

## Research Article

# Agronomic Evaluation and Yield Performance of Selected Barley (*Hordeum vulgare* L.) Landraces from Jordan

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Barley (*Hordeum vulgare* L.) landraces collected previously from main production areas across Jordan are expected to perform well under stressful environments. In this study, the agronomic performance of 10 Jordanian barley landraces and three local cultivars was evaluated in two locations for two growing seasons. Clear significant variations for all studied traits were observed among the selected genotypes, environments, and their interactions. The local cultivar Rum and Baladi landrace showed the best yield performance, while Herawi and Nabawi landraces produced the lowest yield across all environments. Clustering analysis using genotypic data from the iSelect 9k SNP barley array showed a clear grouping based on row type with 100% similarity level between the Syfi and Arabi landraces. The characterized Jordanian landraces can be used to improve barley resilience against climate change and associated conditions and are recommended in breeding programs to improve productivity under dry conditions.

## 1. Introduction

In dry areas across the Mediterranean basin, barley (*Hordeum vulgare* L.) is considered the crop of choice for many poor-resource farmers and it is commonly used for human consumption, animal feed, and malting [1]. In addition, barley cultivation is gaining increased interest throughout the world due to its high nutritional value and low glycemic index [2]. In Jordan, barley is cultivated mainly under rainfed conditions in marginal areas receiving annual precipitations less than 300 mm [3]. In such areas, barley cultivation faces many challenges and associated conditions with climate change, which already resulted in drier environments, thus affecting crop productivity [4].

Barley domestication from its wild progenitor (*H. vulgare* subsp. *spontaneum*) started nearly 11,000 years ago in

the southern regions of the western horn of the Fertile Crescent [5]. Diversity studies indicate the existence of high genetic variation among collected wild and domesticated barley germplasm from this region [3, 6, 7]. Genetic analysis of a 6000-year-old barley landrace excavated from Yoram cave nearby the Dead Sea using whole-exome sequencing showed a close relatedness of the ancient sample with current existing landraces in the region [8]. This might indicate that the primary center of origin and domestication of barley lies within this region, although other studies support the occurrence of other domestication events in eastern areas of the Fertile Crescent and the Horn of Africa [9, 10]. Recently, the utilization of genome-wide scanning and targeted resequencing technologies revealed that modern cultivated barley contains a mosaic of fragments from wild barley populations originated from different sites in the Fertile Crescent region [11, 12]. This led to the

hypothesis that the progenitor of modern domesticated barley is a mosaic of highly admixed ancestral populations of wild barley [13].

As a consequence of early domestication of barley by local farmers, landraces were originated and were used for thousands of years before modern breeding programs were introduced [14, 15]. They played and still play a major role in the maintenance of genetic variation of modern barley germplasm. Such farmers' varieties usually have limited geographic range, are diverse within particular types, are adapted to local conditions, and possess great potential to tolerate multiple stresses [16]. In arid and semiarid areas of east Mediterranean region, barley landraces are usually cultivated in marginal, low-input, and drought-prone environments and are commonly subjected to different abiotic stresses including drought [17]. Barley landraces domesticated and evolved in these dry areas of the Fertile Crescent are adapted to growth-limiting factors with great resilience and yield stability [16–18]. For this reason, it is important to conserve local barley landraces, to understand their adaptation to adverse climates as well as to identify QTLs and genomic regions that have adaptive roles against multiple stresses [15–18].

In many studies, Jordanian barley landraces were subjected to comprehensive analysis to assess their genetic diversity and agronomical performance under dry conditions. For instance, a collection of 150 Jordanian barley landraces deposited in the International Center for Agricultural Research in Dry Areas (ICARDA) were evaluated under rainfed conditions [18]. This panel was found to possess high genetic diversity with a clear grouping based on row type of the collected samples. Furthermore, genome-wide association mapping identified several markers associated with multiple traits under dry conditions. A clear clustering based on collection site was observed in Jordanian barley landraces analyzed by using intron splice junction markers that were able to separate them from wild barley populations [19]. In another study, no major significant change in genetic diversity was found in seed samples of Jordanian landraces collected in 1981 when compared with recent collection in 2012 from the same collection sites [20].

The conservation of Jordan barley landraces is urgently needed knowing that their natural habitats are subjected to degradation due to urbanization activities and climate change. In Jordan, the number of *ex situ* conserved barley accessions in global collections is considerably low when compared with neighboring countries [7]. Assessing the agronomical performance of historic landraces conserved in Gene Banks might identify new germplasm with improved adaptation to dry conditions. Therefore, the main objective of this study is to evaluate the agronomical performance of 10 historic and *ex situ* conserved Jordanian barley landraces in comparison with three improved lines under contrasting environments. Furthermore, assessment of their genetic diversity was performed by using the 9k Illumina iSelect SNP assay. Understanding the genetic diversity within Jordanian landraces and their yield performance is important for their future use in breeding

programs to improve yield and stability under dry conditions.

## 2. Materials and Methods

**2.1. Plant Material.** In this study, 13 barley genotypes were used that included two Jordanian barley cultivars (Mutah and Rum), an improved line from ICARDA (Arta), and 10 historic Jordanian landraces retrieved from two international Gene Banks (Table 1). The historic set included eight landraces from the USDA-ARS national small grains collection and two landraces from the Plant Gene Resources of Canada. The landraces were collected during the period from 1955 to 1979, before the registration of Rum, the first barley variety to be released in Jordan in 1986 (Table 1). The seeds of the three checks were certified in the National Agricultural Research Center (NARC), Jordan.

