Identification and multi-environment validation of resistance against broomrapes (*Orobanche crenata* and *O. foetida*) in faba bean (*Vicia faba*)

D. Rubiales[1,*], F. Flores[2], A.A. Emeran[3], M. Kharrat[4], M. Amri[5], M.M. Rojas-Molina[6], J.C. Sillero[6]

[1] *Institute for Sustainable Agriculture, CSIC, Apdo. 4084, E-14080, Córdoba, Spain*

[2] *E.T.S.I. La Rábida., Univ. Huelva, 21819 Palos de la Frontera, Spain*

[3] *Faculty of Agriculture, Kafr El-Sheikh University, 33516-Kafr El-Sheikh, Egypt*

[4] *Carthage University, INRAT, Rue Hédi Karray, 2080 Ariana, Tunisia; present address: ICARDA, Rabat, Morocco*

[5] *Regional Field Crop Research Center of Beja, Tunisia*

[6] *IFAPA Centro Alameda del Obispo, Apdo. 3092, 14080 Córdoba, Spain*

**Correspondence*: Diego Rubiales
Institute for Sustainable Agriculture, CSIC, Apdo. 4084, 14080 Córdoba, Spain.
Tel: + 34 957499215; Fax: + 34 957499252; E-mail: diego.rubiales@ias.csic.es

ABSTRACT

Broomrapes are weedy root parasitic plants that severely constrain faba bean (*Vicia faba*) production. The most widely distributed species affecting faba bean is *Orobanche crenata*, although *O. foetida* is of local importance in Tunisia. After long and extensive breeding efforts made in several countries only moderately resistant cultivars are available to farmers. In an attempt to identify new sources of resistance a germplasm collection of 483 *V. faba* accessions was screened for resistance to *O. crenata* under field conditions in Córdoba, Spain. Stability of resistance of the 37 most resistant accessions was further tested in a multi-location experiment in Egypt, Tunisia and Spain over three field seasons. Resistance to *O. foetida* was also tested in Tunisia.

Although complete resistance was not found, and in spite of significant genotype x environment interaction revealing instability of the phenotypic expression across environments, this study allowed the identification of a number of accessions showing significant levels of resistance that was stable across environments. Cultivar Baraca and accessions V-1268, V-1302, V-1301, V-268, V-231, V-319 and V-1272 were the most resistant and stable across environments, and what is most interesting, being resistant both to *O. crenata* and *O. foetida*.

Key words: breeding, grain legume, parasitic weed, disease resistance

1. Introduction

Faba bean (*Vicia faba* L.) is a cool season legume crop valued as a source of protein in human diets, as fodder and a forage crop for animals, and for available nitrogen in the biosphere (Jensen et al., 2010; Rubiales, 2010). However, in spite of these advantages faba bean acreage has declined due to low and unstable yields as well as incidence of diseases (Stoddard et al., 2010). The major constraint for faba bean cultivation in the Mediterranean area and west Asia is broomrape infection (Pérez-de-Luque et al., 2010; Maalouf et al., 2011). Broomrapes are root parasitic weeds which are completely dependent on the host due to the lack of chlorophyll and functional roots. Several broomrape species can infect faba bean, crenate broomrape (*Orobanche crenata* Forsk.) being the most damaging and widespread (Parker, 2009; Rubiales and Fernández-Aparicio, 2012). Fetid broomrape (*O. foetida* Poir.) is of importance on faba bean only in Beja region of Tunisia (Kharrat et al., 1992) although it has recently also been found in Morocco infecting common vetch (*V. sativa* L.) (Rubiales et al., 2005).

Several measures are available for broomrape management, including cultural practices and chemical control (Rubiales et al., 2009). However they are not always fully effective or applicable in low input crops such as faba bean (Pérez-de-Luque et al., 2010). One of the most suitable control options is the development of resistant cultivars (Rubiales et al., 2006). However, resistance against broomrape in legumes is difficult to access, scarce, of complex nature and of low heritability, and these factors complicate resistance breeding (Cubero and Hernández, 1991; Román et al., 2002b; Rubiales et al., 2009; Gutiérrez et al., 2013). As a result, only cultivars with moderate levels of resistance to *O. crenata* or/and *O. foetida* are available to farmers (Kharrat et al., 2010; Pérez-de-Luque et al., 2010; Maalouf et al., 2011). The fact that at least two clearly distinct *Orobanche* species can infect faba bean has important implications in resistance

