistence farmers in Jordan. Nevertheless, the continued cultivation of this landrace and other indigenous crop genetic resources of Jordan are potentially threatened and could be lost before they are adequately collected and thoroughly evaluated.

Very little is known about the genetic diversity and morphological variability present in barley landraces in Jordan, a country experiencing loss of biodiversity, because of replacement of landraces with modern cultivars, land fragmentation and frequent drought period during last decades.

Therefore, the present study was carried out to assess phenotypic diversity among Jordanian barely landraces (*Hordeum vulgare* L.) collected from two diverse environments.

MATERIALS AND METHODS

Collection of landrace accessions: Collection missions were carried out in two target areas: Ajlun and Muwaqqar. Ajlun represents the semi-humid ecosystem in Jordan with a latitude of 32°20' N and a longitude of 35°45' E with an elevation range from 500-1250 m above sea level (Ehrenberg and Randall, 1990). While Muwaqqar represents the semi-arid ecosystem in Jordan which is located 30 km southeast of Amman at a latitude of 31°84' N and longitude of 36°6' E with an elevation of 625-1110 m. Long term average annual precipitation for Ajlun is 583 and 177.3 mm for Muwaqqar. Within each area, many sub-sites were selected to represent the possible range of topography, climate and species concerned. A total of 33 accessions, representing the local varieties, were collected during September, October and November 2000 (Table 1).

Experiment description: Thirty-three collected accessions were grown in the Jubeiha Agricultural Research Station at the University of Jordan during 2000/2001 growing season. The study area has latitude of 32°N and longitude of 35°E with an elevation of 980 m above sea level and has a mean annual rainfall of about 491 mm.

Table 1: Locations and numbers of barley landraces collected

Area	Sub-site (villages)	No. of samples	Accessions No.
Ajlun	Al Wahadina	4	1,2,3,10
Ajlun	Samta	1	4
Ajlun	Hawd al Qala	4	5,6,9,33
Ajlun	Al Maydan	2	7,8
Ajlun	Skhra	2	11,12
Ajlun	Rajib	2	13,14
Muwaqqar	Qaryyat Salim	4	15,16,17,18
Muwaqqar	Adh-Dhuhayba ash-Sharqiyya	3	19,20,21
Muwaqqar	Maghair Mahanna	4	22,23,24,25
Muwaqqar	An-Nuqayra	4	26,27,28,30
Muwaqqar	Z-Zumaylat	1	29
Muwaqqar	Rujm ash-Shami al Gharbi	2	31,32
Total No. of samples		33	

Triple lattice design (6×6) with three replications was used. Thirty-three collected accessions and three long-term checks were included in this experiment. The check cultivars were Harmal, Zanbaka and Arta. The order of treatments was randomized within blocks that were randomized within each replication. Each replication contains six blocks spaced by 0.5 m apart. Each block consist of six treatments and each treatment is composed of four rows spaced by 25 cm apart.

Germination test was carried out before planting and seeding rate was adjusted according to germination percentage. Seeds were hands sown in plots of one m² at a seeding density of 200 plant m⁻² which is equivalent to 100 kg ha⁻¹. To avoid border effects, no space was left between plots. Planting was under typical farmer's conditions, when possible, i.e., rainfed with no fertilizers, no pesticides and no herbicides. Date of sowing was 15, Jan/2001, while date of harvesting was 7, June/2001. Weeds were removed by hands as they appeared in the field.

The following traits were measured or scored through out the growing season or after harvesting:

Days to heading: Number of days from emergence to the day when the awns appeared in 50 % of the plot.

Days to maturity: Number of days from emergence to the day when the peduncle is completely discoloured.

Grain filling period: As the difference in number of days between days to maturity and days to heading.

Thousand-kernel weight: Weighting 1000 seeds in grams.

Growth habit: Refers to the angle between the axillary tillers of a plant and it's actual or imaginary upright main stem. It is best scored when there are about five tillers and was divided into five classes: (1) Erect, (2) Semi-erect, (3) Intermediate, (4) Semi-prostrate and (5) Prostrate.