**2.2. Field Experiments.** The barley genotypes were grown for two growing seasons (2011–2012 and 2012–2013) in two locations. The first location was the University of Jordan (JU) campus research station located in Jubeiha in the middle of Jordan (32°00'40" N, 35°52'24" E; elevation: 990 m; average annual precipitation: 505 mm) and representing a humid area. The second location was Jordan University of Science and Technology (JUST) campus research station located in Ramtha in the north of Jordan (32°28'47.4" N, 35°59'11.1" E; elevation: 520 m; average annual precipitation: 217 mm) and representing a dry area. The soil type in both locations was analyzed and found to be clay loam. The tested genotypes were sown on the last week of December in each growing season and cultivated under rainfed conditions without any supplementary irrigation. The seeding rates were adjusted according to the results of a seed germination test to obtain a plant density of 150 plants/m<sup>2</sup> from the viable seeds. All field trials followed a fallow in the crop rotation and were established using hand broadcasting following conventional tillage performed with chisel plough and disk harrow. In each location, experimental plots were managed following the standard agricultural practices including fertilizer application to match the crop needs, weed control (hand weeding and herbicides against broadleaf weeds), and pesticide use against main pathogens. Climate stations at the field sites were used to record precipitation and temperatures during the growing seasons. For experimental design, a randomized complete block design (RCBD) with four replications was used. Each block area included 30 rows (0.5 m apart and 2 m long) and each two adjacent rows represents a tested genotype distributed randomly in the block with two rows cultivated with Rum as borders.

**2.3. Traits Measurements.** Days to heading (HD) was recorded as the number of days from emergence to the day when 50% of plants had full spikes. Days to physiological maturity (MD) was recorded as the number of days from emergence to the day when 90% of the plants have reached the physiological maturity stage (no green tissue remained in 90% of the plants of each plot). Grain filling period (GFP) was

TABLE 1: Name and origin of barley genotypes used in this study.

Landraces				
Name	Accession number	Receiving date	Row type	Holding institute
Arabi	PI 223130	1955	2	USDA-ARS National Small Grains Collection
Baladi	PI 223131	1955	6	USDA-ARS National Small Grains Collection
Habashi	PI 223142	1955	6	USDA-ARS National Small Grains Collection
Herawi	PI 223143	1955	6	USDA-ARS National Small Grains Collection
Konari	PI 223145	1955	6	USDA-ARS National Small Grains Collection
Nabawi	PI 223146	1955	6	USDA-ARS National Small Grains Collection
Syfi	PI 233837	1956	2	USDA-ARS National Small Grains Collection
Weah	PI 371819	1972	2	USDA-ARS National Small Grains Collection
Jordan1	CN 7339	1979	6	Plant Gene Resources of Canada
Jordan2	CN 7340	1979	6	Plant Gene Resources of Canada
Checks				
Name	Developing institute	Releasing date	Row type	Pedigree
Rum	CIMMYT/ICARDA	1986	6	Harbinger-Arivat × Attiki
Mutah	ICARDA	2004	2	Roho/Arabi × Abiad/6250
Arta	ICARDA	1994	2	A single spike selection from a field of Arabi Abiad

calculated by subtracting MD from HD. Plant height (PH in cm) was measured for each genotype at maturity from the ground to the spike tip on three selected plants that were randomly distributed within the rows. Peduncle length (PL in cm) were measured as the distance from the flag leaf collar to the base of the spike on the same main stem of the plants used to measure plant height. When the PL value is negative, this indicates that the spike remained inside the sheet of flag leaf.

At the end of field experiments, plants were harvested at maturity and the following traits were recorded: grain weight (GW in  $\text{g/m}^2$ ) was recorded as average of grain weight measured after all harvested spikes were separated and threshed and the grains were cleaned from chaff. Total weight (TW in  $\text{g/m}^2$ ): dry weight of the whole harvested plants (including straw and spikes). Spikes number (SN) of all plants harvested. Grain number of harvested grains were counted using seed counter. Thousand kernel weight (g): calculated by dividing the grain number by grains weight multiplied by 1000. Harvest index (%): calculated by dividing grain weight over total weight multiplied by 100.

The drought susceptibility index (DSI) was calculated as described in [21] using two different approaches. The first approach was based on using GW after the classification of environments into high-yielding environments (HYE) and low-yielding environments (LYE) [22, 23]. In the second approach, the DSI analysis was carried out using GW based on location GW mean where JU represented a humid area and JUST represented a dry area.

**2.4. Molecular Analysis.** For genomic DNA extraction, leaf tissue samples from each genotype were harvested from three-week-old seedlings that were grown under controlled growth chambers conditions with temperature range between 22 and 24°C under short-day photoperiod (10 h light: 14 h darkness). Total genomic DNA extraction was performed using the CTAB (cetyltrimethylammonium bromide) method [24]. For SNP genotyping, the Illumina Infinium iSelect 9k SNP barley array, which includes a set of 7864 gene-based SNPs markers

considered to be independent with high confidence rate, was used as described previously [18]. The genotyping assay was conducted in TraitsGenetics GmbH (Gatersleben, Germany) facilities as a service. Markers with allele frequency <5% or missing data >10% were removed from further analyses.

