Theor Appl Genet (1989) 78:857–862

Ecotypes and genetic divergence among sympatrically distributed populations of *Hordeum vulgare* and *Hordeum spontaneum* from the xeric region of Jordan

A. A. Jaradat

Department of Agricultural Sciences, Jordan University of Science and Technology, P.O. Box 3030 Irbid, Jordan

Received May 22, 1989; Accepted July 31, 1989
Communicated by P. M. A. Tigerstedt

Summary. The progeny of paired samples of *Hordeum vulgare* L. and *Hordeum spontaneum* C. Koch. collected from Jordan's xeric region was used in this study. Statistical analyses of seven easily measured morphometric traits were used to elucidate the relationships and distances between populations of both species, to detect any ecogeographical races, and to study the interrelationships and adjustments in the morphometric traits under study. Flag leaf area and plant height were the two most important discriminating variables which totally separated *Hordeum vulgare* from *Hordeum spontaneum* and accounted for 85.3% of total phenotypic variance in the collection. Cluster analysis indicated that the level of divergence among populations of both species was considerably different. Populations of *Hordeum vulgare* clustered at a maximum Euclidean distance of 2.08, while the maximum distance at which populations of *Hordeum spontaneum* clustered was 1.49. Three ecotypes each of *Hordeum vulgare* and *Hordeum spontaneum* were identified. These ecotypes corresponded to the environmental range of the collection sites. The interrelationships between the seven morphometric traits were adjusted in different ways as revealed by the principal components analysis. Sampling from the different clusters identified in this analysis is expected to increase the allelic diversity for selection and breeding purposes.

Key words: *Hordeum vulgare* – *Hordeum spontaneum* – Divergence – Ecotypes

Introduction

One solution to the genetic impoverishment of crop germ plasm is genetic conservation and utilization in breeding of the vast genetic variation found in natural populations of wild progenitors and landraces of cultivated plants (Nevo et al. 1986). Present-day crop cultivars and their wild progenitors are linked through an evolutionary continuum. Therefore, these wild progenitors are important sources for crop improvement. Jordan, a country in the Near East Fertile Crescent, represents a sizable part of the center of origin and diversity for barley (Harlan and Zohary 1966), where a wealth of genetic diversity has been built up throughout the evolutionary history of the species (Nevo et al. 1984).

Wild barley (*Hordeum spontaneum* C. Koch.), the immediate progenitor of cultivated barley and landraces and primitive cultivars of the domesticated species (*Hordeum vulgare* L.) from the Fertile Crescent are of particular interest for barley breeders (Plucknett et al. 1983). These genetic resources are the least conserved and exploited category in barley breeding and improvement. However, impressive advances in crop improvement have been reported for a number of crops with genes contributed by landraces (Chang 1985) and wild taxa (Frankel and Soule 1981; Chang 1985).

Hordeum spontaneum populations from parts of the Fertile Crescent were found to be highly variable and to differ from each other in their level of variation (Brown et al. 1977; Nevo et al. 1984, 1986). Moreover, regular introgression from *Hordeum vulgare* was reported in *Hordeum spontaneum.* This phenomenon is well documented in nature (Zohary 1969), especially in populations of both species sympatrically distributed in wadi bottoms in parts of the Fertile Crescent. *Hordeum spontaneum* populations from these sites are essentially wild plants modified only slightly by occasional introgression with cultivated landraces of barley (Harlan and Zohary 1966). However, the rates of introgression are relatively lower in xeric than in the more mesic regions (Brown et al. 1977).

Morphological variation was reported from parts of the Fertile Crescent for agronomic traits in *Hordeum spontaneum* (Snow and Brody 1984) and barley landraces (Ceccarrelli 1984; Jaradat et al. 1987). Diversity in both species sympatrically distributed in the xeric parts of Jordan was assessed for a number of isozyme systems (Jana et al. 1987) and qualitative traits (Jaradat 1989). *Hordeum spontaneum* expressed a higher level of diversity for the qualitative but not for the isozyme traits. However, it has been shown (Giles 1984) that enzyme variation does not necessarily reflect phenotypic polymorphism, even if the latter are pronounced.