breeding, reinforcing the need to search for additional sources of resistance and to test their stability. Selection of genotypes with adequate broomrape resistance is also strongly affected by the genotype × environment interaction (GEI) (Maalouf et al., 2011). GEI results bring about discrepancies between expected and realized responses to selection due to an upward bias in the estimation of genetic variances, as shown by Haussmann et al. (2001) for *Striga*. This makes it difficult to predict the behaviour of the accessions in different situations reinforcing the need for multi-environmental testing of stability of disease resistance in faba bean. GGE biplot analysis has been previously proven useful to identify and characterize disease resistance and yield stability of breeding material in field trials (Villegas-Fernández et al., 2009, 2011; Fernández-Aparicio et al., 2012; Rubiales et al., 2012; Flores et al., 2012, 2013; Sánchez-Martín et al., 2014) taking advantage of the discrimination power versus representativeness view of the GGE biplot effective in evaluating test environments.

The objective of this work was to identify sources of broomrape resistance in faba bean germplasm and to test their stability against the two major broomrape species reported on faba bean (*O. crenata* and *O. foetida*). For this purpose, multi-year and multi-location field experiments were carried out.

2. Materials and methods

A faba bean germplasm collection consisting of 483 accessions maintained at IFAPA, Córdoba, Spain was screened for *O. crenata* resistance under field conditions at Córdoba in a preliminary screening during 2002-2003 cropping season (November-June). Susceptible (Brocal and Prothabon) and resistant (Baraca) cultivars (Pérez-de-Luque et al., 2010) were included as checks. Ten seeds of each accession were sown along one

linear meter–long row, rows being 70 cm apart without replication. Each test row was surrounded by 4 rows of the susceptible check faba bean cv. Brocal. Hand weeding of weeds other than broomrape was carried out when required, but no herbicides were applied to avoid interference with broomrape development. The number of emerged broomrapes per host plant was determined at crop maturity. Data were expressed relative to the broomrape infection of the adjacent rows of the susceptible faba bean cv. Brocal (Rubiales et al., 2006).

Thirty seven accessions were selected from the 2002-2003 trial on the basis of their resistance and seed availability and were subjected to a multi-environment screening over three consecutive seasons (2003-2004, 2004-2005 and 2005-2006) at 4 contrasting locations across the Mediterranean basin (Córdoba, Spain; Kafr El-Sheik, Egypt; and Loubna and Beja, Tunisia) (Table 1). Experimental fields in these locations were selected based on their known history of high and uniform infestation of *O. crenata* (Córdoba, Kafr El-Sheik and Loubna) or of *O. foetida* (Beja). In all these locations, due to their mild winter and hot spring, faba beans are autumn sown. Cultivars Prothabon and Brocal, and Baraca were included as susceptible and resistant checks, respectively. Ten seeds of each accession were sown along a one linear meter row with three repetitions. Each test row was surrounded by 4 rows of the susceptible check faba bean cv. Brocal as described above. At crop maturity, the final number of emerged broomrape plants per host plant was recorded. Data were expressed relative to the broomrape infection of the susceptible faba bean check cv. Brocal. The plots at Córdoba were covered with an insect proof mesh ensuring self-pollination for seed multiplication.

A combined analysis of variance was conducted to determine genotypic differences and genotype x environment interactions for infection by each broomrape species (*O. crenata* and *O. foetida*). Data were approximated to normal frequency

distribution by means of arcsin square root transformation. Environments were defined as the combination of “cropping season” and “location”; each site in a given year was a separate environment. Information for the tested environments is given in Table 1.

GEE biplot analysis show the factors (G and GE) that are important in genotype evaluation and that are also the sources of variation in GEI analysis of multi-environment trials data (Yan, 2001; Yan et al., 2000). Biplot analysis of Genotype x Environment interaction (GE) are particularly appropriate when using cultivars or breeding lines which after several cycles of selection may be reasonably considered as fixed (Yang et al., 2009). Heritability-Adjusted Genotype plus Genotype x Environment interaction (HA-GGE) biplot was used, since it takes into consideration the differences in heritabilities (H) (data not shown) between environments. HA-GGE is the most appropriate method for visual evaluation of the test environments and genotypes (Yan and Holland, 2010; Flores et al., 2013). Thus, HA-GGE biplot analysis based on percentage of broomrape infection on each accession relative to the susceptible check was conducted for graphical analysis of GE interaction and to identify accessions that could be valuable for faba bean breeding programs. The GE two-way tables were first centred with the respective environment means, multiplied by √H and then divided by the SD (standard deviation) of the respective environment (Yan and Holland, 2010). The HA-GGE biplot shows the first 2 principal components (PC1 and PC2) derived from the previous two-way table of each trait to singular value decomposition (Yan, 2001; Yan et al., 2000). Singular value partitioning is achieved by providing a scaling factor *f* to obtain alternative genotypes and environment scores. We chose the most straightforward variant called symmetric scaling ($f = 0.5$) since it bears most of the properties associated to other scaling methods (Yan, 2002).

