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Adaptation of One-Flowered Vetch (*Vicia articulata* Hornem.) to Mediterranean Rain Fed Conditions

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Abstract: One-flower vetch (*Vicia articulata*) was widely cultivated in the Mediterranean Basin in the past but is currently underutilized. Valuable germplasm collections are stored in genebanks, which are poorly characterized. In an attempt to explore adaptation of landraces we performed a multi-environment field testing, showing the availability of valuable resources for crop development, with average yield across environments ranging from 651 to 1102 kg/ha. Environmental factors and significant Genotype-by-Environment (G*E) interaction hampers selection of superior genotypes. Heritability-Adjusted Genotype plus Genotype-by-Environment interaction (HA-GGE) biplot performed here allowed to focus on the G and G*E interaction components relevant to cultivar evaluation. Landraces Va-38 and Va-85 were identified as the highest yielding landraces, being also the most stable over the environments. Two additional groups of landraces with relatively high yield were also identified but showing little stability across environments, with landraces Va-91 and Va-103 performing better in Córdoba, whereas Va-1, Va-2 and Va-66, did in Escacena. Córdoba appeared as a useful environment for selection, being representative, discriminant, and reproducible. Differences in precocity among landraces had little effect on yield in any of the studied environments. Temperature was the climatic factor most influential on yield as shown by Non-Metric Multidimensional Scaling (NMDS) analysis. High temperatures after flowering being the most limiting factor for yield. Increased radiation during and after flowering were beneficial, with rain having little effect.

Keywords: vetches; *Vicia*; yield; Genotype-by-Environment interaction

1. Introduction

The genus *Vicia* is a member of the legume tribe *Vicieae* along with *Lathyrus*, *Lens*, *Pisum* and *Vavilovia*. It comprises approximately 190 species [1] that as a whole are well placed to help meet the increased global demand for animal feed and to provide crops for a diversity of farming systems. The most widely cultivated species are *V. faba* L. (faba bean), *V. sativa* L. (common vetch) and *V. villosa* Roth (hairy vetch), but there are many other vetches that are cultivated as minor seed, forage or fodder crops, including *V. ervilia* Willd. (bitter vetch), *V. pannonica* Crantz. (Hungarian vetch), *V. narbonensis* L. (Narbon vetch) or *V. benghalensis* L. Among these, one-flower vetch (*Vicia articulata* Hornem., syn. *V. monanthos*) has been widely cultivated for fodder or green manure or for seeds for fattening feed for ruminants. It grows well in low fertile soils, is drought tolerant and frost resistant [2]. Seeds can also be eaten alone or mixed with lentils by humans. In fact, it is known by farmers to be more rustic and productive than lentil although a bit inferior in taste. It has a Mediterranean distribution and was widely cultivated in the past, but currently it is underutilized and threatened by extinction [2,3].

For instance, in Spain its cultivation has been reduced from 160,000 ha in 1960 to current virtual disappearance [4].

There is therefore an urgent need to characterize existing germplasm preserved in genebanks [5,6] and to submit them to breeding in order to exploit the potential of the species. In order to study the adaptation of the crop to Mediterranean rain fed farming systems, landraces selected from previous unpublished studies were submitted to multi-environmental testing. Genotype-by-Environment (G*E) interactions complicates the prediction of the performance of the accessions, thus hampering proper selection. Multi-environment trials are conducted yearly, and mean performance is often calculated as an average of cultivar performance over years and locations, but comparisons of mean performance are not sufficient for cultivar evaluation unless a due understanding of genotype by environment interactions is added. HA-GGE biplot (Heritability Adjusted Genotype plus Genotype-by-Environment Interaction) removes the statistical main effect of the environment and focuses on the genotype and genotype by environment interaction components relevant to cultivar evaluation [7].

The objectives of this research were to evaluate the performance and stability of yield among one-flowered vetch accessions.