**2.5. Data Analysis.** The analysis of variance (ANOVA) and associated data (broad-sense heritability ( $H^2$ ), pairwise correlation Pearson coefficients ( $r$ ), and coefficient of variation (CV%)) for yield and yield-component traits were statistically analyzed using GenStat software Edition 15 [25]. Mean separation was analyzed using least standard error of the differences between means (LSD) test at 0.05 level of probability. The GGE biplot software [26] was used to analyze the specific adaptation, superiority, and stability of the genotypes across tested environments for the GW and TW traits. In addition, the GGE biplot software was used for the principle component analysis (PCA) to extract multitrait relationships within the inference space of genotype variation for each environment. Each environment represents a location-growing season combination that resulted in four environments: Humid2012 (JU-2011/2012), Dry2012 (JUST-2011/2012), Humid2013 (JU-2012/2013), and Dry2013 (JUST-2012/2013).

For genetic diversity analysis, SNP markers were transformed into a binary matrix that was used to generate a genetic distance matrix-based Dice similarity coefficient and a dendrogram was constructed based on the UPGMA (unweighted pair group method with arithmetic average) algorithm using the SHAN module of NTSYS-pc software, Version 2.2 package [27].

### 3. Results

**3.1. Growing Seasons and Weather Conditions.** Analyzing weather data during the two growing seasons from meteorological stations in the two locations indicated that mean maximum and minimum temperatures for the first three months of growing seasons (January to March) were higher

in 2012/2013 growing season when compared with 2011/2012 except for January minimum temperatures in JUST location (Table 2). For the rest of the growing seasons, April and June maximum temperatures in 2011/2012 showed higher values in both locations. Maximum and minimum temperatures in May were higher in 2012/2013 than in 2011/2012 (Table 2).

In Jordan, the average annual rainfall distribution is concentrated in December, January, February, and March, with each month receiving 18%, 24%, 21%, and 19% of the total annual amount, respectively [28]. In the first growing season (2011/2012), both locations received higher amounts of rainfall when compared with the long-term average where JU location received 563 mm (11.5% above annual average) while JUST received 303 mm (40.3% above annual average) (Table 2). The rainy season in 2011/2012 terminated earlier than expected as no precipitation was received in April, May, and June in both locations accompanied with high temperatures at the same period that resulted in terminal drought conditions. During the 2012/2013 growing season, both locations received more than the long-term average of rainfall by 5.5% (533 mm) in JU and 9.5% (237 mm) in JUST (Table 2). However, 61% and 58% of the total rainfalls were received in January alone in JU and JUST, respectively, causing uneven distribution patterns. At JUST location, severe drought conditions were encountered with low amounts of precipitation (16 mm) received after January contributing 6.75% (16 mm) from the total amount of received rainfall (237 mm). On the other hand, the JU location received 86 mm (16%) after January (Table 2) with a clear dry period in March (2 mm) accompanied with higher mean temperatures when compared with the 2011/2012 growing season.

**3.2. Performance of Barley Genotypes under Field Conditions.** The combined ANOVA showed high significant ( $p < 0.01$ ) differences for all tested traits among environments, genotypes, and genotype  $\times$  environment interaction ( $G \times E$ ) (Table 3). For genotypes' effect, high significant differences ( $p < 0.01$ ) were observed for all traits indicating the presence of genetic variabilities in the agromorphological traits among the tested genotypes. For environments' effect, high significant differences ( $p < 0.01$ ) were also observed for all traits indicating the presence of variations among the tested environments. For broad-sense heritability ( $H^2$ ), HD and MD gave the highest estimates (0.93), while PL and GFP produced the lowest values (Table 3).

For genotypic effect, the mean values of the GW of tested genotypes ranged 208.9 g/m<sup>2</sup> for Nabawi landrace to the significantly highest mean value (533 g/m<sup>2</sup>) recorded in Rum, which was followed by the Baladi landrace (498.2 g/m<sup>2</sup>) (Table 4). Eight genotypes produced GW mean values above the overall average (374.3 g/m<sup>2</sup>). No significant differences were found in GW mean values between the two-row barley genotypes, while clear significant differences exist among the six-row genotypes (Table 4). Based on environmental grouping, the mean values of the GW ranged 265.4 g/m<sup>2</sup> for Dry2013, which was significantly the lowest, to 421.9 g/m<sup>2</sup> recorded in Humid2012 (Table 4). Clear

significant differences were observed between dry and humid environments and within dry for GW trait mean values, with a clear reduction in Dry2013 due to severe drought conditions. For combined analysis, the highest GW mean value (595.4 g/m<sup>2</sup>) was observed in Rum in Humid2012 environment, while the lowest mean value (91.5 g/m<sup>2</sup>) was observed in Nabawi in Dry2013 (Table 4).

For TW, Rum cultivar produced significantly the highest mean value (1445.05 g/m<sup>2</sup>) among tested genotypes followed by Jordan2 (1336.70 g/m<sup>2</sup>) and Baladi (1301.10 g/m<sup>2</sup>) landraces (Table 4). On the other hand, Arta produced the lowest TW mean value (882.50 g/m<sup>2</sup>), although it was not significantly different from Herawi landrace (898.40 g/m<sup>2</sup>) and Nabawi (962.00 g/m<sup>2</sup>) mean values. For environments, the mean values of the TW ranged 987.50 g/m<sup>2</sup> for Dry2013, which was significantly the lowest, to 1264.10 g/m<sup>2</sup> recorded in Humid2012, which was not significantly different from Dry2012 (1244.80 g/m<sup>2</sup>) (Table 4). A clear significant difference was observed between seasons for TW trait mean values, with a clear reduction in mean values in 2013 compared with 2012. For combined analysis, the highest TW mean value (1621.50 g/m<sup>2</sup>) was observed in Rum in Humid2013 environment, while the lowest mean value (700.40 g/m<sup>2</sup>) was observed in Arta in Dry2012 (Table 4).

