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Variation in yield component, phenology and morphological traits among Moroccan bitter vetch landraces *Vicia ervilia* (L.) Wild.

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In this study, 19 ecotypes of *Vicia ervilia* (L.) Willd. sampled in the traditional agro ecosystems of the Rif mountains (Northwestern of Morocco) were investigated for their characteristics related to germination, phenology, morphology and yield. A large variation was determined among these traditional populations, especially among productive capacity traits as harvest index, which variation can reach up to 40%. The extent of phenotypic variation suggests an important genetic diversity. Indeed, despite a limited geographical scale, we have highlighted population differentiation linked to the production basin origin. Moreover, these populations are characterized by a short lifespan, the absence of dormancy together with precocity, which give to these landraces an interesting adaptive potentiality in the search for alternative crops tolerant to aridity and temperatures raises predicted by ongoing global changes.

Key words: *Vicia ervilia,* landraces, Rif, ecotypes, genetic resources, alternative crops.

INTRODUCTION

Vicia ervilia (L.) Willd. is an ancient legume whose domestication starting during the Neolithic and the Bronze Age, in the eastern Mediterranean, in Anatolia and northern Iraq (Zohary and Hopf, 2000). In the western Mediterranean its presence has been reported in various archaeological sites in the Iberian Peninsula and northern Morocco (Zapata et al., 2004; Pena-Chocarro et al., 2013). It is part of Mediterranean species that have been marginalized since the 16th and 17th century with the introduction of new crops from the New World (Hernandez-Bermejo and Gonzales, 1994). Currently, it is a minor crop grown around the Mediterranean basin and

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the Near East. It is distributed in southern of Europe, west and central Asia and Northern Africa (GRIN, 2008). In the Mediterranean region the overall socio-economic transformations and their impacts on agricultural production systems have been shown to result in real risk of genetic erosion of the diversity of several crops species, including *Vicia* (IBPGR, 1985).

V. ervilia world production is estimated around 800,000 tons per year with a mean yield of 1600 Kg/ha (FAO, 2013). The primary use of the bitter vetch grains is cattle feeding (Enneking, 1995; Francis et al., 1999), recent studies recommended it as protein and energy source (Abdullah et al., 2010). Indeed, seeds contain up to 22 to 28.5% of protein, 18,196 MJ / kg of gross energy and 12.967 MJ / kg of metabolic energy (Fernandez-Figares et al., 1993; Aletor et al., 1994; Yalcin and Önol, 1994; Farran et al., 2005; Sadeghi et al., 2009a). It can also be used as a fodder plant (Abd El-Moniem et al., 1988; Turk, 1999). Its use in human food is rare due to its toxicity (Sadeghi et al., 2009b) and it has only been reported during starvation periods (Enneking et al., 1995).

In Morocco, it is a minor crop in the northern part of the country where it is cultivated in the regions of Aarbaoua, Ouazzane, Pre-Rif, Rif, Fez and Taza (Foury, 1954), but its cultivation has declined for decades. Indeed, the cultivated areas were already stagnating in the 80s and 90s around 20,000 ha (Bounejmate, 1997) reaching nowadays 10,000 ha (FAO, 2013). The varieties used are local populations maintained by traditional farming practices within traditional agro-ecosystems of the Rif Mountain (Hmimsa and Ater, 2008; Ater and Hmimsa, 2008). This local selection resulted in ecotypes adapted to the local agro-climatic conditions (Foury, 1954; Francis et al., 1994; Enneking et al., 1995; Bounejmate, 1997) whose genetic resources assessment has nearly never been done (Francis et al., 1994; Van de Wouw et al., 2001) for limited *Vicia* studies.