2. Materials and Methods

2.1. Plant Material and Experimental Design

The one-flowered vetch population comprised 13 landraces selected from a previous field screening of a larger collection [5 and Rodríguez-Conde, unpublished] that were grown at nine contrasting location–year environments (Table 1). At each location, a randomized complete block design with three replications was used. The experimental unit consisted of three parallel one m long rows per accession separated 35 cm, with 10 plants per row. Sowing took place by middle December each season, according to local practice. Weeds were controlled by hand weeding. Days to flowering (DF) was estimated in three environments by weekly recording the date in which 50% of the plants of each plot had at least one fully opened flower. The harvest of the plants took place by late May to early June, depending on the environment.

2.2. Statistical Analysis

A combined ANOVA for randomized complete-block designs within each year-location environment was carried out using SAS® 9.3 (SAS Institute Inc.). F ratios, used to test effects for randomized complete block experiments combining location-year environments (Table 1), were determined according to [8]. Prior to each ANOVA, tests for normality and equality of variance were conducted for each dependent variable.

To determine stability and identify superior accessions across environments, the HA-GGE biplot analyses was conducted [9–13], comprising seasons 2009 to 2013, since it takes into consideration any heterogeneity among environments by giving weights to the test environments proportional to their root square heritability. As the environments have different heritabilities (data not shown) for the same trait, HA-GGE biplot is most appropriate for visual evaluation of the test environments and genotypes [9]. Analyses were made with the SAS® 9.3 (SAS Institute Inc.) program developed by [14] to graph GGE biplots.

The G*E two-way tables were first centered with the respective means for the environments, multiplied by $\sqrt{H}$ and then divided by the SD of the respective environment [9].

The general model for HA-GGE biplots is:

$$p_{ij} = \left(\overline{y}_{ij} - \mu_j\right)\sqrt{H_j}/s_j = \sum\nolimits_{k=1}^{t} \lambda_k \alpha_{ik} \gamma_{jk} + \varepsilon_{ij} \tag{1}$$

where p_{ij} represents the G*E two-way table of GGE effects with $i = 1, \ldots, m$ genotypes and $j = 1, \ldots, e$ environments, which is decomposed into $k = 1$ to t principal components (PC), with $t \leq \min(e, m - 1)$.

$\overline{y}_{ij}$ is the cell mean of genotype i in environment j; μj is the mean value in environment j. $\sqrt{H_j}$ is the square root of the heritability in the environment j and s_j is the standard deviation of the distribution of genotype means within environment j. λ_k is the Eigenvalue of the principal component analysis (PCA) axis k, α_{ik} and γ_{jk} are the genotype and environment principal component scores for axis k, t the number of principal components retained in the model and ε_{ij} is the error term.

The HA-GGE biplot shows the first two principal components (PC1 and PC2) derived from the previous two-way table of yield to singular value decomposition [15,16].

Singular value partitioning is achieved by providing a scaling factor f to obtain alternative accessions and environment scores. We chose the most straight forward variant called symmetric scaling (f = 0.5) since it bears most of the properties associated to other scaling methods [7].

The *target environment axis* abscissa (TEAa) is represented by a straight line drawn through the biplot origin and the average environment, which was defined by the mean ordinates of all environments in the biplot. The main effects of genotypes (G) are represented by the projection of genotypes onto this axis. These projections provide us with the contribution of each genotype to G, so genotypes may be ranked along the TEA abscissa, with the arrow pointing to higher mean yield. The TEA ordinate (TEAo) shows the contribution of each genotype to the interaction G*E, thus giving information about the genotypic stability or instability (consistency or inconsistency across environments). The best genotype would be that with the highest yield (higher positive projection on TEAa) and the highest stability, i.e., projection on TEAo close to 0 [16]. Similarly, the ideal environment would be the one showing a high projection value onto the TEA abscissa (more discriminating of principal effects of genotypes) and a small absolute projection value onto TEAo (more representative of all the tested environments) [16].