For other traits, mean values of tested genotypes across different environments are shown in Figure 1. For HD and MD, Mutah cultivar was significantly the fastest growing genotype across all environments, whereas Herawi landrace was the latest. In addition, Nabawi landrace showed a delayed HD and MD under dry environments, while Herawi was the latest under humid environments (Figure 1). For PH, clear significant differences were observed between dry and humid environments, with a clear reduction in Dry2013. Rum cultivar was significantly the tallest genotype, while Nabawi was significantly the shortest. For SN, two-row genotypes tend to produce the highest mean values (Figure 1), with Arabi landrace producing the highest overall mean value of 545.8 spikes, which was not significantly different from Syfi landrace. On the other hand, Nabawi landrace produced the lowest mean value of SN, although it was not significantly different from Herawi. For GN, Rum cultivar produced the highest mean value in Dry2013 followed by Baladi with no significant differences between both genotypes. On the other hand, Herawi and Nabawi produced the lowest GN mean value in Dry2013. For TKW, Nabawi produced significantly the lowest mean value across different environments, while for HI, Arta produced significantly the highest mean value in Humid2012 (Figure 1).

**3.3. Drought Susceptibility Index.** For DSI analysis that is based on the grand mean of GW over all environments (374.29 g/m<sup>2</sup>) (Table 4), the environments were classified into high-yielding environments (HYE), which included Humid2012, Humid2013, and Dry2012, and LYE that included Dry2013 that had a GW mean value below the grand mean [22, 23]. In addition, the DSI analysis was carried out based on the location GW mean values where JU represented a humid area and JUST represented a dry area. Based

TABLE 2: Actual rainfall (mm on monthly basis) and average maximum (max.) and minimum (min.) temperatures (°C on monthly basis) in the two locations over two growing seasons.

Average temperatures	JU				JUST			
	2011-2012		2012-2013		2011-2012		2012-2013	
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
December	14.7	3.1	15.0	5.8	15.8	3.6	15.8	6.8
January	10.1	2.7	12.7	2.9	12.2	4.1	12.8	2.9
February	11.5	2.9	14.2	5.3	13.0	3.4	15.3	4.3
March	14.4	4.0	19.1	7.2	16.5	4.8	20.4	7.5
April	24.1	8.9	20.8	8.4	24.9	9.6	22.0	8.6
May	26.7	12.7	27.3	13.6	28.90	14.3	29.5	13.8
June	30.9	19.3	28.7	17.1	33.80	17.0	30.7	16.5
Actual rainfall	2011-2012		2012-2013		2011-2012		2012-2013	
December	47.5		85.5		12.2		51.0	
January	132.0		327.5		79.3		136.8	
February	131.0		59.8		74.4		8.3	
March	210.0		2.0		53.7		0.3	
April	0.0		23.9		0.2		5.8	
May	0.0		0.0		0.0		1.7	
June	0.0		0.0		0.0		0.0	
Total annual rainfall	563		533		303		237	

TABLE 3: Mean squares from combined ANOVA of 13 barley genotypes grown across four different environments.

Source of variation	DF	HD	MD	GFP	PH	PL	TW	SN	GN	GW	TKW	HI
Env. Rep stratum												
Environment	3	248.8**	3365**	2143**	5228**	592**	754275**	43170**	6.08 <sup>e+07**</sup>	282149**	1047.6**	846.4**
Error	12	1.64	4.99	2.15	12.47	3.11	19930	4339	1.46 <sup>e+06</sup>	3165	1.75	4.519
Gen. Env. Rep stratum												
Genotype	12	484.0**	480.4**	43.79**	633.5**	13.61**	389281**	130072**	3.02 <sup>e+07**</sup>	138836**	429.4**	411.4**
G × E	36	34.1**	34.9**	32.5**	132.8**	13.7**	155308**	13251**	1.17 <sup>e+07**</sup>	20174**	75.2**	78.274**
Error	144	1.2	1.2	1.7	10.2	2.4	11740	1872	1.36 <sup>e+06</sup>	1977	1.7	5.1
Total	207											
Grand mean		107.40	141.16	33.72	56.78	-4.7	1162.0	395.9	8801	374.3	41.85	31.53
H <sup>2</sup>		0.93	0.93	0.26	0.79	0.00	0.60	0.89	0.61	0.85	0.83	0.81
CV (%)		1.00	0.78	3.89	5.62	32.97	9.27	10.93	13.27	11.88	3.12	7.15

DF, degree of freedom; Env, environment; Gen, genotype; \*\*significant at  $p < 0.01$ .

on HYE and LYE grouping, DSI values ranged 0.25 that was recorded in Baladi to 1.84 recorded in Weah landrace followed by Nabawi (1.79) with eight genotypes producing DSI values less than 1, which indicate good tolerance against drought conditions (Figure 1). Using location GW mean values to calculate DSI, the values ranged 0.4 that was recorded in Baladi to 1.91 recorded in Arta followed by Weah landrace (1.58). Furthermore, six genotypes produced DSI values more than 1 including all two-row genotypes, which was not the case with the HYE-LYE-based analysis (Figure 1). In both scenarios, the DSI values of Rum and Baladi were consistent and among the lowest values indicating their good drought tolerance potential and putative adaption to dry environments. On the other hand, Weah and Jordan2 landraces were poor performers under drought conditions producing DSI values above 1 in both analyses (Figure 1).

**3.4. Correlation between the Agronomic Traits.** Significant correlations ( $p < 0.01$ ) were found between GW with all

tested traits except for the MD (Table 5). Total weight was positively correlated with all tested traits except for HD, MD, and GFP. A significant positive correlation was found between HD and MD, and both traits showed a significant positive correlation with PH. Plant height showed a significant positive correlation with all traits except for SN (Table 5). TKW and HI showed significant positive correlation with all tested traits except for MD.