In accordance with previous expectations (FAO, 2006; IPCC, 2007), the Mediterranean climate regions have experienced perceptible temperature rise in temperatures and periods of severe drought under climate change effect during the last decades (Bindi and Olesen, 2011; Supit *et al.,* 2010). Thereby, rainfed agriculture is now facing high risks and uncertainties (Trnka et al., 2011). Northern Africa is one of these endangered areas that might undergo a 20% rainfall decrease and an increase of annual mean temperature reaching 0.2°C per decade (Nefzaoui et al., 2012). Such environment require various strategies in operating systems and agricultural policies to cope with these threats. Among the productions of economical importance, forage production and animal feeding, are vulnerable strategic sectors to these changes (Hopkins, 2012). Within this frame the exploration of alternative crops has become a priority (López-I-Gelats and Bartolomé, 2012). Selecting forage species adapted to climate change requires the evaluation of plant genetic resources for species that display a high tolerance to drought and high temperatures. Among these candidate species, priority should be given to ancient locally grown species, supposedly more adapted to local conditions (Berger et al., 2002; Van de Wouw et al., 2001) rather than introducing new crops. Among the cultivated species that belong to the genus *Vicia*, *V. ervilia* is a very promising species with good adaptive abilities, especially tolerance to aridity, that can be cultivated with low rainfall level (Foury, 1954; Maxted, 1995; Abd El-Moneim and Saxena, 1997). It is a short life-cycle species; it can be used for both grain production or forage, and show good nutritional quality. Hence the goals of this study were to provide a first assessment of morphological diversity, phenology variations and components of productivity among local populations of *V. ervilia* in Morocco. This is a relevant contribution to the assessment of the productive potential and diversity of this species, in a goal of its valorization.

MATERIALS AND METHODS

Plant material

A survey was conducted in the main principal growing area of *V. ervilia* in Morocco, located in the Tingitane peninsula, in the western Rif (Figure 1). Seeds of 19 populations distributed between different regions were sampled directly from the field (Table 1). The seeds were sampled from individuals randomly chosen along a diagonal line crossing the fields. In parallel, surveys were conducted with farmers to determine the origin of the seeds sown and circuit of exchanges. On average 5 farmers are interviewed by locality.

Experimental device

For each population, 30 seeds were used for germination test. The seeds are disposed in petri dishes, imbibed with distilled water and placed in the dark. Germination time (GT), precocity (PR) and percentage of germination (PG) were noted. Plants used for phenotypical measures were grown in a greenhouse in pots with potting soil as a substrate. For each population, ten replicates were sown (10 pots) with one plant per pot. The device was then composed of 190 randomized pots. Twenty quantitative characters related to morphology, phenology and production were measured (Table 2). Harvest index (HI) was calculated by dividing seed yield (SY) by the total biomass (BY) multiplied by 100.

Used software

Descriptive statistics and analysis of variance (ANOVA) were performed by SPSS Statistics (17.0). The principal component analysis (ACP) was performed by XLSTAT (13.1). The location map of the surveyed population was carried out by using the DIVA-Gis software (5.2).

RESULTS AND DISCUSSION

Seed origin

87.5% of the farmers we could interview used their own local seeds from previous harvests. When using other