No information is available on the genetic divergence among and within sympatrically distributed populations of both barley species as detected by morphometric trait measurements. This research was undertaken to elucidate the relationships and distances between populations of both barley species collected from the xeric region of Jordan, to detect any ecogeographical races (ecotypes), and to study the interrelationships and adjustments in seven morphometric traits in these populations.

Materials and methods

A subsample of 520 individual plants each of barley landraces (*Hordeum vulgare* L.) and wild barley (*Hordeum spontaneum* C. Koch.) was derived from the progeny of an original collection of the two sympatrically distributed *Hordeum* species in the xeric region of Jordan (Jaradat 1989).

Collection sites extended from Mafraq in the North to Rashadiah in the South. Three major regions in that xeric part of the country were covered. These were: (1) northern steppe and steppe desert, (2) southern mountains, and (3) desert wadi bottoms, where cultivated and wild barley rely on stored soil moisture from water harvesting. Detailed geographic and ecological information about collection sites is available in Jaradat (1989).

A randomized complete block design with two replicates and ten progenies in each experimental unit (plot) was used. The progeny of a single plant is referred to as a family. A number of morphometric traits was recorded on individual plants within each plot, however, only data on the same morphometric traits measured on the original collection (Jaradat 1989) were used in this study.

Statistical analysis

The original collection of both barley species was assembled from nine sites in three regions (Table 5). The following measurements were recorded at physiological maturity on a maximum of ten plants per family: flag leaf length, flag leaf width, flag leaf sheath length, awn length, peduncle length, plant height, and number of fertile tillers/plant. Flag leaf size was calculated from flag leaf length and width.

Data were checked for homogeneity, then subjected to a hierarchical analysis of variance (Sokal and Rohlf 1981) using the subroutine Hierarch in MSTAT statistical package (MSTAT 1987). A linear additive model describing the hierarchal components of the form:

$$\chi_{ijkl} = \mu + \alpha_i + \beta_j + \delta_k + \sigma_1 + \varepsilon_{ijkl} ,$$

was used, where μ is a general mean, α_i, β_j, σ_k, and δ_l are effects due to regions, locations within regions, species within locations, and families within species, respectively. ε_{ijkl} is a random effect (error). In order to estimate these effects for each species separately, a linear additive model of the form:

$$\chi_{ijk} = \mu + \alpha_i + \beta_j + \delta_k + \varepsilon_{ijk}$$

was used, where μ is a general mean, α_i and β_j are as above, β_k is the effect due to families within locations, and ε_{ijk} is a random effect (error).

The genetic portion of the phenotypic variability that can be attributed to each component in the additive linear models described earlier was estimated from the expected means of squares in the analysis of variance. Discriminant analysis (SAS 1988) was used to determine whether it is possible to separate the collection at the species or at a geographical level by linear combinations of the phenotypic measurements.

A hierarchical cluster analysis was run on the means of the seven variables using the CLUSTER program of SAS (1988). The distances between clusters are reported in a dendrogram as standardized Euclidean distances.

A principal components analysis was performed on the standardized data matrix to study the interrelationships and adjustments in the seven morphometric traits in this collection.