**3.5. PCA and GGE Biplot Analysis.** The PCA was used to extract multivariate relationships within the inference space of genotype variation for each environment (Figure 2). The PCA extracted two major components that accounted between 54% and 75% of the total variation for each environment. In general, a clear grouping based on row type was observed, in particular a clear grouping of the two-row genotypes that was observed across all environments (Figure 2). Strong associations between HD and MD were observed in the PCA plot where both traits were loaded negatively in PC1 and PC2. Strong associations between

TABLE 4: Grain weight (GW) and total weight (TW) mean values of 13 barley genotypes across four tested environments.

Genotype	GW					TW				
	2012		2013		Means	2012		2013		Means
	JU	JUST	JU	JUST		JU	JUST	JU	JUST	
Arabi	401	362	516	303	396	1256	1212	1385	942	1199
Arta	365	287	592	278	381	820	700	1245	764	883
Baladi	495	488	546	464	498	1218	1436	1370	1181	1301
Habashi	543	477	291	273	396	1571	1364	1064	932	1233
Herawi	279	286	238	129	233	981	991	822	799	898
Jordan1	450	449	375	276	388	1270	1256	1173	1173	1218
Jordan2	530	555	526	221	458	1472	1563	1248	1064	1337
Konari	346	348	311	246	312	1066	1140	1018	1236	1115
Mutah	422	357	458	295	383	1238	1156	1219	1064	1169
Nabawi	292	284	168	92	209	1222	1200	650	776	962
Rum	595	550	557	430	533	1568	1522	1622	1110	1455
Syfi	362	304	499	314	370	1234	1116	1323	1049	1180
Weah	405	364	341	129	310	1518	1526	836	748	1157
Mean	422	393	417	265	374	1264	1245	1152	988	1162
	Location mean		Year mean			Location mean		Year mean		
	JU	419	2012	408		JU	1208	2012	1255	
	JUST	329	2013	341		JUST	1119	2013	1072	

For GW  $LSD_{(0.05)}$  values, 31.81 was used to compare between genotypes means, 17.64 was used to compare between environments means, and 63.61 was used to compare means for combined analysis. For TW  $LSD_{(0.05)}$  values, 78.01 was used to compare between genotypes means, 43.27 was used to compare between environments means, and 156.03 was used to compare means for combined analysis.

GW and TW (positively loaded in PC1) were observed in humid environments and to less extent in dry environments.

To analyze genotype-specific adaptation to specific environment, the GGE biplot was used to provide a two dimensional approximation of genotype effect plus  $G \times E$  interaction (GGE). The associated visual presentation of the genotypes and environments, their positions relative to the other genotypes per environments as well as nearness between genotypes and environment for GW and TW are shown in Figure 3. Using the means across all environments and considering all the genotypes, the first two principal components (PC) accounted for a total of 93% and 87% variations for GW and TW, respectively. For GW, Humid2012, Dry2012, and Dry2013 environments fell in the sector in which Rum cultivar was the vertex genotype, which means that Rum was the best genotype in these environments (Figure 3(a)). The Humid2012 environment fell in the sector in which Baladi landrace was the vertex genotype, meaning it was the best genotype for this environment. For TW, all environments fell in the sector in which Rum was the vertex genotype, which indicates it was the best genotype for all tested environments (Figure 3(b)). For GW, the GGE biplot analysis identified Baladi and Rum to have the highest average GW, while Nabawi and Herawi had the lowest (Figure 3(c)). For stability measured by projection to the Average-Tester Axis  $y$ -axis, Arta and Habashi were the least stable genotypes, while Konari, Herawi, and Rum were the most stable. For TW, the GGE biplot analysis showed Rum to have the highest average TW, while Herawi had the lowest. The stability analysis identified Rum as the most stable genotype, while Weah and Arta were the least stable genotypes (Figure 3(d)).

**3.6. Molecular Analysis.** Molecular markers' data generated by using 9k SNP chip were used to analyze the relatedness between the 13 barley genotypes (Figure 4). At similarity coefficient 62%, a clear separation of genotypes into two clusters was observed that was based on the row type. This clustering indicates the existence of good genetic variation among barley genotypes used in this study. The genetic similarity among the 13 genotypes ranged from 62% to 100% (Figure 4). Group 1 included all two-row barley genotypes with Arabi and Syfi landraces showing a similarity level of 100% indicating they are identical genotypes. The second group included all six-row genotypes with Baladi and Herawi showing a similarity level of 94%, while Weah showed the lowest similarity with other genotypes and was the most diverse.

#### 4. Discussion

In marginal Mediterranean environments, inconsistent climatic conditions associated with heat and drought stresses are considered major constrains for cereal production [29]. In Jordan, the mean values of annual temperatures in the last three decades increased significantly and were accompanied with precipitation reduction and its uneven distribution [20]. Therefore, the utilization of well-adapted and resilient germplasm in breeding programs are considered a useful strategy to compact climate change-associated conditions such as drought and heat stresses [30]. Landraces possess desirable traits for marginal areas such as resistance against diseases and pests, tolerance to drought and heat stresses, and stable performance under low soil fertility and dry conditions [31]. For instance, evaluating 150 Jordanian barley landraces from ICARDA GeneBank identified

