Identification and multi-environment validation of resistance to rust (Uromyces viciae-fabae) in Vicia faba

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Abstract

A germplasm collection of 484 accessions of *Vicia faba* was screened for resistance to rust (*Uromyces viciae-fabae*) under field conditions. Accessions varied in the levels of rust infection, although no complete resistance was identified. Stability of resistance of the thirty-nine most-resistant accessions was tested in a multi-location experiment in Austria, Egypt, Tunisia, United Kingdom and Spain over three additional field seasons. Genotype x environment interaction accounted for 43% of the sum of squares of the multi-environment evaluation, revealing instability of the phenotypic expression across environments. This might hamper the efficiency of selection suggesting the need for selection in different environments. Three possible mega-environments were discerned in the studied area, Mediterranean (Spain, Tunisia and Egypt), Oceanic (UK) and Continental (Austria). Córdoba (Spain) and Kafr El-Sheik (Egypt) showed as ideal environments for rust resistance screenings within Mediterranean environment. Several

accessions (300, 303, 311, 313, 720, 1196 and 1271) were grouped as moderately to highly resistant in the three defined mega-environments. These accessions showed clear differences both in terms of reduced disease severity and high stability, which make them good candidates for international faba bean breeding programs. Concerning each mega-environment, accessions 300 and 311 were the most resistant and stable ones across the Mediterranean one, followed by accessions 720, 1022, 1272, 1320 and BPL261. On the contrary other accessions (313, 452, 481 and 1196) were the most resistant in Oceanic and Continental environments. However, 452 and 481 were susceptible in the Mediterranean mega-environment. This contrasting performance across the environments was also supported by contradictory performance of the checks BPL261 and Baraca in Oceanic and Continental environments, suggesting differential virulence in rust populations, which deserves further attention.

Keywords: Disease resistance; faba bean; genotype x environment interaction; rust.

Introduction

Faba bean (*Vicia faba* L.) is a major food and feed legume crop (Rubiales, 2010; Flores *et al.* 2012). The importance of diseases as major constraint in faba bean production has become increasingly evident during the past decades (Stoddard *et al.* 2010). *Uromyces viciae-fabae* (Pers.) J. Schröt is the causal agent of faba bean rust. This pathogen is present in almost every area of the world where faba beans are grown. Serious damage to faba bean crop has often been reported in the past (Hiratsuka 1933; Rademacher 1934; Kispatic 1949; Mohamed *et al.* 1981; Lapwood *et al.* 1984), but are aggravated in

recent years especially in the Middle East, North Africa, Europe and China (Sillero *et al.* 2006).

Chemical treatments, such as fungicides and inducers of systemic resistance, have been reported along time to control faba bean rust (Yeoman *et al.* 1987; Murray and Walters 1992; Marcellos *et al.* 1995; Emeran *et al.* 2011; Sillero *et al.* 2012). The management of adequate cultural practices (Fernández-Aparicio *et al.* 2006) has also been suggested to reduce rust attacks on the crop. However, the use of genetic resistance is the most desirable, environment friendly and efficient strategy (Sillero *et al.* 2010). Several sources of resistance have been identified and subsequently used in breeding programs although none resulting in complete resistance (Sillero *et al.* 2010). Information on the genetic basis of the resistance is limited, and both polygenic and major gene inheritances have been suggested (Rashid and Bernier 1986; Stoddard and Herath 2001; Avila *et al.* 2003; Torres *et al.* 2006; Adhikari *et al*. 2016). Moreover, the suggested existence of the physiologic specialization in *U. viciae-fabae* (Conner and Bernier 1982; Emeran *et al.* 2001; Rojas-Molina *et al.* 2006) might imply that the use of single resistance genes in cultivars would likely not result in long term rust control. Unfortunately, insufficient efforts have been made to discern the existence of races and their regional occurrence which deserves urgent monitoring. Stability and durability of resistance is one of the most important concerns for breeders (Rubiales *et al.* 2011), which reinforces the need to search and characterize additional sources of resistance.

An understanding of the causes of genotype (G) x environmental (E) interactions (GE) can help in identifying superior genotypes for recommendation to farmers. Usually, a large number of genotypes are tested over a number of sites and years, and it is often difficult to determine the pattern of genotypic performance across

environments. Numerous methods have been used in the search for better understanding the interactions. Although strategies may differ in overall appropriateness, different methods usually lead to the same or similar conclusions for a given dataset (Flores *et al.* 1998). Yan *et al.* (2000), using a sites regression model (SREG) combined Genotype (G) and Genotype-Environment (GE) interaction, proposed a GGE biplot, constructed from the first two principal components derived from singular value decomposition (SVD) of the environment-centered data. In the present study multi-year and multi-location field experiments were conducted to identify sources of rust resistance in faba bean germplasm and to test their stability across environments.