Early growth vigor: A visual assessment of growth vigor scored six weeks after emergence. It was recorded in categories: (1) excellent, (2) medium, (3) good, (4) poor and (5) very poor.

The previous traits were recorded on plot basis, while the remaining traits were recorded on single plant basis. Five plants were selected randomly from the central rows of each plot. The emergence date was on 7, Feb. the number of days to heading and maturity was counted according to that date.

Plant height (cm): The height of the plants at maturity is measured, from ground surface to tip of head excluding awns.

Grain yield per plant: The grain yield for each plant was weighted (g).

Biological yield per plant: The total dry matter (grain and straw) for each individual plant was weighted.

Spike weight: Weighting the main spike.

Spike length: Length in cm of the main spike of the tallest culm.

Awn length: Was measured from the tip of the main spike to the end of the awns.

Spikelets per spike: No. of spikelets per spike.

Grains per spike: No. of grains per spike.

Spike density: Was calculated as the ratio between the no. of spikelets per spike over spike length.

Statistical analysis

Estimation of phenotypic diversity index (H): Mean (\bar{X}) and standard deviation (S) were calculated for each quantitative trait. The two statistical values were used to classify the traits into three categories groups:

Group 1: Less than or equal to $\bar{X}-S$.

Group 2: Greater than $\bar{X}-S$ to less than $\bar{X}+S$.

Group 3: Greater than or equal to $\bar{X}+S$.

Shannon's information statistic ($h_{s,j}$) as described by Hutcheson (1970) was used to describe phenotypic diversity. The following formula was used for calculating $h_{s,j}$ for the j^{th} trait with (n) states or classes:

$$h_{s,j} = -\sum_{i=1}^n P_i \ln P_i, \quad \text{for } n = 1, 2, 3$$

Where P_i is the relative frequency in the i^{th} category of the j^{th} trait.

The minimum value of the index is zero for a monomorphic trait. The value of the index increases with the rise in polymorphism and reaches the maximum value when all phenotypic classes have equal frequencies.

The average diversity (H') over k character was estimated as the following:

$$H' = \sum_{i=1}^k h_{s,j}/k$$

The diversity index (H') has been extensively used to estimate phenotypic diversity in germplasm collection of barley (Tolbert *et al.*, 1979) and used for measurement and comparison of geographical pattern of phenotypic diversity in germplasm collection of wheat (Jaradat, 1992; Yang *et al.*, 1991; Jain *et al.*, 1975).

Cluster analysis: The relationship between collected accessions or measured parameters was studied using cluster analysis that results in the grouping of variables into clusters. Distances between clusters were analyzed and reported as a Dendrogram of Euclidean distances.

RESULTS

Estimates of diversity indices (H): Variation or polymorphism was common, in varying degrees, for most traits, thus, indicating a wide variability among Jordanian landraces. Estimates of (H') for individual traits are presented in Table 2. These estimates ranged from 0.0 (monomorphic) for growth habit to 1.0 (highly polymorphic) for plant height. While most traits showed high ($H' > 0.60$) levels of polymorphism, a few of these traits (e.g., growth habit and early growth vigor) displayed low H' estimates (0.00 and 0.12), respectively. Days to

Table 2: Phenotypic diversity index (H') of 15 characters for 32 collected barley accessions from Jordan

Traits	Diversity index (H')
Days to heading	0.78
Days to maturity	0.48
Grain filling period	0.51
Thousand kernel weight	0.92
Biological yield/plant	0.84
Grain yield/plant	0.83
Plant height	1.04
Spike length	0.9
Awn length	0.83
Spike weight	0.96
#Spikelets/spike	0.83
Grain/spike	0.77
Spike density	0.77
Early growth vigor	0.12
Growth habit	0
Average diversity index	0.71±0.05

maturity ($H' = 0.48$) and grain filling period ($H' = 0.51$) gave intermediate level of diversity. High levels of diversity were obtained from other characters (Table 2). The higher level of polymorphism was obtained by the traits: plant height, ($H' = 1.0$), spike weight ($H' = 0.96$), thousand kernel weight ($H' = 0.92$) and spike length ($H' = 0.90$).