Results and discussion

Classification of the whole collection by discriminant functions (SAS 1988) separated *Hordeum vulgare* totally from *Hordeum spontaneum* and revealed distinct geographical groupings in both species. The two discriminant functions used in Fig. 1 accounted for 85.3% of the total phenotypic variance. Flag leaf area was the single most important discriminating variable in function one. Plant height was the most important discriminating variable in function two. Although Fig. 1 shows that *Hordeum spontaneum* populations are biologically distinct from those of *Hordeum vulgare* there was considerable overlap among *Hordeum spontaneum* populations from Wadiabiad, Qatranah I, and Qatranah II, on the one hand, and among populations from Mafraq and Mansurah, on the other. The first three locations are close to each other and represent the wadi-bottom ecotypes. *Hordeum spontaneum* populations from Abubana, Rashadiah, and Fjaij were distinctly different and represented the high-elevation ecotype. *Hordeum vulgare* populations were more distant from each other and the only

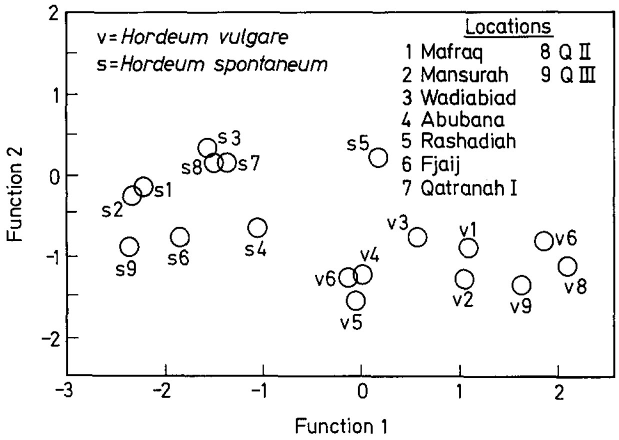

Fig. 1. Two-dimensional plot of the dispersion of *Hordeum* populations collected from Jordan, based on two functions derived through discriminant analysis (v = *Hordeum vulgare*, s = *Hordeum spontaneum*)

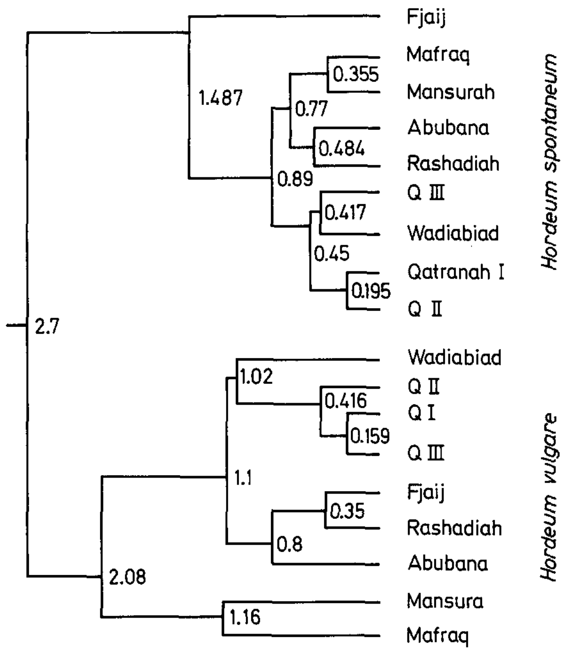

Fig. 2. Dendrogram of normalized Euclidean distances among populations of *Hordeum vulgare* and *Hordeum spontaneum* collected from Jordan

overlap was between those of locations Abubana and Fjaij.

The single linkage and Euclidean distance method of cluster analysis was utilized to quantify the distances between these populations. Taxonomic distances, expressed as normalized Euclidean distances, between populations from different locations are presented in Fig. 2. *Hordeum vulgare* populations were more distant from one another compared to populations of *Hordeum spontaneum*. The maximum distance at which populations of *Hordeum vulgare* clustered together was 2.08, while populations of *Hordeum spontaneum* clustered at a maximum distance of 1.49. The higher level of diversity in *Hordeum vulgare* populations is probably due to a lack of seed selection practices, combined with some degree of cross pollination. This must have sustained and further developed the diversity of these barley landraces. A similar conclusion was reported for wheat landraces (Poiarkova and Blum 1983).