Inspection of the angle formed by two environmental vectors (r) in an HA-GGE biplot visually conveys the genetic correlation between both environments, thus, an acute angle (close to 0°) implies a high positive genetic correlation (Yan and Holland, 2010). In a HA-GGE biplot the projection of the vector of an environment onto the Target Environment Axis abcissa (TEA_a) defined by Yan (2001) should approximate $r\sqrt{H}$ (Fig. 1 in Yan and Holland, 2010) which is an overall measure of the usefulness of an environment (Allen et al., 1978). On the other hand, the smaller the angle between years (r) within a test location, the more repeatable the test location, and, the smaller the angles between years within a test location and the TEA_a, the more representative the test location (Yan et al., 2011). Genotypes and environments were displayed in the same plot (see Fig. 1 in Flores et al., 2013 for more details). This HA-GGE biplot could identify broadly adapted genotypes that offer stable performance across all environments, as well as genotypes that perform well under specific environments. The relative infection average and stability of the genotypes were examined, thus the infection average of the genotypes is approximated by the projections of their markers onto the TEA abcissa and the stability is measured by their projection to the TEA ordinate. The greater the absolute length of the projection of a genotype, the less stable it is.

In order to characterize testing sites in terms of environmental factors, a correlation matrix-based PCA was performed on the environment x covariate two-way table (Table 1). This biplot of the first two PC axes were used to visualise the environmental factors characterising the different testing sites and help interpret the interaction patterns observed in the GGE analysis.

Data derived from biplots were tested statistically by non-parametric bootstrapping for constructing 95% confidence intervals on the basis of empirical distributions of estimated parameters. Because singular value decomposition needs to be

done on a balanced data set, we randomized (with replacement) only either columns or rows (but not both), keeping the other fixed (Yang et al., 2009). This resampling process was repeated 1000 times to provide accurate estimates of confidence intervals (Yang et al., 1996).

Analyses were performed using SAS® 9.3 (SAS Institute Inc.) program for graphing GGE biplots developed by Burgueño et al. (2003).

3. Results

Preliminary screening performed on the whole faba bean collection in 2002-2003 at Córdoba, Spain revealed a broad range of response to *O. crenata* among the tested material (Fig. 1). Infestation level was high and uniform across the plot, with averages of 9.3 and 9.7 emerged broomrapes per plant of the susceptible checks cv. Brocal and Prothabon, respectively. Resistant check cv. Baraca showed only 10.6% relative infection (0.8 broomapes/plant). Eighty six accessions showed moderately low levels of infection (< 50% of the check). A number of accessions with low infection died prematurely (V-238, V-592, V-1070 and V-1195). This was suspected to be due to high susceptibility, as confirmed by digging and observing the high number of non-emerged tubercles. Therefore, in order to avoid this confounding effect only 37 accessions with low infection, good plant appearance and good pod setting were selected to be further evaluated in different locations throughout 3 consecutive cropping seasons (Table 1). These results are presented in Tables 3 and 4, where relative infection values and ranking position of each accession are given.

The combined analysis of variance showed that relative infection was significantly affected by environments (E) and genotypes (G), which explained 13 and 34% of the treatment sum of squares for *O. crenata* and 2 and 62% for *O. foetida* (Table

2). Genotype x environment interaction (GE) significantly explained 53 and 36% of the total variation, respectively (Table 2). The presence of GE interaction complicates the selection process as GE interaction reduces the effect of genotypes by confounding their performance through minimizing the association between genotypic and phenotypic values. Performance and stability of genotypes were visualized graphically through HA-GGE biplot (Figs. 2 and 3). The partitioning of G + GE through HA-GGE biplot analysis showed that the first two principal components were significant factors for both traits that explained 70% to 85% of total G + GE sum of squares, suggesting that a biplot of PC1 and PC2 adequately approximates the environment-standarized data (Table 2). According to Yang et al. (2009) the first two PCs should account for approximately 60% of the (G + GE) variability and the combined (G + GE) effect should account for >10% of the (E + G + GE) variability before claiming the usefulness of biplots. These conditions were observed for both biplots, the lowest PC1 + PC2 sum was 70% for test environment for *O. crenata* (Fig. 2), and the (G + GE) / (E + G + GE) ratio was much higher than 10% (87% for *O. crenata* and 98% for *O. foetida*) for both biplots.