Cluster analysis: Cluster analysis was performed with the quantitative data only according to Weltzien (1989). The similarity matrix was constructed using Euclidean distances to assess the phenotypic relatedness among the 32 barely accessions and the three long term-checks. The mean similarities indices ranged from 0.60 to 0.99 between accessions. All accessions showed an average of 0.81, which means that the accessions share an average of 81% of their agronomic traits.

The dendrogram of the similarity of 35 barely accessions resulted in 10 sub-clusters based on the means of quantitative data (Fig. 1). The results showed

that landraces from the same collection site did not tend to cluster together in the same cluster, whereas landraces of the same row type or the same subtaxa tend to cluster together in the same cluster regardless of the collection site. Therefore, corresponding to the barley row-type, the 32 accessions and three checks clustered into two main clusters; the first cluster consists of accessions, which have two-row type, including the three checks (Harmal, Zanbaka and Arta). The second cluster consists of all accessions of six-row type even collected from Ajlun or Muwaqqar. The first main cluster consists of five main sub-clusters that ranged in similarity from 0.99 to 0.78. While the second main cluster consists also of five sub-clusters, that ranged in similarity from 0.96 to 0.84. The three-long term checks are clustered in the first main cluster; Harmal are more similar to many of the collected two row types while Zanbaka and Arta are clustered in a separate cluster for each one. It was interesting to distinguish one of the accessions (No. 26), which has its

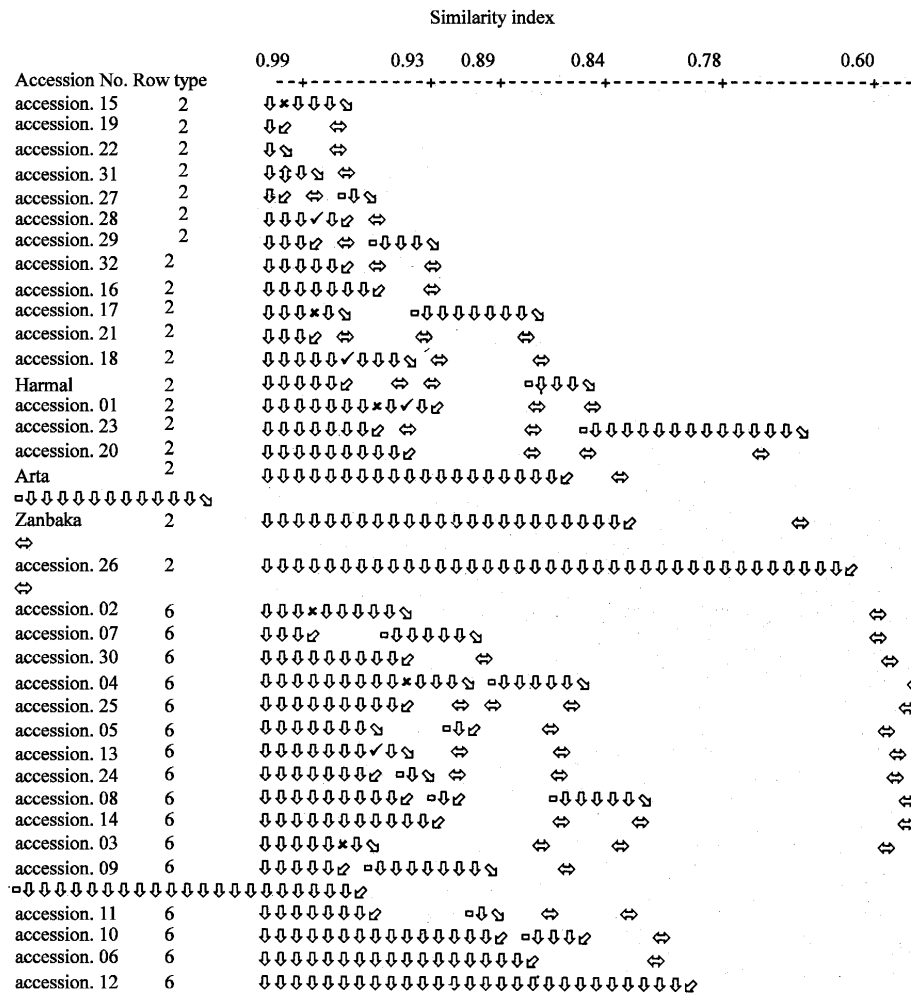


Fig. 1: Dendrogram for the 32 collected accessions and the three long-term checks based on similarity coefficient of Euclidean distances