Climate data for each location [maximum, minimum and average temperature, maximum, minimum and average humidity, accumulated radiation, evapotranspiration and accumulated rain during pre-flowering, at flowering and post-flowering period, also during the growing season] were obtained from the Agencia Estatal de Meteorología (AEMET) (https://datosclima.es/Aemet2013/LocalizacionEstaciones.php). The influence of environmental factors on yield was studied by submitting each climatic parameter to a Non-Metric Multidimensional Scaling (NMDS) ordination [17].

Table 1. Description of the environments (combination of location and season) of the trials for the multi-environment study. Summary climatic data corresponding to each growing season are provided.

Environments	Location	Soil Type	Soil pH	Latitude	Longitude	Altitude (m.a.s.l)	Growing Season	Average T_{max} (°C)	Average T_{min} (°C)	Rain (mm)
CORD09	Córdoba Spain	Cambisol	8–8.5	37°50′ N	4°50′ W	90	2008–09	21.7	7.9	279
CORD10	Córdoba Spain	Cambisol	8–8.5	37°50′ N	4°50′ W	90	2009–10	21.3	9.4	626
CORD11	Córdoba Spain	Cambisol	8–8.5	37°50′ N	4°50′ W	90	2010–11	22.9	10.7	378
CORD12	Córdoba Spain	Cambisol	8–8.5	37°50′ N	4°50′ W	90	2011–12	22.5	7.6	145
CORD13	Córdoba Spain	Cambisol	8–8.5	37°50′ N	4°50′ W	90	2012–13	20.8	8.6	480
ESC11	Escacena Spain	Fluvisol	7 –7.5	37°25′ N	6°15′ W	88	2010–11	22.1	11.8	407
ESC12	Escacena Spain	Fluvisol	7–7.5	37°25′ N	6°15′ W	88	2011–12	21.9	10.1	124
ESC13	Escacena Spain	Fluvisol	7–7.5	37°25′ N	6°15′ W	88	2012–13	20.5	9.6	427
ESP3	Espiel, Spain	Fluvisol	7.5–8	38°11′ N	5°01′ W	548	2012–13	18.2	5.6	366

3. Results

The combined analysis of variance for grain yield and flowering date revealed that all main effects (environments (E), genotypes (G) and G*E interaction) were statistically significant (Tables 2 and 3). Environment explained for 78% of the total variation (G + E + GE interaction sum of squares) in yield, whereas G and G*E interaction accounted for 8% and 14%, respectively (Table 2). For flowering date, E, G and G*E interaction accounted for 24%, 58% and 18%, respectively (Table 3). When fitting the HA-GGE model, the first two PCs for yield and days to flowering explained 75% and 94%, respectively, of total G + GE interaction, and (G + GE)/(E + G + GE) yielded a value of 22% and 75% (Figures 1 and 2). This fulfilled the requirements of [18], who established that for a biplot to be useful, the first two PCs should be higher than 60% and (G + GE)/(E + G + GE) ratio should be higher than 10%.

Table 2. Analysis of variance for yield (kg/ha) of 13 genotypes of *V. articulata* in 9 environments (DF: degrees of freedom; SS: sum of squares; MS: mean square; G*E: term of the genotype-by-environment interaction).

Source	DF	SS	MS	% Respect (E + G + GE) SS
Environment (E)	8	72350709	9043838 ***	78
Replication	18	4149747	230541	
Genotype (G)	12	6983148	581928 ***	8
G*E	96	13141006	136885 ***	14
Error	216	14693165	68024	
Total	350	111317775		

*** Significant at $p < 0.0001$ level of probability.

Table 3. Analysis of variance for flowering date of 13 genotypes of *V. articulata* in 3 environments (DF: degrees of freedom; SS: sum of squares; MS: mean square; G*E: term of the genotype-by-environment interaction).