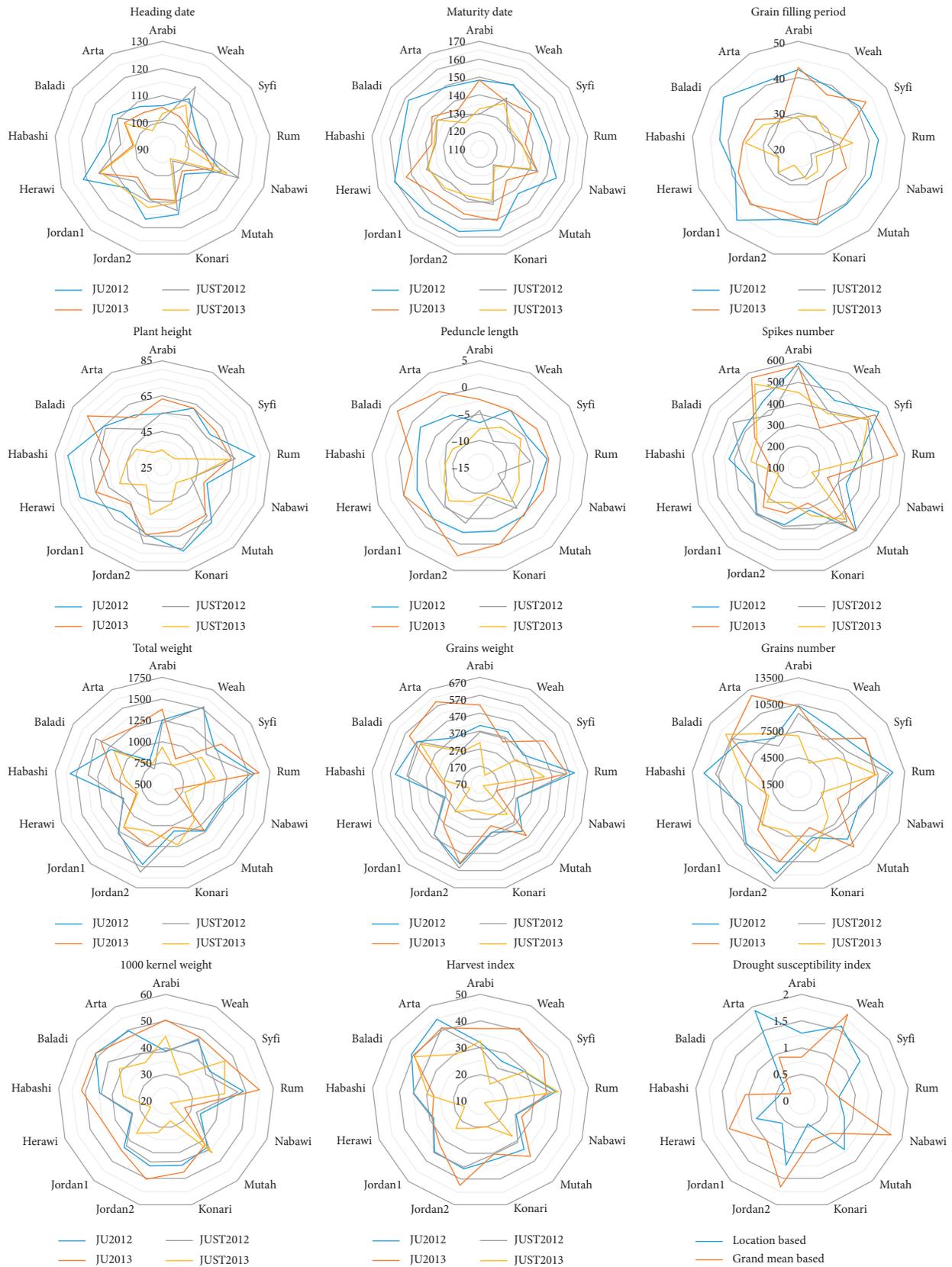


FIGURE 1: Agronomical performance of 13 barley genotypes evaluated in four tested environments as represented in 11 recorded traits and drought susceptibility index analysis based on location and grand mean.