Table 3: Canonical variables, Eigen values, percent of variation and cumulative variation among different morphological characters of

Character	CAN 1	CAN 2	CAN 3	CAN 4	CAN 5
Grain yield/plant)	0.288	1.01	0.418	-0.134	0.058
Plant height	-0.914	-0.577	0.436	1.482	1.186
Awn length	-0.043	-0.751	0.073	-1.257	1.012
Spike weight	0.842	1.783	-2.235	-0.838	-2.201
grain/spikelet	0.275	-1.185	1.46	1.529	1.412
Eigen value	11.876	0.566	0.433	0.285	0.165
Variance (%)	89.1	4.2	3.3	2.2	1.2
Cumulative variation (%)	89.1	93.3	96.6	98.8	100

own separate cluster, which means, it has agronomic characters that differs from other collected accessions. Also accessions No. 1 and 11, collected from Ajlun showed good performance in grain yield and other agronomic characters, which make them ideal to be used in breeding programs.

Canonical analysis: The canonical discriminant analysis was performed with the standardized mean values for each of the quantitative traits (Table 3). The first canonical variable, on average, explained 89.1% of the total variation, while the second, third, fourth and fifth variables accounted for, 4.2, 3.3, 2.2 and 0.3% of the total variation, respectively.

The first canonical variable coefficient shows that spike weight, grain yield/plant and grains/spikelets are the major discriminating coefficient among clusters, while in the second canonical variant spike weight and grain yield/plant have played a major role in separating clusters. The third, fourth and fifth canonical variant give the role of separation for the grains/spikelets. The first two canonical variant accounted 93.3% of the total variations, this conclude that spike weight and grain yield/plant traits are more variables than the others.

DISCUSSION

The higher level of polymorphism was obtained by the yield related traits confirm high level of diversity. However, a low H' estimates may reflect unequal frequencies of different classes rather than the absence of the desirable class for a particular trait. These results were agreed with those of Jaradat (1992). Average diversity (H') estimated on traits evaluated in this study was 0.71 ± 0.05 . However, when only drought-related traits were considered (Yang *et al.*, 1991; Jaradat, 1992), H' estimate dropped slightly to 0.70 ± 0.08 . Average estimate of diversity was higher than that reported by Jaradat (1989) for barley landraces collected in Jordan during summer of 1986 ($H' = 0.55 \pm 0.13$), which was based on seven morphometric and 13 qualitative traits, none of them included in our study. This low diversity calculated by