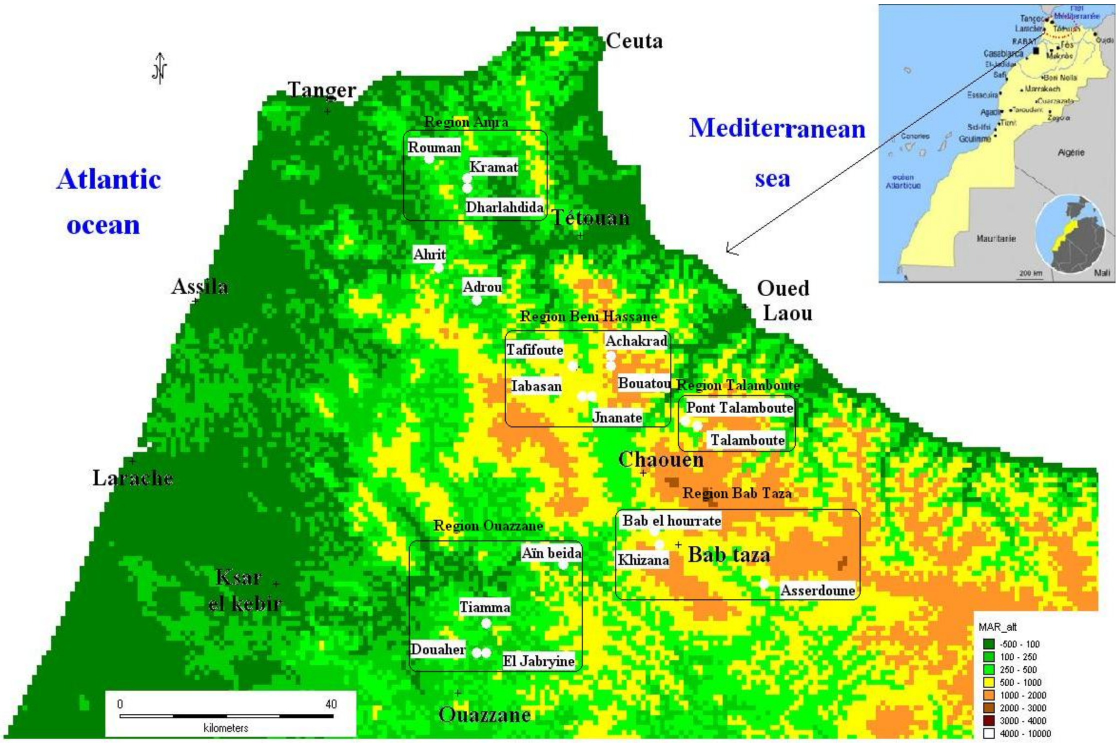

Figure 1. Location of the surveyed sites with background of altitude above sea level (edited by Diva-Gis).

Table 1. Localization of sampled populations

Region	Locality name	Longitude East	Latitude North	Altitude (m)
	Kramat	05'34,780	35'40,638	301
Anjra	Dharlahdida	05'34,307	35'39,893	373
	Rouman	05'38,098	35'32,080	175
Ahrit	Ahrit	05'33,511	35'38,990	224
Adrou	Adrou	05'33,236	35'25,308	566
	Achakrad	05'29,985	35'23,729	468
	Tafifoute	05'22,355	35'23,507	647
Beni Hassane	Bouatou	05'25,355	35'221,507	713
	Jnanate	05'23,301	35'17,876	720
	Iabasan	05'25,504	35'16,551	819
Talamboute	Talamboute	05'18,888	35'18,262	504
	Pont Talamboute	05'20,992	35'18,979	419
	Khizana	05'14,750	35'03,583	825
Bab Taza	Bab el hourrate	05'04,150	34'59,348	492
	Asserdoune	05'03,178	34'59,797	644
	Ain Beida	05'24,647	35'01,393	185
Ouazzane	Tiamma	05'32,011	34'55,654	136
	Douaher	05'33,317	34'52,120	228
	El Jabriyine	05'32,703	34'52,914	170

Table 2. List of the measured characters.

Character type	Character	Code
Germination	Germination time (Days)	GT
	Precocity (Days)	PR
	Percentage of germination (%)	PG
Morphology	Plant height (mm)	PLH
	Stem diameter (mm)	SD
	Length of the first internode (mm)	LFI
	Number of leaf per plant	NLPL
	Leaf Length (mm)	LL
	Leaf width (mm)	LW
	Pod Length (mm)	POL
	Pod width (mm)	POW
Phenology	First flowering date (Days)	FF
	Duration of flowering (Days)	F
	Days of pod maturity (Days)	FDPOM
	Maturity (Days)	M
Productivity	Total biomass (g/plant)	BY
	Seed yield (g/plant)	SY
	Harvest index (%)	HI
	Number of thallus per plant	NTPL
	Number of pods per plant	NPOPL
	Pod yield (g)	POY
	Number of seeds per pod	NSPO
	Hundred seeds weight (g)	HSW

seeds than their own, they either used seeds from other farmers in the same village (dchar) or bought some at the local "souk" (Weekly rural market). Therefore, the seeds used have a short geographical circuit. Indeed, the souk geographical range of attraction is limited (Troin, 1975) and usually which corresponds to the mountainous regions of catchment areas. We can then consider that the seeds are specific to each catchment area with a very limited circulation and exchange level in relation to materialized connections like major roads.

Seed quality

It was important to check the seed quality used by farmers. For this, we conducted germination tests. The results showed that the majority of seeds have an excellent germination capacity and are therefore of good quality. Indeed, average germination rate for all populations is about 96% with a level of variation being less than 7%. However, comparison of populations showed a significant differences among them. The lowest rate of germination was found in Asserdoune (73.33%) and Bab el hourrate (86.67%). At the opposite 9 populations showed a 100% germination rate and 8 populations ranged between 93.3 and 96.7%. With regard to the precocity (PR), the seeds of *V. ervilia* revealed precocious with rapid germination and lack of dormancy. The range of variation of precocity varied between 1 and 2 days (Table 3) with slight differences were observed between the different populations, with two (Kramat and Dharlahdida) showing a quicker germination, starting during the second day. The total time of the germination (GT) is short, varying between 2 and 3 days.