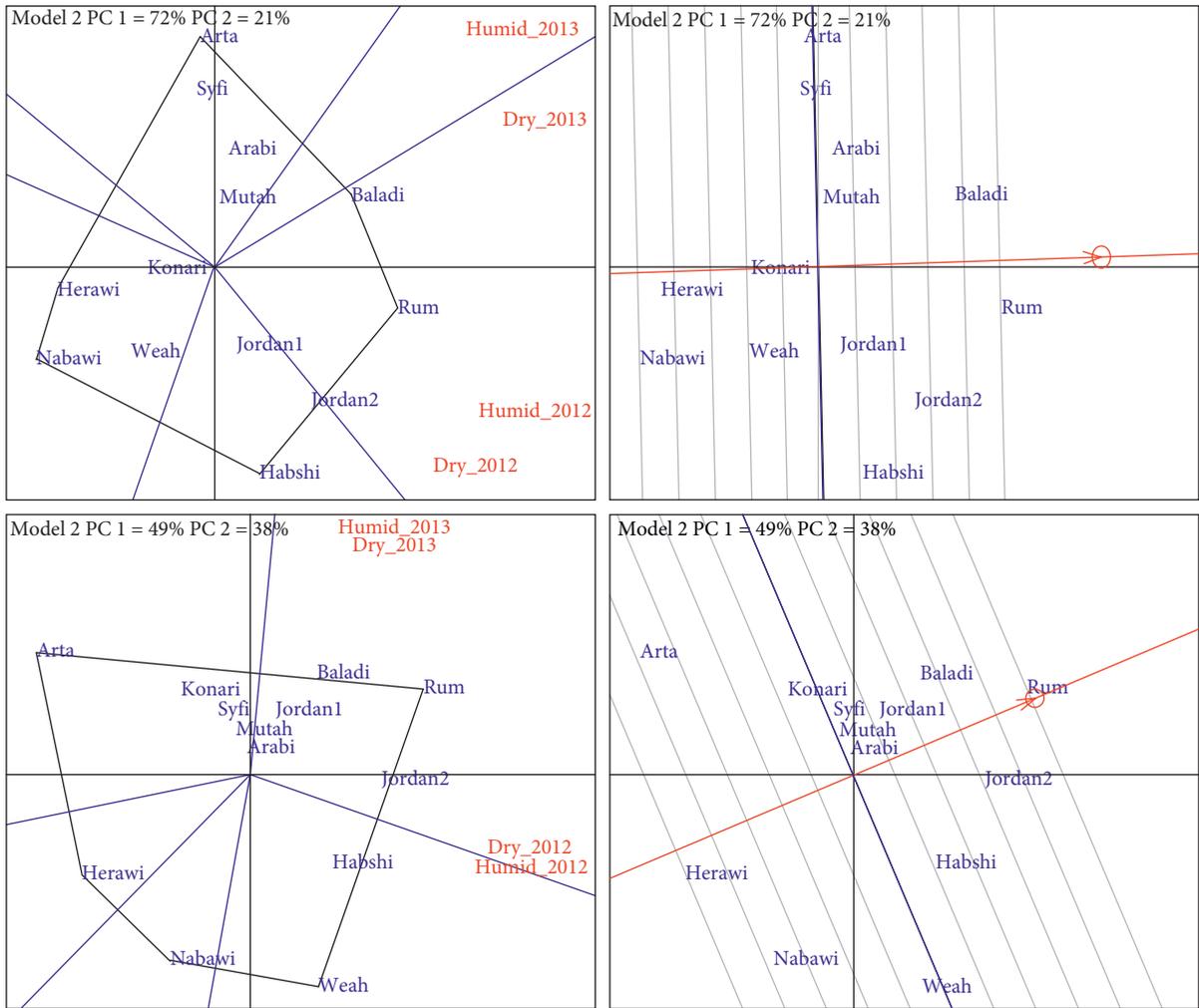


FIGURE 3: GGE biplot analysis for GW (a) and TW (b) and stability analysis using biplot for GW (c) and TW (d) of 13 barley genotypes grown across four different environments.

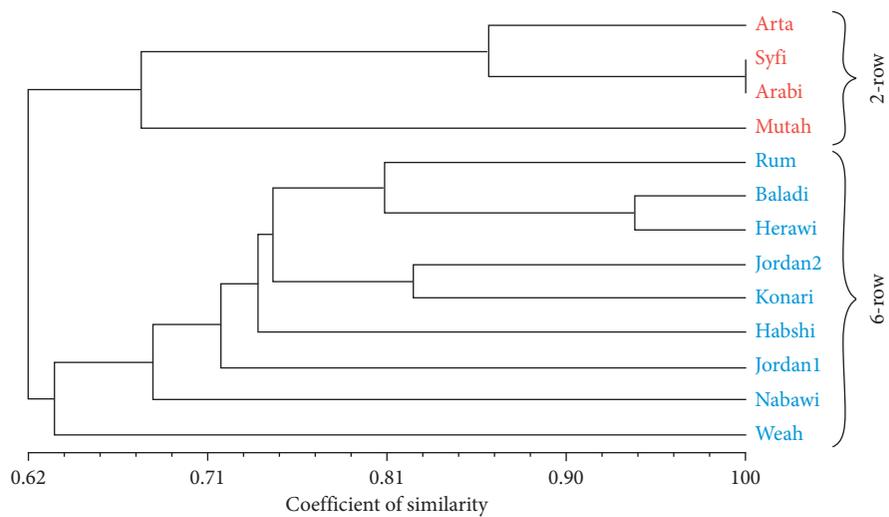


FIGURE 4: A dendrogram of relationships among 13 barley genotypes using DICE genetic similarity and UPGMA clustering method.

close relatedness with it, while Mutah is a released cultivar that includes Arabi in its pedigree (Table 1). Based on indigenous knowledge of local Jordanian farmers, Arabi is a

common name for most dominant two-row landraces in the north of Jordan, while Syfi (means sword-shape in Arabic) is the common name of the most domain two-row barley

landrace cultivated in the middle and south of Jordan (Karadsheh and Al-Abdallat, unpublished data). Baladi, which means local in Arabic, is commonly used by framers to describe a local landrace in a given area. On the other hand, six-row landraces are not well speared in main cultivated areas and they are called Argadi in northern parts of Jordan, while in the middle and south regions, they are called Konari and in this study, none of the six-row landraces showed 100% similarity with Konari.

In the six-row group, Weah was the most diverse landrace and formed a clear outgroup from other landraces. This particular genotype was collected separately with two other genotypes described as improved breeding lines in a separate mission in 1972. Therefore, it is not clear whether Weah is a true Jordanian landrace or considered an introduced breeding material. However, its divergence from other Jordanian landraces might indicate that it is not indigenous to the region. The second most divergent landrace in the six-row group was Nabawi, an exotic naked barley landrace from Jordan. In a genetic diversity study of barley accessions from North Africa, naked barley genotype formed a separate subgroup from hulled subgroups, which support the findings of this study [32].