Source	DF	SS	MS	% Respect (E + G+ GE) SS
Environment (E)	2	1088	544 ***	24
Replication	6	67	11	
Genotype (G)	12	2560	213 ***	58
G*E	24	787	33 ***	18
Error	72	281	4	
Total	116	4783		

*** Significant at $p < 0.0001$ level of probability.

3.1. *Evaluation of Environments for Yield*

The genotypic variability in each environment is represented by the length of its vector (line connecting it with the origin). A short vector (environment close to the origin) would mean a non-discriminating environment in which all genotypes perform similarly. These environments were CORD09, ESC11 and ESP13. Conversely, CORD12 was the most discriminating based on their vector length, followed by ESC12, COR11 and COR13. In addition to exhibiting a high level of discrimination, an ideal test location should also be representative of the target growing region. The environments whose vectors form the smallest angles with TEAa (CORD12, followed by ESP13, COR09 and COR11) will be the most representative. Córdoba was therefore the most useful environment in which to select for yield, as characterized by the acute angles with TEA that were indicative of representativeness of the environment, and the long vectors that were indicative of its high level of discrimination capability. There were also acute angles between the vectors that corresponded to the different seasons, indicating that yields in this environment were very reproducible (Figure 1).

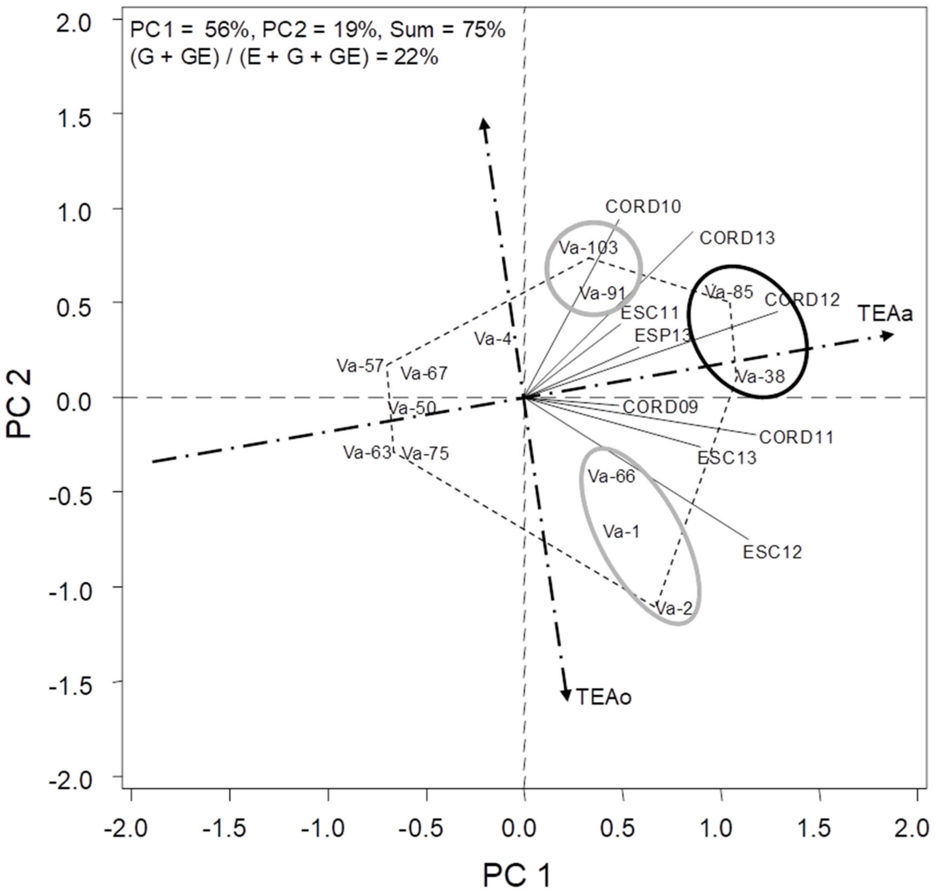

Figure 1. HA-GGE biplot based on yield (kg/ha) of 13 selected one-flowered vetch accessions in 9 environments (combination season-location).