Phenology

The phenological stages observed focused on both the first flowering date and pod formation. From the agro-climatic point of view, they are interesting and major adaptive traits. Our results showed a low level of variation among populations, the results being very homogeneous and the analysis of variance not showing any significant effect (Table 4). On average it took 47 days for the appearance of the first flowers (FF) and maximum flowering (F) is reached between 48 and 50.36 days. In

Table 3. The main parameters of the seeding measure for 19 populations of *Vicia ervilia*.

Code	Mean ± SD	Min.	Max.	CV (%)
GT (Days)	2.26±0.45	2.00	3.00	19.99
PR (Days)	1.10±0.31	1.00	2.00	28.53
PG (%)	95.96±6.53	73.33	100.00	6.81

SD: Standard deviation, Min: Observed minimal value, Max : Observed maximal value, CV: Coefficient of variation.

Table 4. The parameters of the phenology measures for 19 populations of *Vicia ervilia*.

Code	Mean ± SD	Min.	Max.	CV (%)	F Pop.	F Reg.
FF (Days)	47.42±0.5	47.00	48.00	1.07		
F (Days)	48.89±0.71	48.00	50.36	1.45	1.52NS	2.41*
FDPOM (Days)	72.26±0.45	72.00	73.00	0.63		
M (Days)	73.25±0.28	72.71	73.70	0.40	1.49NS	2.79*

SD: Standard deviation, Min: Observed minimal value, Max: Observed maximal value, CV: Coefficient of variation, F Pop.: Report of variance of the pop effect, df (18). F Reg.: Report of variance of the region effect, df (6). NS : Not Significant, * Significant at p=0.095, ** Significant with p=0.090, *** Significant with p=0.099.

comparison with other studies, we can consider our populations as early flowering, and not spread out in populations of northern Morocco. Indeed, Larbi et al. (2011) measured an average of 93 days to detected the first flower and Mebarkia and Abelguerfi (2007) estimated it as 72.5 days. Regarding pod formation (FDPOM) and their maturities (M), the populations studied presented an early production with few variation among them (CV< 1%). Indeed, the first pods were observed after 72 days and the maximum of maturity was observed between 72.71 and 73.70 days. The values reported in other studies are much higher and show a later production date estimated to 141 days (Larbi et al., 2011) and 127.4 days (Mebarkia and Abelguerfi, 2007). The large difference between these values and those obtained in our study can be explained by the fact that we grow our plants in greenhouse contrary to these previous studies that have led experimental crops fields. Another important result is the low level of variation among populations, the differences observed in these parameters not exceeding 3 days. The only significant variations are among regions, while the study of Larbi et al. (2011) showed a 17 days difference among accessions to reach flowering and 40 days to pod maturity, Saxena et al. (1993) also revealing differences (14 days to flowering and 18 days to pod maturity). The difference in the level of variability is most certainly explained by the range of the genetic basis of the populations included in the study. Indeed, the previous studies used accessions from the ICARDA collection that come from a broader geographical area than our study with its narrow geographical sampling. However, the ecotypes of northern Morocco are interesting, since they are precocious and have a short cycle. This is an important character in the agro-climatic level in the Mediterranean context with erratic weather and tending towards aridity.

Morphology

To get an idea of the variability of shapes and sizes within and among populations, we studied traits related to plant (height, length of internodes, stem diameter), leaves (length, width) and pods (length, width) morphologies. The results obtained showed a variable level of variation depending on the characters (Table 5). For example, the coefficient of variation for pods characters is around 10%, without any significant variation either at the inter-population level or inter-regions. The average size of pods (18.9 mm of long to 4.7 mm of width) is similar to that observed by Berger et al. (2002). There is therefore no differentiation for this character. Conversely, width leaves showed a highly significant variation both at inter-pop and inter-regions levels. The height of the plants also showed also significant variations. The average height (PLH) measured is 525.83 mm with a variation between the populations of Bab el hourrate (457.5 mm) and those of Ain Beida (586.5 mm). This variation is larger than the 329 mm observed by Berger et al. (2002). The importance of these phenotypic variations predicts a significant genetic variation despite the geographical scale reduced, leaving open the possibility for varietal selection.

Production

The traits related to biomass production and grains are of great agronomical interest. The importance of the

Table 5. The parameters of the morphology and productivity measures for 19 populations of *Vicia ervilia*.