The Jordanian landraces used in this study were collected at three different time points before the release of Rum, the first cultivar in Jordan, with the oldest mission dated back to 1955-1956. Seven landraces were collected in the first mission and therefore were considered ancient landraces when compared with the recently studied ICARDA material, which was collected after 1977 [18]. In a recent study, no major changes in genetic diversity parameters were identified between Jordanian barley landraces collected from the same locations at two different time points (1982 and 2012) [20]. Besides, more phenotypic homogeneity was observed among sites in 2012 when compared with 1982 with no clear correlation between climate change and the observed genetic and phenotypic variations. Clustering analysis of landraces used in this study using NtSys software with Jordanian barley landraces from ICARDA Gene Bank identified G140 to be identical to Jordan1, G090 and G105 to be identical to Syfi and Arabi, and G119 and G143 to be identical to Konari (data not shown). The rest of this study material was considered more diverse and did not cluster with any of the ICARDA material [18] and they showed similar grouping pattern as shown in Figure 4. This complex picture might indicate that the on-farm maintenance of some old landraces by Jordanian farmers is still prevalent [20]; however, there is a need to find whether the rest of them were lost and to validate this assumption, there is a need to collect new material and compare it at the morphological and molecular level with *ex situ* conserved germplasm.

High significant genotypic differences ( $p < 0.01$ ) were observed for all traits under all environments indicating the existence of a high genotypic variation and diversity among the tested genotypes. Similar results were observed by Shakhathreh et al. [33], who found significant genetic variation among 87 barley genotypes for different traits across

different environments in Jordan. In addition, the  $H^2$  values for several traits were found to be high (above 0.6), indicating that the observed variations are most likely related to genetic differences. Such high  $H^2$  values were observed previously in 50 Romanian and Italian barley cultivars tested across different environments [34]. Significant differences were also observed between the Jordanian landraces when compared with the released varieties for all tested traits. This is also supported by the molecular analysis where a clear genetic variation existed between tested genotypes, although a clear relatedness between two-row landraces and cultivars exists, which is expected due to the use of these landraces as parents in ICARDA breeding program (Table 1; Figure 4).

The high levels of variability for studied traits in the tested genotypes indicate their importance as a valuable genetic resource for breeders to develop new cultivars adapted to dry environment. For instance, Mutah cultivar (includes the Arabi landrace in its pedigree) was found to be the earliest in heading date and the most stable two-row genotype for GW when compared with Arabi/Syfi and Arta genotypes. In addition, GW was correlated significantly and negatively with heading date (Table 3). Similar results were observed by [35] where a clear GW stability was observed in Keel, an early flowering Australian cultivar, when compared with Arta. Mutah was the fastest genotype to flower under dry conditions when compared with Arabi/Syfi and Arta (Figure 1). This is somehow expected as Mutah was improved to carry spring alleles associated with accelerated heading date, when compared with allelic variant associated with delayed heading date existing in Arta [36].

The significant effect of environments on different traits indicates the existence of differences among growing conditions in the two locations and growing seasons as reflected in temperature and water availability (Table 2). The most pronounced effect of drought conditions on tested material was observed in JUST location in 2012-2013, which resulted in 40% reduction in GW overall mean when compared with JU location in 2013 (Table 4), while in 2011-2012, no pronounced effect on GW was observed between the two locations. Such year-to-year variation was found to have stronger effect on agronomical performance of barley plants under dry environments when compared with variations across contrasting locations [35]. In this perspective, DSI analysis based on GW means of location grouping was different for DSI based on GW grand mean for selected genotypes such as Arta, Arabi/Syfi, Nabawi, and Herawi (Figure 1). This inconsistency in discriminating drought-tolerant genotypes using DSI was reported previously with winter wheat accessions from the USDA-ARS National Small Grains Collection [37]. GGE biplot analysis identified Rum and Baladi landraces as the best and most stable genotypes for GW and TW across the tested environments. Rum is the oldest registered cultivar in Jordan that was bred for dry environments by CIMMYT/ICARDA and it is to date preferred by many Jordanian farmers. Its preference by local Jordanian farmers is due to its stability across different environments and its high yielding potential under rainfed conditions [38].

## 5. Conclusions

In conclusion, a high level of genetic variation exists for all tested agromorphological traits measured in a historic collection of Jordanian barley landraces evaluated. Such genetic variation was observed at the molecular level using SNP markers' analysis with a clear grouping based on row type and breeding activities. Rum cultivar and Baladi landrace outperformed many landraces across different environments and particularly for GW and TW that are of special importance to farmers in dry regions. The exotic Jordanian barley landraces described in this study can be used in future breeding activities to improve productivity in marginal areas throughout the world.

## Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare no conflicts of interest.

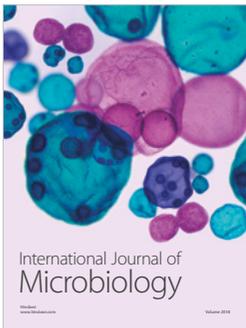
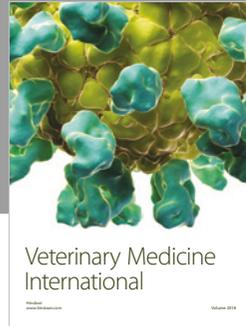
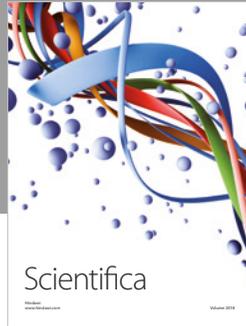
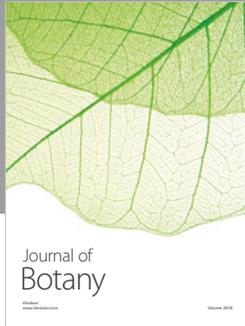
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