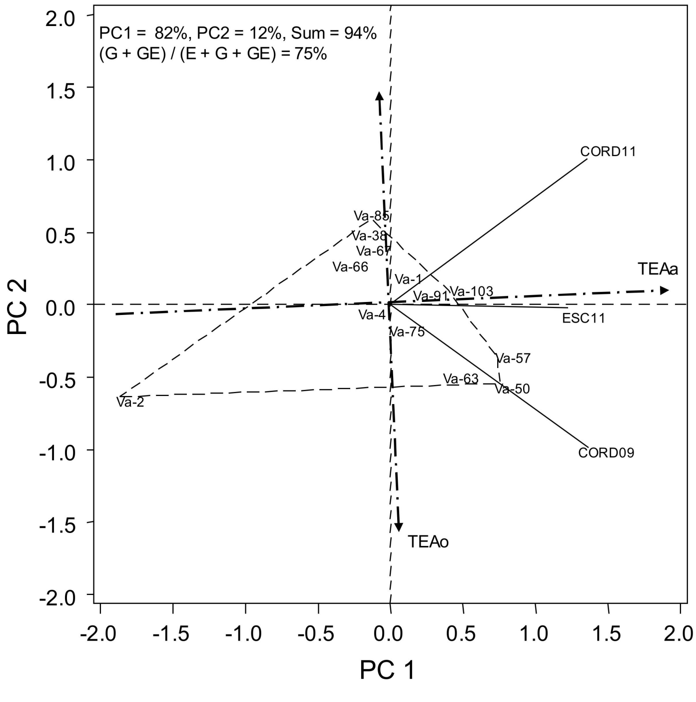

Figure 2. HA-GGE biplot based on flowering date of 13 selected one-flowered vetch accessions in 3 environments (combination season-location).

3.2. Average Performance and Stability of Genotypes for Yield

Average yield across environments of landraces ranged from 651 to 1102 kg/ha, with and average mean of 837 kg/ha (Table 4). In order to evaluate the accessions in terms of high yielding ability, the "TEA" view of the biplot was examined. In this way, the best characteristics of ideal genotypes should be high performance and stability. These characteristics may be inferred from the biplots since projection of an accession over the average environment axe indicates its mean performance across all environments, and its projection over the TEAo indicates its stability [16]. Figure 1 shows that Va-38 and Va-85 were the highest yielding landraces on average (high absolute primary scores, TEAa), that were also relatively stable over the environments (small absolute secondary scores, TEAo) [16]. In contrast, landraces Va-50, Va-57 and Va-63, yielded poorly at all environments. Landraces Va-91 and Va-103, and Va-1, Va-2 and Va-66, formed two groups with similar principal effects (similar yield), but of little stability across environments. Va-91 and Va-103 performed better in CORD10 and CORD13, whereas Va-1, Va-2 and Va-66, did in ESC12 and ESC13.

Table 4. Mean grain yield (kg/ha) of 13 one-flowered vetch landraces grown at 9 location–year environments.

Accession	**CORD 09**	**CORD 10**	**CORD 11**	**CORD 12**	**CORD 13**	**ESC 11**	**ESC 12**	**ESC 13**	**ESP 13**	**Mean**
Va-1	1082	695	553	687	368	705	1483	630	1783	887
Va-2	1370	913	798	529	140	747	1849	599	1938	982
Va-38	1269	854	845	798	586	961	1434	524	2650	1102
Va-4	849	916	463	617	325	893	562	497	1725	761
Va-50	831	982	280	267	205	691	513	374	1609	639
Va-57	807	1218	375	196	264	567	487	295	1702	657
Va-63	1290	692	302	280	255	598	487	310	1706	658
Va-66	940	851	680	602	381	829	967	666	1802	857
Va-67	940	1184	267	333	295	596	749	443	1701	723
Va-75	871	857	447	276	225	542	726	290	1624	651
Va-85	1158	1360	823	680	526	984	1266	531	1906	1026
Va-91	1118	980	547	678	551	962	998	379	2569	976
Va-103	1147	1769	422	717	297	949	793	537	1979	957
Mean	1052	1021	523	512	336	771	947	467	1900	837
SE	52.2	55.5	40.5	36.9	25.6	53.0	74.6	27.1	88.0	29.9