Jaradat (1989) compared with our results, is due to the fact that qualitative variation is less limited by natural selection than is quantitative variation, which is highly affected by selection, environment and affects survival and production. However, the high diversity indices where agreed with Jaradat *et al.* (2004a) where the total genetic variation (H_T) for quantitative (0.717) and qualitative (0.533) traits were recorded and differed significantly in Batini barley landrace. Also the high level of phenotypic diversity in these landraces is higher than phenotypic diversity levels reported for larger germplasm collections of landraces in the primary (e.g., Jaradat *et al.*, 1987; Parzies *et al.*, 2000) and secondary (e.g., Demissie and Bjornstad, 1996) centers of genetic diversity. Also, diversity estimate for barely landraces in this study is higher than that reported for the Middle East Region (0.40 ± 0.00) (Tolbert *et al.*, 1979), which was based on 5 qualitative characters, one of them (growth habit) was included in our study or 0.534 on the basis of nine phenotypic markers in Ethiopian landraces (Kebebew *et al.*, 2001). However, it was argued (Bjornstad *et al.*, 1997) that phenotypic diversity does not reflect a random and chromosomally balanced sample of genetic variation. Therefore, this high phenotypic diversity may not reflect a higher average diversity at biochemical or molecular levels (Lefebvre *et al.*, 1991). Nevertheless, the functional genetic diversity (i.e., based on morphological traits deliberately targeted for selection by farmers) (Koehner *et al.*, 2002) and population differentiation (based on phenotypic markers) were found to be higher when compared with biochemical markers in cultivated barley (Jaradat *et al.*, 1987), wheat (Tsegaye *et al.*, 1996) and sorghum (Dje' *et al.*, 1998). Moreover, Donini *et al.* (2000) and Koebner *et al.* (2002) found in a detailed study of genetic diversity in wheat and barley varieties released in the UK during the 20th century that, the average diversity per trait for four biochemical and molecular traits was only 20 to 70% of the average diversity per morphological trait; these differences were attributed to the multigenic nature of most individual phenotypic markers and hence variation at more than one locus is being analyzed.

The wide range of similarity indices showed high polymorphism among landraces and large amount of phenotypic variation exists among the landraces. However the landraces of six-row types are more related to each other than landraces of two-row types, which ranged from 0.98 to 0.78. This wide range indicated the presence of higher variability among the two-row typed of barley, which originated mainly under harsh conditions, than six-row barely, which are grown in more humid areas. These results are in agreement with Jaradat *et al.* (1996) who found that the drier regions of Jordan harbor higher

levels of variability for most morphological and yield-related traits. The similarity coefficient values suggested that the resemblance among accessions within the same cluster is high compared to the coefficient values between accessions from different clusters (Migdadi, 2001). This means that landraces in the same sub-cluster share the same quantitative traits.

On average, the first and second principal component, explained the 49.8 and 26.9% of the total variation in the seven subpopulations of barley land races. The most important traits found to explain the variation were seed weight, 1000 kernel weight and number of seeds per spike for principle component one and spike length and number of spikelets per spike in the second principal component (Jaradat *et al.*, 2004a).

Landrace cultivation has been discouraged in many developing countries because of low yield potential and susceptibility to diseases. Although exotic barley cultivars out-yield local landraces under good management practices in selected testing sites (Ceccarelli *et al.*, 1987), local landraces usually out-yield the exotic material under the low input conditions that predominate in subsistence farming systems. Jordanian barely landraces have higher level of diversity in desirable traits that can be exploited in breeding programs. Phenotypic polymorphisms were common, in varying degrees, for most traits as indicated by a wide phenotypic variation among barely landraces and clustering of different accession were not based on locations of collection, but mainly according to the row types of barley.