Morphological variation in the seven traits was utilized to detect ecotypes in both barley species. Three clusters (ecotypes) each of *Hordeum vulgare* and *Hordeum spontaneum* were identified. Analysis of variance and summary of statistics for these ecotypes are presented in Tables 1–4. The three *Hordeum vulgare* clusters differed significantly from each other for all morphometric traits. The first cluster is characterized by less than average flag leaves, awns, plant height, and number of tillers. These are characteristics of the mountainous ecotype. Small leaves in this environment ensure slow growth until the danger of frost is passed (Evans and Dunstone 1970). Most members (80%) in this cluster came from the three mountain locations. The second cluster is similar to the first, except that it is characterized by average plant height and above average number of tillers. Sixty-five percent of its members came from the northern desert locations. In such a harsh environment, plants tend to produce as many tillers as possible to ensure a minimum number of seed for survival (Poiarkova and Blum 1983). This is in agreement with findings at a regional level where tillering capacity of the plants increased as the aridity increased (Brody 1983). The third cluster is characterized by large leaves, tall plants, and above average number of tillers. The majority of its members (95%) came from wadi-bottom locations, where plants rely on stored soil moisture. Large leaves are required for rapid grain filling in this environment as stored soil moisture is being rapidly depleted (Poiarkova and Blum 1983, Snow and Brody 1984). Members of this ecotype are expected to harbour genes or gene complexes for a higher water use efficiency.

The three *Hordeum spontaneum* clusters (ecotypes) differed significantly from each other for all traits except for flag leaf area. They resembled, in their morphological variation and collection sites, those of *Hordeum vulgare*. The first cluster is characterized by short plants and less than average number of tillers. The second is characterized by average plant height and average number of tillers, while the third is characterized by robust plants and above average number of tillers. These traits, which differ among and within populations of both species, are geographically structured and originated by natural, or man-made selection as adaptations to the environments in which they evolved. This localized variation is a char-

Table 1. Analysis of variance for three clusters (ecotypes) of *H. vulgare* from Jordan

Variable	Between clusters SS	*DF*	Within clusters SS	*DF*	F	Probability
Flag leaf area	45.116	2	139.702	23	3.714	0.040
Awn length	28.261	2	42.070	23	7.725	0.003
Flag leaf sheat	84.694	2	47.615	23	20.455	0.000
Peduncle length	210.150	2	192.476	23	12.556	0.000
Plant height	840.962	2	465.445	23	20.778	0.000
No. of tillers	271.031	2	106.534	23	29.257	0.000

Table 2. Summary statistics (minimum, mean, maximum, and standard deviation) for three clusters (ecotypes) of *H. vulgare* from Jordan

Cluster	Flag leaf area (cm^2)	Awn length (cm)	Flag leaf sheat (cm)	Peduncle length (cm)	Plant height (cm)	No. tillers
1 min.	5.88	11.80	5.2	18.20	45.41	7.3
mean	7.90	14.57	7.93	24.20	52.41	9.09
max.	13.14	16.60	10.20	28.20	58.20	11.70
s.d.	1.60	1.34	1.35	2.88	3.94	1.32
2 min.	3.80	12.80	4.30	20.60	57.00	17.60
mean	7.37	14.70	5.52	23.68	61.42	18.22
max	10.24	16.00	8.00	28.20	65.10	19.30
s.d.	2.41	1.20	1.47	2.79	3.67	0.70
3 min.	6.84	14.60	9.70	28.40	58.30	7.90
mean	10.73	16.94	11.03	30.49	65.00	12.24
max.	17.27	18.30	13.90	34.80	75.60	18.40
s.d.	3.34	1.15	1.29	2.30	5.06	3.34

Table 3. Analysis of variance for three clusters (ecotypes) of *H. spontaneum* from Jordan