3.3. HA-GGE Biplot for Days to Flowering

The earliest landrace was Va-2 (Table 5) with the longest negative projection on TEAa (Figure 2). Va-50, Va-57, Va-63 and Va-103 were the latest ones. Concerning environments, Córdoba was the most discriminant as shown by the longest projections on TEAa, whereas ESC11 was more representative as shown by the smaller angle with TEAa. Pearson correlations between yield and flowering date with genotype as a weighting variable revealed a not significant and low and negatives r values (from −0.15 to −0.49) for the three environments which yield and flowering date were registered, which suggests a little association between both traits. This is in agreement with previous observations on different environments and accessions (Rodriguez-Conde, personal communication).

Table 5. Days to flowering of 13 one-flowered vetch genotypes grown at 3 location–year environments.

Accession	CORD09	CORD11	ESC11	Mean
Va-1	116.0	120.7	114.7	117.1
Va-2	102.7	102.7	103.7	103.0
Va-38	111.7	121.7	111.0	114.8
Va-4	119.7	121.7	109.7	117.0
Va-50	126.7	121.3	118.3	122.1
Va-57	126.0	122.7	117.3	122.0
Va-63	126.3	121.7	112.0	120.0
Va-66	111.0	118.7	111.0	113.6
Va-67	111.7	120.3	112.0	114.7
Va-75	119.3	120.7	112.0	117.3
Va-85	111.3	122.0	112.0	115.1
Va-91	119.3	122.0	113.0	118.1
Va-103	119.3	122.7	115.7	119.2
Mean	117.0	119.9	112.5	116.5
SE	1.132	0.837	0.716	0.594

3.4. NMDS Analysis

Biplot from NMDS analysis gave a stress value of 0.019, indicative of an excellent fit [19]. This allowed a nice separation of the environments (Figure 3) where the highest yields were achieved (CORD09, CORD10, ESC12 and ESP13), to the right, from those giving lower yields, to the left. Temperature arises as the most influential parameter on yield in these highest yielding environments, as shown by their vectors closer to x axis, to the left side. Coefficients of correlation among yield and climatic parameters revealed the same results, with negative effect of Tmin at flowering (R = −0.62), of Tmax at flowering (R = −0.68) and postflowering (R = −0.46). Radiation during and after flowering has a small positive influence in yield (R = 0.28). However, rain showed little influence (R = 0.01 at pre-flowering, R = −0.10 at flowering, R = −0.14 at postflowering).