All locations were associated with each other and no independent groups of locations could be identified (Fig. 2 and 3). This suggests that the covered growing region represents a single mega-environment. This makes test environment and genotype evaluations meaningful and they should only be conducted within mega-environments (Yan et al., 2007).

HA-GGE biplot involving all the year-location combinations for *O. crenata* is shown is Figure 2 According to Allen et al. (1978) and Yan and Holland (2010) genetic correlation (r) and the square root heritability (√H) should be considered simultaneously for the assessment of the usefulness of a test environment. According to this, a test environment is not useful if its √H is very low or its genetic correlation with TEA is small

or negative (r wide or obtuse). Egyp04 had the longest vector, indicative of the highest √H, followed by Spain03, Spain04 and Egyp03. The projection of each test environment onto TEA (r√H) gives an estimation of its usefulness in selecting superior genotypes for the covered growing region. According to this criterion, Egyp03 and Spain03, Spain04 and Spain05 locations (Fig. 2) were the most useful locations for selecting superior genotypes. Therefore, Córdoba was the most repeatable location across years, as shown by the acute angle among years within a test location and the TEA_a (Yan et al., 2011). Córdoba can be regarded as highly representative for the covered growing region, and can be classified as Type I according to Yan et al. (2011), being considered the ideal test locations for use as a core test location for the region target. This type of test location is crucial for a breeding program particularly for early generation selection when amount of seed is limited and insufficient for multilocation trials. No significantly different mega-environments (ME) could be derived from the analysis as there was an overlap of the 95% confidence intervals between environments studied. PCA based on the environment x covariate two-way table (data not shown) accounted for 87% of the total variation. A simple visualization of biplot indicates a separation of the locations at Egypt, Spain and Tunis along the second PC axis by growing season minimum and average maximum temperatures. Therefore, this PCA biplot would suggest 3 mega-environments (PCA-ME) (Zhang et al., 2013). However, in this analysis we also used the non-parametric bootstrapping technique to construct confidence intervals (CI) on the basis of the empirical distribution of estimated parameters (Yang et al., 2009). The CIs of individual environments scores on either PC1 or PC2 axis all overlapped (not shown), suggesting the groups suggested by visual observations would not be statistically different. This trend was also observed in the HA-GGE biplot involving all the year-location

combinations (Fig. 2). Therefore, no significantly different mega-environments could be derived from both these analysis.

Figure 2 is also the most appropriate for genotype evaluation as well as for test environment evaluation. HA-GGE biplot for relative infection of *O. crenata* shows a six-sided polygon formed by the union of the identified genotypes that were further away from the biplot origin, so-called vertex genotypes (V-1068, V-932, V-997, V-975, V-377 and Baraca). The projection of the genotype vector onto the TEA axis abscissa (TEA_a), with an arrow pointing to a greater value based on their mean performance across all environments within target region. The double arrowed line (TEA_o) separates entries with below–average means from those with above-average means. According to this, the accessions more resistant to *O. crenata* in all the tested environments were those more distantly located on the left to TEA_o vector in Fig. 2, this is, Baraca, V-1268, V-319, V-1302, V-1196, V-252, V-1301, V-268, V-231, V-245, V1272, V-1271, V-1375, V-927, V-294, V-903, V-324 and V-930 (Table 4). For simplicity, we provide actual values of infection in each location only on these more resistant accessions (Table 3).

However, it is important to consider genotypic stability in addition to mean values of infection. The projection to the TEA_o vector, regardless of the direction, expresses the genotypes' contributions to GE, and thus estimates the genotypic stability (consistent rank across environment). The shorter the absolute length of the projection of a genotype on TEA_o, this is, the closer it is to TEA_a vector, the more stable it is. Therefore, among the more resistant accessions Baraca, V-1268, and V-319 were the most stable ones, followed by V-1302, V-1196, V-1301, and V-252 (Fig. 2). In fact, all these seven accessions displayed relative *O. crenata* infection averages < 80% across all environments tested. Indeed Baraca and V-1268 were significantly different from any other cultivar and also between them according to the bootstrap analysis. According to

the 95 % confidence intervals, no significant difference was recorded between the accessions V-319, V-1302, V-1196, V-1301, and V-252.