Variable	Between clusters SS	*DF*	Within clusters SS	*DF*	F	Probability
Flag leaf area	7.693	2	103.550	23	0.855	0.438
Awn length	30.019	2	57.834	23	5.969	0.008
Flag leaf sheat	622.260	2	137.239	23	52.142	0.000
Peduncle length	1,670.250	2	318.094	23	60.384	0.000
Plant height	3,532.409	2	641.677	23	63.307	0.000
No. of tillers	41.153	2	96.211	23	4.919	0.017

Table 4. Summary statistics (minimum, mean, maximum and standard deviation) for three clusters (ecotypes) of *H. spontaneum* from Jordan

Cluster	Flag leaf area (cm^2)	Awn length (cm)	Flag leaf sheat (cm)	Peduncle length (cm)	Plant height (cm)	No. tillers
1 min.	1.35	8.50	8.10	23.60	48.30	3.5
mean	3.62	10.00	10.43	25.65	49.55	6.37
max.	6.58	11.30	12.00	27.00	52.00	10.70
s.d.	2.28	1.00	1.40	1.44	1.46	2.73
2 min.	1.00	9.20	12.80	29.00	57.10	3.80
mean	4.46	11.15	17.19	33.69	63.73	7.58
max	7.35	14.80	23.00	40.80	72.80	11.40
s.d.	2.15	1.58	2.63	3.52	4.64	1.85
3 min.	1.20	11.00	22.90	43.50	76.10	8.00
mean	3.25	13.16	25.43	48.93	84.54	10.00
max.	5.70	16.10	28.50	55.60	93.50	12.30
s.d.	1.38	1.52	1.91	4.22	6.66	1.48

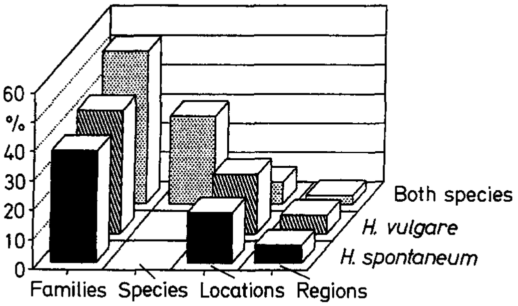

Fig. 3. Genetic portions of total phenotypic variability due to various levels of sampling hierarchy of *Hordeum vulgare* and *Hordeum spontaneum* collected from Jordan

acteristic of self-pollinated species (Allard and Kannenberg 1967).

The interrelationships between these morphometric traits are adjusted in different ways as revealed by the principal components analysis. Three principal components (Table 5) explained 80.91%–92.71% of the variability in these populations. Apart from the association, in one principal component, of plant height, peduncle length, and flag leaf sheath, the rest of the morphometric traits varied in the way they formed the rest of the principle components, and the amount of variation explained by the remaining principal components varied considerably. This reflects differences in magnitude of trait associations both among species and locations. Such a spatial (Damania and Jackson 1986) and temporal (Briggs and Shebiski 1971) variation in principal components is not unusual and reflects specific adaptation to, or interaction with, the environment.

It can be seen from Fig. 3 that differences between species at the same location account for a large portion (30%) of the total genetic variability. However, differences between families within populations accounted for 50% of the total genetic variation. The portion due to regions (3%) and locations (7%) was relatively low. Separate estimates for each species followed the same trend. The esitmates in Fig. 3 are averages for all seven morpho-

Table 5. Principle components, percent variance, and cumulative variance explained for seven morphometric traits as influenced by collection site