Code	Mean ± SD	Min.	Max.	CV (%)	F Pop.	F Reg.
PLH (mm)	525.83±31.62	457.50	586.50	6.01	2.51**	2.41*
SD (mm)	2.04±0.18	1.74	2.39	9.14	1.56NS	3.33**
LFI (mm)	33.71±7.33	22.25	58.81	21.76	2.25**	1.43NS
NLPL	13.18±1.03	11.20	14.70	7.86	1.31NS	2.05NS
LL (mm)	106.19±23.44	91.52	200.57	22.08	1.32NS	2.92*
LW (mm)	18.9±2.2	15.54	22.48	11.65	2.99***	5.82 ***
POL (mm)	18.92±1.9	14.83	21.18	10.08	1.63NS	1.29 NS
POW (mm)	4.77±0.39	4.03	5.26	8.23	1.41NS	1.75NS
BY (g/plant)	4.52±0.33	3.84	5.23	7.33	1.81*	3.58**
SY (g/plant)	0.34±0.11	0.13	0.60	34.48	1.29NS	2.31*
HI (%)	6.16±2.46	2.50	10.62	39.95	1.41NS	2.711*
NTPL	2.51±0.19	2.10	2.80	7.87	1.17NS	0.88NS
NPOPL	4.81±1.3	2.10	6.70	27.06	1.24NS	1.55NS
POY (g)	0.07±0.02	0.03	0.13	31.03	1.64NS	2.76*
NSPO	2.92±0.29	2.37	3.50	10.10	1.35NS	1.76NS
HSW (g)	3.92±0.44	3.30	5.09	11.32	-	-

SD: Standard deviation, Min: Observed minimal value, Max: Observed maximal value, CV: Coefficient of variation, F Pop.: Report of variance of the pop effect, df (18). F Reg.: Report of variance of the region effect, df (6). NS: Not Significant, * Significant with p=0,095, ** Significant with p=0,090, *** Significant with p=0,099.

variation is dependent on the character studied (Table 5), ranging from 39.9% for the harvest index (HI) to only 7.33% for the biomass (BY). The biomass estimated by the weight of the plants (BY) is the only character showing a significant population effect. Conversely, at the among-region level, the differentiation seems to be more important and we observed significant variations for 4 characters: biomass (BY), seed production (SY), harvest index (HI) and the weight of the pod (POY). Differentiation between the different ecotypes appears to be distance-related and due (at least partially) to the isolation between crop areas. For the harvest index (HI), the values varied between 2.50% (Asserdoune) and 10.62% (Rouman) with a mean of 6.16% and a high coefficient of variation (39.95%). Compared to other available data, this estimation appears to be relatively high compared to those given by Berger et al. (2002) and Larbi et al. (2011) and less than that the one obtained by Saxena et al. (1993). This difference could be explained by the difference in experimental devices and the way to calculate the HI. Regarding the weight of the pod (POY), the mean value is 0.07 g with a CV of 31.03%. Although the population effect is not significant, the difference observed between some populations is important, for example between Bab el hourrate (0.03 g) and Achakrad (0.13 g).

Structuring variability

In order to explain the variance in the data using all measured parameters, we conducted a principal component analysis (PCA). This analysis was performed with a matrix encompassing the 19 surveyed populations of *V. ervilia* and 23 characters considered in the study. An important part of the information is correlated with the first two factorial axes containing 44.58% of the total variance (Table 6). Variance expressed by the axis 1 (31.70%) is mainly due to the strong correlation (Table 6) between traits related to production, such as size and weight of pods and seeds. Axis 2 (12.88%) is rather correlated with characters related to phenology and morphology. The population projection in the factorial plan (1, 2) (Figure 2) show a structuring gradient along axis 1 with a negative side isolating the 3 populations sampled in the region of Bab Taza. The remaining populations clustered in an opposite pole, forming a gradient along axis 2, the populations of Anjra (north) to that of Ouazzane (south) and an intermediate position with other populations (Beni Hassane and Talamboute). This gradient structured populations according to reproductive parameters, such as phenology, morphology and germination. This structure has a geographical meaning, corresponding to a differentiation related to both distance and physical isolation. Indeed, the region of Bab Taza belongs to an isolated area with relatively few connections with other regions, thus limiting possibility of exchanges. Other populations belong to more connected regions, where seeds trade would be more easily among them. The fact that it is a mountainous area with rugged terrain may explain the differentiation on such a small scale.