Figure 3 shows the HA-GGE biplot involving three years at Beja, Tunisia location selected because of its unique infestation by *O. foetida*. The long length (high √H) of the three vectors (TUN03FO, TUN04FO and TUN05FO) and the acute angles they make with the TEA vector (r√H) show that the three years were representative, indicating their usefulness in selecting for *O. foetida* resistance. The high angle between TUN04FO and the other two vectors is indicative of low repeatability. Beja location should therefore be considered of Type II according to Yan et al. (2011), being still useful, particularly at the multilocation test stage. These data were supported by the 95% confidence interval graphs that pointed out TUNO4FO as significantly different from the others.

Figure 3 also illustrates the graphic comparison of the relative performance and stability of accessions. According to this, accessions V-1268, Baraca, V-1302, V-1301, V-231, V-377, V-268, V-329, V-1068, V-319, V-1272, V-929, V-169, V-1008, V-252, V-511, V-1375, V-1271, V-1013, and V903 appeared as the best performing genotypes in terms of average *O. foetida* infection, in decreasing order as shown by their distance to the left of TEA_o vector. However, stability varied greatly, being low in V-329, V-1271, V-1272, and V-1375 as shown by their longer projection on TEA_o. Accessions farther distant to the left to TEA_o (indicative of lower infection) and closer to TEA_a (indicative of stability) such as V-1268, Baraca, V-1302, V-1301, V-231 and V-377 should be the top six preferred ones for their future utilization in *O. foetida* resistance faba bean breeding. The 95% confidence interval showed two significantly different groups, Baraca and V-1268, and V-1302, V-1301, V-231 and V-377, whose members did not differ within them.

Table 3 shows the actual values of relative broomrape infection of the accessions genotypes with below-average means by the projections of their markers onto the TEA of the HA-GGE biplots for each broomrape species. They are ranked in Table 4, where it is shown that most of the accessions resistant to *O. crenata*, showed also good resistance to *O. foetida* as shown by the fact that 70% of the genotypes with below-average means matched for both *Orobanche* species. Baraca, V-1268, V-1302, V-1301, V-268, V-231, V-319 and V-1272 are among the most resistant accessions to both *Orobanche* species. Accessions V-1196 and V-245 were exceptions and although they were among the more resistant ones to *O. crenata*, they were above-average mean for *O. foetida*.

4. Discussions

Resistance to broomrape is a major priority for faba bean breeding. In spite of the many efforts made by national and international programs, the levels of resistance available are low and of litle stability across environments (Pérez-de-Luque et al., 2010; Maalouf et al., 2011). Resistance against broomrape is a particularly difficult character to asses as it is highly influenced by environmental factors (Ter Borg et al., 1994; Pérez-de-Luque et al., 2004; Grenz et al., 2005; Rubiales et al., 2006; Fernández-Aparicio et al., 2009a; Maalouf et al., 2011). The complexity of broomrape evaluation and the polygenic nature of the trait turn breeding for resistance into a challenging process (Sillero et al., 2010). This has made selection more difficult and has slowed down the breeding process.

Identified defensive responses in faba bean against *Orobanche* seem to operate after attachment and penetration into the host tissues but prior to the development of the haustorium (Pérez-de-Luque et al., 2007), being based on callose depositions and lignification of host pericycle and endodermal cells. Low induction of seed germination

was considered to be rare in faba bean against both *O. crenata* and *O. foetida* (Ter Borg et al., 1994; Pérez-de-Luque et al., 2010) although resistant lines showing no-induction of broomrape seed germination have been recently described (Abbes et al., 2009; Fernández-Aparicio et al., 2012).

Both *O. crenata* and *O. foetida* populations are known to be very heterogeneous (Román et al., 2001, 2002a, 2007; Vaz Patto et al., 2008). Although variation among *O. crenata* populations in the ability to parasitize different faba bean accessions have been suggested (Radwan et al., 1988), no physiological races have been reported. This might be due to the lack of selection pressure by the host due to the lack of highly resistant cultivars. Therefore, it is feasible that new parasitic biotypes could be selected for virulence when challenged by the widespread use of highly resistant cultivars. In fact, a new race of *O. crenata* was suggested attacking resistant vetches (Joel, 2000). This putative race seems to have been selected by the frequent culture of the resistant vetch cultivar in the area. However, there are no further reports on its virulence or actual spread.