Region	Location	PC1	Variance (%)	PC2	Variance (%)	PC3	Variance (%)	Cum. variance
Northern desert	Mafraq	No. tillers Awn length	30.38	Flag leaf area	27.0	Plant height Peduncle length Flag leaf sheath	31.7	89.08
	Mansurah	Peduncle length Flag leaf sheat Plant height No. tillers	41.2	Flag leaf area	24.49	Awn length	19.76	85.45
Southern mountains	Abubana	Flag leaf area Flag leaf sheat Peduncle length	41.95	Plant height No. tillers	38.3	Awn length	12.25	92.5
	Rashadia	Peduncle length Plant height Flag leaf sheath	38.79	Flag leaf area	29.54	Awn length No. tillers	16.56	84.89
	Fjaij	Flag leaf area Awn length	39.5	Plant height No. tillers	17.75	Peduncle length Flag leaf sheath	29.6	86.85
Desert wadi-bottoms	Wadiabiad	Peduncle length Flag leaf sheath Plant height	37.98	Flag leaf size Awn length	33.04	No. tillers	16.9	87.92
	Qatrana I	Peduncle length Flag leaf sheath Plant height	54.3	Flag leaf area No. tillers	21.85	Awn length	14.55	90.7
	Q II	No. tillers Flag leaf area	34.17	Peduncle length Flag leaf sheath Plant height	40.68	Awn length	17.86	92.71
	Q III	Flag leaf area Awn length	28.0	Peduncle length Flag leaf sheath Plant height	30.58	No. tillers	22.33	80.91

Table 6. Mean squares for seven morphometric traits and level of significance for each source of variation in a collection of paired samples of *Hordeum* from Jordan

Trait	Regions	Locations	Species	Families	Error
Flag leaf length	6,153.2**	314.94^{ns}	166.07**	21.37**	3.72
Flag leaf width	12.57**	1.13^{ns}	0.62**	0.08**	0.02
Awn length	5,584.2**	311.24*	63.97*	22.29**	4.28
Flag leaf sheath	5,015.6**	1,384.84*	737.74*	319.55**	14.43
Peduncle length	5,210.9*	1,563.26**	309.39*	135.14**	7.61
Plant height	10,975.3*	2,195.30^{ns}	1,980.34*	963.07**	43.04
No. tillers	2,019.5**	226.65^{ns}	76.14^{ns}	39.14*	8.21

^{ns} not significant
* significant at the 5% level of probability
** significant at the 1% level of probability

metric traits. The averaging was justified on the bases of similarity in magnitude of values within each level of the sampling hierarchy (Table 6). However, in a study which involved *Hordeum spontaneum* only, Brown et al. (1978) estimated that 17% of the allozyme diversity in populations of the species was due to regions, 32% to populations within regions, and 51% to within populations.

Broad-sense heritability estimates in both species were calculated by a full-sib family method for all seven traits (Falconer 1981) and are presented in Table 7. This pattern of heritability is consistent with earlier studies (Freeman and Dowker 1973; Giles and Edwards 1983) in which low heritabilities were reported for traits closely associated with reproductive fitness. A noticeably high h^2 estimate in both species was found for awn length.

It was demonstrated that plant morphometric characters such as the ones used in this study can be used successfully to discriminate between species, populations within species, and ecotypes within populations of *Hordeum vulgare* and *Hordeum spontaneum*. A similar

Table 7. Broad-sense heritability (h^2) estimates for seven morphometric traits in paired samples of *Hordeum vulgare* and *Hordeum spontaneum* populations collected from Jordan

Trait	Broad-sense heritability estimate	
	Hordeum vulgare	*Hordeum spontaneum*
	$\bar{x}$ s.e	$\bar{x}$ s.e
Flag leaf length	0.11±0.15	0.13±0.17
Flag leaf width	0.14±0.10	0.19±0.10
Awn length	0.42±0.11	0.36±0.19
Flag leaf sheath	0.12±0.09	0.20±0.10
Peduncle length	0.19±0.12	0.17±0.14
Plant height	0.18±0.14	0.14±0.17
No. of tillers	0.29±0.15	0.20±0.17

conclusion was reported earlier for wheat (Porceddu and Scarascia Mugnoza, 1983). A substantial divergence between and within *Hordeum* species was detected by morphometric measurements. It is expected that allelic diversity for selection and breeding purposes, for these and probably other agronomically useful traits, can be increased by sampling from the different clusters (ecotypes) identified in this analysis.

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