REFERENCES

- Bjornstad, A., A. Demissie, A. Kilian and A. Kleinhofs, 1997. The distinctness and diversity of Ethiopian barleys. *Theor. Applied Genet.*, 94: 514-521.
- Brush, S.B., 1995. In situ conservation of landraces in center of crop diversity. *Crop Sci.*, 35: 346-354.
- Ceccarelli, S., S.M. Grandi and J.A.G Van Leur, 1987. Genetic diversity in barley landraces from Syria and Jordan. *Euphytica*, 36: 389-405.
- Demissie, A. and A. Bjornstad, 1996. Phenotypic diversity of Ethiopian barleys in relation to geographical regions, altitudinal range and agro-ecological zones: As an aid to germplasm collection and conservation strategy. *Hereditas*, 124: 17-29.
- Dje', Y., M. Ater, C. Lefebvre and X. Vekemans, 1998. Patterns of morphological and allozyme variation in sorghum landraces of Northwestern Morocco. *Genet. Res. Crop Evol.*, 45: 541-548.
- Donini, P., J.R. Law, R.M.D. Koebner and J.C. Reeves, 2000. Temporal trends in the diversity of wheat. *Theor. Applied Genet.*, 100: 912-917.
- Ehrenberg, R.E. and R.R. Randall, 1990. *Gazetteer of Jordan*. 2nd Edn., Washington, D.C.
- Hutcheson, K., 1970. A test of comparing diversities based on the Shannon formula. *J. Theor. Biol.*, 29: 151-154.
- Jain, S.K., C.O. Qualset, G.M. Bhatt and K.K. Wu, 1975. Geographical patterns of phenotypical diversity in a world collection of durum wheats. *Crop Sci.*, 15: 700-704.
- Jaradat, A.A., S. Jana and L.N. Pietrzak, 1987. Collection and evaluation of cereal genetic resources in Turkey and Jordan. *Rachis*, 6: 12-14.
- Jaradat, A.A., 1989. Diversity within and between populations of two symmetrically distributed *Hordeum species* in Jordan. *Theor. Applied Genet.*, 78: 653-656.
- Jaradat, A.A., 1992. Estimates of phenotypic diversity and trait associations in durum wheat landraces from Jordan. *J. Genet. Breed.*, 46: 69-76.
- Jaradat, A.A., M.M. Ajlouni and M.A. Duwayri, 1996. Genetic Resources of Cereals and their Wild Relatives in Jordan: Revisited. In: *Plant Genetic Resources of Jordan*. Jaradat, A.A. (Ed.). Proceedings of National Seminar, 2-4 August 1994, Amman, Jordan, pp: 69-70.
- Jaradat, A.A., M. Shahid and A. Al-Maskri, 2004a. Genetic diversity in the Batini barley landrace from Oman: I. Spike and seed quantitative and qualitative traits. *Crop Sci.*, 44: 304-315.
- Jaradat, A.A., M. Shahid and A. Al-Maskri, 2004b. Genetic diversity in the Batini barley landrace from Oman: II. Response to salinity stress. *Crop Sci.*, 44: 997-1007.
- Kebebew, F., Y. Tsehaye and T. McNeilly, 2001. Morphological and farmers cognitive diversity of barley (*Hordeum vulgare* L. [Poaceae]) at Bale and North of Ethiopia. *Genet. Res. Crop Evol.*, 48: 1-10.
- Koebner, R.M.D., P. Donini, J.C. Reeves, R.J. Cooke and J.R. Law, 2002. Temporal flux in the morphological and molecular diversity of UK barley. *Theor. Applied Genet.*, 106: 550-558.
- Lefebvre, V., A. Palloix and M. Reives, 1993 Nuclear RFLP between pepper cultivars (*Capsicum annuum* L.). *Euphytica*, 71: 189-199.
- Migdadi, H.M., 2001. Genetic Variation in Some Aegilops Species as Revealed by Morphological and Molecular Techniques. Ph.D Thesis, University of Jordan, Amman, Jordan.

- Nevo, E., 1992. Origin, Evolution, Population Genetics and Resources of Wild Barley, *Hordeum Spontaneum*, in the Fertile Crescent. Barley: Genetics, Biochemistry, Molecular Biology and Biotechnology. Shewry, P.R. (Ed.). CAB International, Wallingford, UK., pp: 19-43.
- Parzies, H.K., W. Spoor and R.A. Ennos, 2000. Genetic diversity of barley landrace accessions (*Hordeum vulgare* sp. *vulgare*) conserved for different lengths of time in ex situ gene banks. *Heredity*, 84: 476-486.
- Tolbert, D.M., C.O. Qualset, S.K. Jain and J.C. Craddock, 1979. A diversity analysis of a world collection of barely. *Crop Sci.*, 19: 789-794.
- Tsegaye, S., T. Tesemma and G. Belay, 1996. Relationships among tetraploid wheat (*Triticum turgidum* L.) landrace populations revealed by isozyme markers and agronomic traits. *Theor. Applied Genet.*, 93: 600-605.
- Weltzien, E., 1989. Differentiation among barley Landraces populations from Near East. *Euphytica*, 43: 29-39.
- Yang, R.C., S. Jana and J.M. Clarke, 1991. Phenotypic diversity and association of some potentially drought-related characters in durum wheat. *Crop Sci.*, 31: 1484-1491.