We found that the accessions most resistant to *O. crenata* tended to be also the most resistant to *O. foetida* (Table 4). This contrasts with the general view that resistance to *O. crenata* was independent from resistance to *O. foetida* (Cubero et al., 1991) although the strategy of selecting for *O. foetida* resistance within breeding material previously selected for *O. crenata* resistance at ICARDA has facilitated the selection of germplasm resistant to both species. Maalouf et al. (2011) also found that within the nurseries composed by faba bean breeding lines with promising resistance to *O. crenata* distributed by ICARDA, there were a number of lines with stable resistance across environments. Unfortunately, they analyzed stability only by environments and referred to *Orobanche* species, not providing detailed information on the ranking of responses to

O. crenata and *O. foetida*. However, the fact that Beja was one of the locations studied, suggests that the reported stability is also against *O. foetida* at least for some of the accessions. In our case, we also performed the multi-location nurseries with accessions previously selected for their response to *O. crenata* at Córdoba. Differing with Maalouf et al. (2011) we selected accessions within a broader germplasm collection not previously submitted to breeding.

Resistance to the two species could be the result of combination of independent resistances. However, there is increasing evidence that this might be due to selection of mechanisms of broad resistance, operative against both species at the same time. In this respect, the co-localization of QTLs for *O. crenata* and *O. foetida* resistances (Díaz-Ruiz et al., 2009; Gutiérrez et al., 2013) reinforces the existence of common mechanisms for resistance to both species. This adds to the findings of Fernández-Aparicio et al. (2012) that low induction of germination was not only operative against *O. crenata* and *O. foetida* but also to the more generalist *Phelipanche aegyptiaca* (Fernández-Aparicio et al., 2009b).

Further studies on the virulence of *Orobanche* populations and characterisation of the resistance mechanisms operative are required to generate new insights into the resistance. The development of *in vitro* protocols to study faba bean/broomrape interaction (Fernández-Aparicio et al., 2012) could improve the phenotypic evaluation by controlling environmental effects. This would allow the dissection of the resistance into specific mechanisms acting in different stages of the infection process (Fondevilla et al., 2010), increasing the accuracy of the assessment and the QTL detection.

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Table 1. Description of the environments (defined as a combination of location and season) of the trials for the multi-environments study. Climatic data (Max. T, Min. T: absolute maximum and minimum temperature, respectively; Av Tmax, Av Tmin: average maximum and minimum temperature; rain) are provided for the growing season.

Environment	Location	Season	Latitude	Longitude	Altitude	Max. T (ºC)	Min. T (ºC)	Av Tmax (ºC)	Av Tmin (ºC)	Rain (mm)
SPAIN02	Córdoba, Spain	2002/2003	37º51' N	4º47' W	117 m	38.5	-2.0	20.8	8.8	504
SPAIN03	Córdoba, Spain	2003/2004	37º51' N	4º47' W	117 m	35.2	-2.5	19.5	7.9	531
EGYP03	Kafr El-Sheik, Egypt	2003/2004	31º05' N	30º56' E	12 m	36.3	2.8	22.8	9.3	71
TUN03CR	Loubna, Tunisia	2003/2004	36°42' N	10°55' E	19 m	40.1	5.9	21.6	13.8	690
TUN03FO	Béja, Tunisia	2003/2004	36°44' N	9°13'E	150 m	32.6	-0.4	23.3	10.3	661
SPAIN04	Córdoba, Spain	2004/2005	37º51' N	4º47' W	117 m	38.0	-8.0	21.1	6.7	201
EGYP04	Kafr El-Sheik, Egypt	2004/2005	31º05' N	30º56' E	12 m	34.0	2.0	22.4	7.9	122
TUN04FO	Béja, Tunisia	2004/2005	36°44' N	9°13'E	150 m	37.2	-0.5	22.8	10.3	727
SPAIN04	Córdoba, Spain	2005/2006	37º51' N	4º47' W	117 m	38.5	-2.0	20.1	8.1	353
EGYP05	Kafr El-Sheik, Egypt	2005/2006	31º05' N	30º56' E	12 m	34.0	1.0	22.5	7.1	62
TUN05FO	Béja, Tunisia	2005/2006	36°44' N	9°13'E	150 m	39.7	-1.3	22.3	10.1	592

Table 2. Genotype (G), environment (E) and genotype by environment interaction (GE) terms for broomrape response trials, 2003 to 2005 years (arcsin square root transformation).

Dataset	Source of variation	df[a]	Mean Squares	Percentage respect (E+G+GE) sum of squares	% of PC1 + PC2[b]
O. crenata	E	6	0.99523***	13	48+22
	G	39	0.39452***	34	
	GE	234	0.10494**	53	
O. foetida	E	2	0.79628*	2	62+23
	G	39	2.25551***	62	
	GE	78	0.66198***	36	

[a] Degrees of freedom

[b] Proportions of the first two principal components derived from singular value decomposition of the environment-centered data.