Conclusions

From a genetic resources management perspective view,

Table 6. Eigen values and contribution of characters to the variance of axis.

Parameter	F1	F2
Eigen values	7.292	2.962
Variance (%)	31.706	12.877
Cumulate variance (%)	31.706	44.583
Contribution of characters (%)		
BY	1.508	12.085
SY	9.237	0.175
HI	6.334	4.310
NTPL	1.532	2.963
NPOPL	4.229	0.030
POL	8.544	0.472
POW	9.977	0.172
POY	9.259	1.329
NSPO	3.082	2.749
HSW	6.083	0.254
FF	4.484	4.333
F	5.734	2.191
FDPOM	0.012	10.386
M	0.108	24.607
PLH	0.774	4.321
SD	1.019	16.083
LFI	2.328	1.103
NLPL	5.126	0.883
LL	1.147	0.472
LW	2.835	7.287
GT	6.980	0.856
PR	1.981	1.411
PG	7.688	1.527

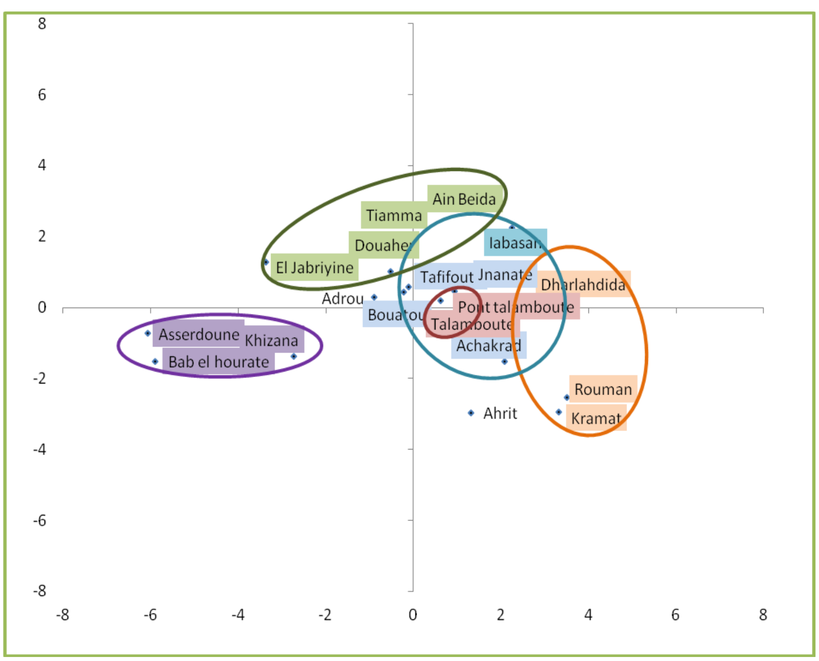

Figure 2. Projection on the plan (1.2) of the principal component analysis (CPA).

this study confirmed the existence of local populations of *V. ervilia,* maintained by farmers in a traditional agriculture frame. The evaluation of the diversity of these populations with a set of characters (germination, phenology, morphology and production) showed the existence of significant variability for several characters, with differentiation between regions representing the various production areas. At a phenotypic level, we showed a significant degree of variability which suggests the existence of significant genetic diversity within and among these populations. An approach using genetic markers will enable us to validate this hypothesis and measure the genetic diversity encompassed within our sampling. At the agronomic level, and in relation with the adaptive potential of these populations in regards to tolerance to drought and high temperature, the populations we studied showed interesting skills for selection. In particular, the lack of dormancy, the germination capacity, the precocity of flowering, pod maturity and a short life cycle are important characters to be selected in this context of global warming and climate change.

From a yield point, surveyed populations present an interesting potential both as a forage crop biomass and grain production. The size and weight of seeds is similar to those observed in other Mediterranean areas. The harvest index is relatively high and shows that these populations are more adapted for grain productions. Traits related to production show some variability and differentiating between regions. However, it would be advisable to check these capabilities by field trials.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Abbreviation: GRIN, Germplasm Resources Information Network; **IBPGR,** International Board for Plant Genetic Resources; **FAO,** Food and Agriculture Organization; **IPCC,** intergovernmental panel on climate change; **ICARDA,** International Centre for Agricultural Research in Dry Areas.

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