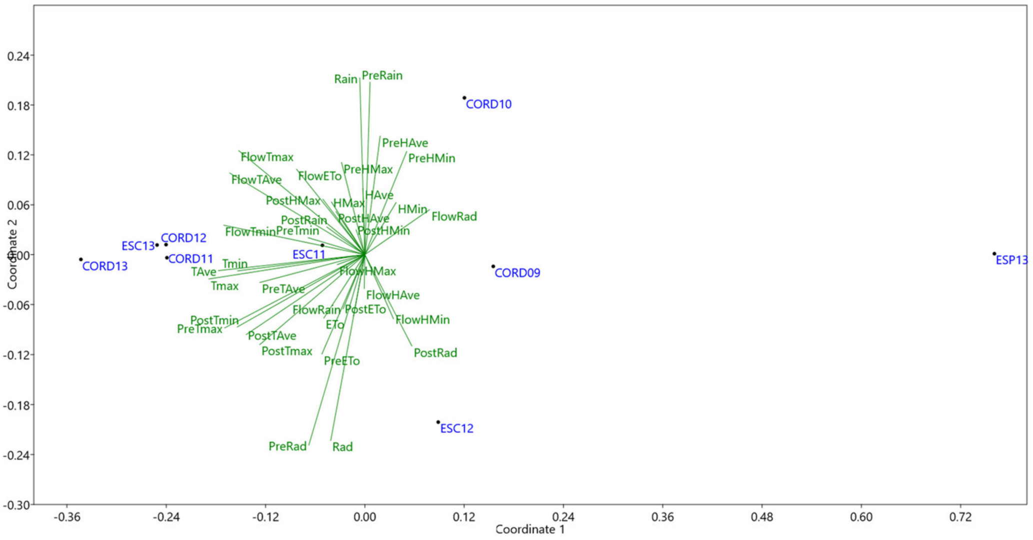

Figure 3. Non metric multidimensional scaling (NMDS) analysis of climate variables including: maximum temperature (Tmax), minimum temperature (Tmin), average temperature (TAve), maximum humidity (Hmax), minimum humidity (Hmin), average humidity (HAve), accumulate Radiation (Ra), Evapotranspiration (ETo) and rain during different growing stages [pre-flowering (Pre), flowering (Flow), post-flowering (Post) and complete growing season] characterizing the nine environments, which are the combination of three localities: Córdoba (CORD), Escacena (ESC), and Espiel (ESP) and 4 years 2009 (09) to 2013 (13), used for phenotyping.

4. Discussion

One-flowered vetch was widely cultivated in the past, being currently underutilized with little effort made on breeding and no cultivar registered. Current interest on revalorization of legume crops put one-flower vetch on focus [20]. In spite of this long term neglect on the crop, valuable landraces exist that might allow cultivation in some areas and can be the bases of breeding of superior cultivars. Previous studies [2,5] have shown a large genetic diversity in morphological and agronomic traits in the species. Germplasm preserved of the underutilized *V. articulata* appears as valuable resources for crop development, reinforcing the need to characterize the adaptability and yield potential of the crop. Our studies showed the potential of the crop in the region, with average yield across environments ranging from 651 to 1102 kg/ha. Multi-environmental field testing showed that environmental factors have a major impact on performance, with a significant G*E interaction, what hampers selection of superior genotypes. GGE biplot performed here allowed to remove the statistical main effect of the environment and to focus on the G and G*E interaction components relevant to cultivar evaluation. Significant genotypic effects showed landraces Va-38 and Va-85 as the highest yielding landraces, being also the most stable over the environments (small absolute secondary scores, TEAo). In contrast two additional groups relatively high yielding landraces showed little stability across environments, with landraces Va-91 and Va-103 performing better in Córdoba, whereas Va-1, Va-2 and Va-66, did in Escacena. Córdoba appeared as a useful environment to select for yield, being representative (acute angles with TEA), discriminant (long vectors), and reproducible (acute angles between the vectors corresponding to different seasons).

Significant differences in precocity were observed among landraces, but this had little effect on yield in any of the studied environment. This is in agreement with previous observations on different environments and accessions (Rodriguez-Conde, personal communication). Temperature was the climatic factor most influential on yield as shown by NMDS analysis (Figure 3). High temperatures after flowering being the most limiting factor for yield. Increased radiation during and after flowering were beneficial, with rain having little effect.

This study shows the HA-GGE biplot as an excellent tool for the visualization of the accessions by trait data and hence to identify the material with interesting traits. Classical breeding would likely yield excellent results in this species by simple selection directly from adapted landraces as a first, most immediate stage to make superior cultivars available to farmers in the short term. This can also serve as parents to start a crossing program for future.

In addition to yield and adaptation, quality is an important trait to consider in future. *V. articulata* straw is similar to other legumes, showing better nutritional quality than cereal straws, making them interesting sources of roughage for incorporation into ruminant diets [21]. *V. articulata* seeds are high in proteins with a balanced amino acid composition [22], however, as other vetches, they have L-canavanine, that reduces its value to feed non-ruminants. This is removed by postharvest processing techniques such as soaking, germination or alkaline heat, making seeds safe in diets. Also, levels of L-canavanine could be reduced by breeding, as variation in L-canavanine content exists in germplasm collections [6]. All this, reinforce the need to characterize existing germplasm and to submit it to breeding.

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