*, **, *** Significant at the 0.05, 0.01, and 0.001 level of probability, respectively.

Table 3. Response to broomrapes of most resistant faba bean accesions (those with below-average means by the projections of their markers onto the Target Environment Axes of the HA-GGE biplots, Fig. 2 and Fig. 3). Relative values expressed as % of the susceptible control.

		O. crenata								*O. foetida*			
		Córdoba Spain 03	KESheik Egypt 03	Loubna Tunisia 03	Córdoba Spain 04	KESheik Egypt 04	Córdoba Spain 05	KESheik Egypt 05	Mean	Béja Tunisia 03	Béja Tunisia 04	Béja Tunisia 05	Mean
Line	Origin												
Cv. Baraca	Spain	22	10	28	5	4	5	22	12	29	15	34	26
V-1268	Pakistan	19	53	119	53	61	51	39	49	24	4	37	22
V-319	Hungary	41	75	115	25	81	63	34	54	79	95	86	87
V-1302	Sudan	59	62	94	47	100	48	69	60	46	22	80	49
V-1196[#]	Spain	47	68	78	77	104	78	47	62	164	85	114	121
V-1301	Sudan	55	61	96	56	107	60	99	67	66	28	70	55
V-231	Palestine	63	67	100	89	69	73	107	71	71	50	65	62
V-268	Ukranie	38	60	58	88	106	112	125	73	92	41	69	67
V-329	Latvia	35	73	81	102	142	110	52	74	76	104	29	70
V-252	Canada	49	49	95	86	136	113	62	74	70	105	114	96
V-245[#]	Canada	38	60	78	86	144	103	91	75	88	135	112	111
V-1271	Russia	12	46	93	78	166	84	128	76	92	121	60	91
V-1375	ICARDA	69	63	81	89	79	138	94	77	103	49	121	91
V-903	Spain	97	85	131	88	114	45	59	77	107	73	110	97
V-211[#]	Belgium	101	59	114	98	162	33	62	79	87	90	121	99
V-169	France	66	65	99	73	194	72	71	80	70	104	90	88
V-294	Bulgaria	94	66	76	87	88	123	109	80	122	74	90	95
V-1068	Afganistan	85	48	97	56	178	122	72	82	97	57	60	71
V-377	Syria	106	73	72	107	65	105	126	82	55	37	78	57
Mean		86	73	98	92	123	107	93	84	99	85	102	95
SD		48	26	34	42	70	66	58		52	52	64	

[#] Genotypes with below-average mean for *O. crenata* (Fig. 2) but with above-average mean for *O. foetida* (Fig. 3)

Table 4. Ranking genotypes with below-average means by the projections of their markers onto the Target Environment Axes of the HA-GGE biplots.

Tested genotypes for *O. crenata*	Tested genotypes for *O. foetida*
Baraca	V-1268
V-1268	Baraca
V-319	V-1302
V-1302	V-1301
V-1196[#]	V-231
V-252	V-377
V-1301	V-268
V-268	V-329
V-231	V-1068
V-245[#]	V-319
V-1272	V-1272
V-1271	V-929[&]
V-1375	V-169
V-927[#]	V-1008[&]
V-294	V-252
V-903	V-511[&]
V-324[#]	V-1375
V-930	V-1271
V-123[#]	V-1013[&]
V-329	V-903
V-1068	V-930
V-404[#]	V-294
V-377	V-932[&]
V-169	
V-211[#]	

[#] Genotypes with below-average mean in Fig. 2 but with above-average mean in Fig. 3
[&] Genotypes with below-average mean in Fig. 3 but with above-average mean in Fig. 2
Top ten genotypes are shaded

Figure 1. Frequency distribution for *O. crenata* relative infection of 483 faba bean accessions evaluated in the field at Córdoba, Spain during season 2002-2003. Susceptible check cv. Brocal infection was established at 100%

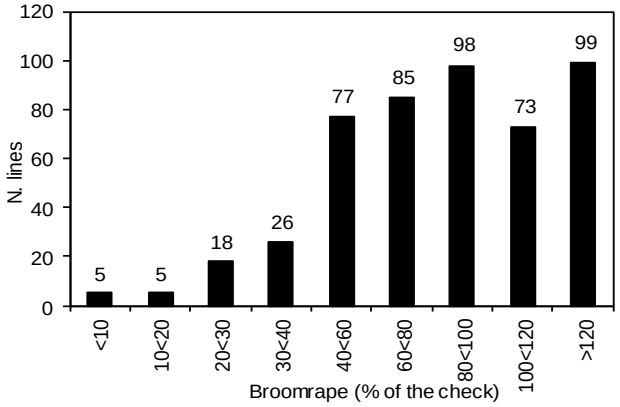

Figure 2. HA-GGE biplot based on *O. crenata* infection of 37 selected faba bean accessions together with a resistant (cv. Baraca) and two susceptible (cvs. Prothabon and Brocal) checks in 7 environments (combination season-location)

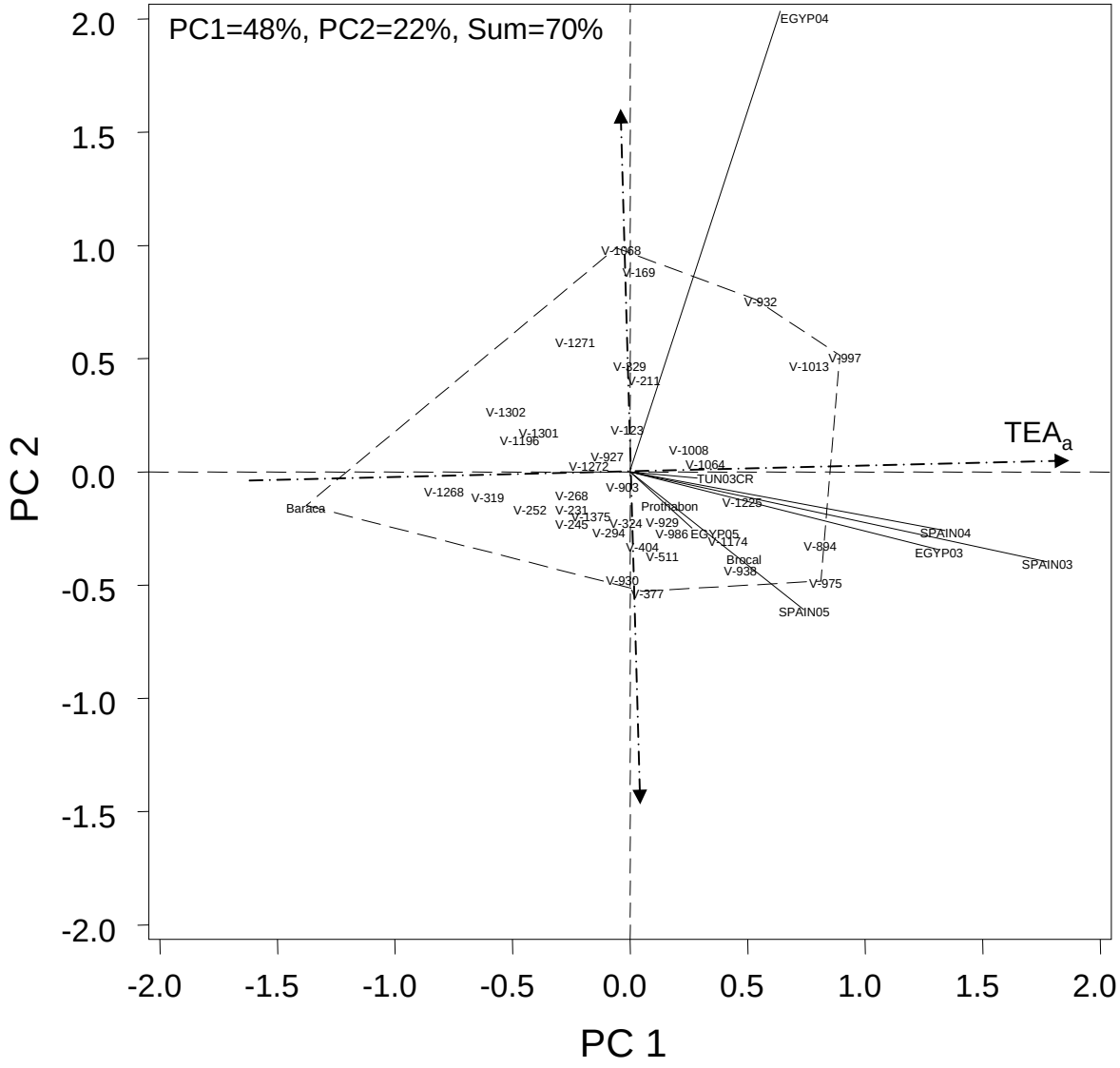

Figure 3. HA-GGE biplot based on *O. foetida* infection of 37 selected faba bean accessions together with a resistant (cv. Baraca) and two susceptible (cvs. Prothabon and Brocal) checks at Beja, Tunisia during three field seasons.