Materials and methods

Field experiments

A germplasm collection of faba bean was first screened for resistance to rust in a single location under field conditions. Selected accessions were further studied in nine field experiments performed during three field seasons, in five different countries.

Initial screening under field conditions

A collection of 484faba bean accessions, originated from all around the world and preserved at IFAPA genebank, was studied for rust resistance under field conditions at Córdoba, Spain. The commercial variety Baraca was used as susceptible check and distributed throughout the trial as disease spreader and as susceptible check. The susceptible line 176 and the partially resistant line BPL261 were also included as checks (Sillero *et al*. 2000). Sowing was performed at mid-November 2002. Each accession was sown in 1-m long row (10 plants per row) with a row spacing of 0.7 m, without replications. Plants were inoculated by mid-March, at flowering stage, by spraying with

an aqueous suspension of urediospores from a *U. viciae-fabae* bulk population collected at Córdoba the previous year in a naturally infected field. The urediospores were suspended in tap water ($1x10^5$ spores mL^{-1}), to which Tween-20 (0.03%, v:v) was added as a wetting agent, to reduce the surface tension of the uredospores and to obtain a homogeneous suspension. Plants were inoculated after sunset to benefit from the darkness and high relative humidity at night.

When rust development started, disease severity (DS) was assessed at two-week intervals by a visual estimation of the leaf area covered with rust pustules. These data were used to calculate the area under the disease progress curve (AUDPC). At plant maturity, infection type (IT) was recorded using the scale of Stakman *et al.* (1962), where IT 0 = no symptoms, IT ; = necrotic flecks, IT 1 = minute pustules barely sporulating, IT 2 = necrotic halo surrounding small pustules, IT 3 = chlorotic halo, and IT 4 = well-formed pustules with no associated chlorosis or necrosis.

Thirty-nine faba bean accessions with lower levels of susceptibility (DS ≤ 60% and AUDPC ≤ 1000) were selected for further field studies.

Multi-environment screening

The thirty nine previously selected accessions were tested in nine different environments, during three growing seasons. The field trials were set up at five locations: Córdoba, Spain (2003/2004, 2004/2005 and 2005/2006), Oued Meliz, Tunisia (2003/2004 and 2004/2005), Kafr El-Sheik, Egypt (2003/2004 and 2004/2005), Gleisdorf, Austria (2005) and Wolverhampton, United Kingdom (2005). Cultivar Baraca and line 176 were included as susceptible checks, and line BPL261 as partially resistant check. A randomised complete block design with three replications was employed. The sowing pattern was the same as described in the first field trial, being

performed during late autumn in the Mediterranean locations (Spain, Tunisia and Egypt) and during spring in the Oceanic (UK) and Continental (Austria) ones. In Córdoba, at mid-March inoculations were performed as described above; in the other locations no artificial inoculations were carried out, as high and uniform disease pressure were known from previous observations on the sites.

At the end of the growing season, at plant maturity stage, the final disease severity (DS), as a visual estimation of the leaf area covered with rust pustules, was recorded in all locations. For each location, a "standardised severity" was calculated by expressing each severity value as a percentage of the highest one in each location; this was made for purposes of graphical comparison of results between locations (Villegas-Fernández *et al.* 2009).

Statistical analysis

A combined analysis of variance was conducted to determine genotypic differences and genotype x environment interactions for disease severity (DS). Data were approximated to normal frequency distribution by means of arcsin square root transformation. Environment was defined as the combination of "season" and "location", and each site in a given year was used as a separate environment (information for the tested environments are given in Table 1). F-ratios used to test effects for randomized complete block experiments combining location-year environments were determined according to McIntosh (1983).

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 disease severity was conducted for graphical analysis of GE interaction and to identify accessions that could be valuable for faba bean breeding programs.

The genotype by environment 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 two principal components (PC1 and PC2) derived from the previous two-way table of each trait to singular value decomposition (SVD) (Yan 2001; Yan *et al.* 2000). The HA-GGE biplot analysis was defined in detail by Yan and Holland (2010) and Rubiales *et al.* (2014).

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 SVD 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).

Results

High susceptibility to rust was the most common response in the collection evaluated under field conditions in 2002/2003 seasons. Most of the accessions were highly

infected, with DS > 60% and AUDPC values > 1200 (Figure 1A, 1B). High IT values, indicating a compatible interaction, were observed on most of the accessions. Only 4 accessions (303, 311, 1196 and 1271) showed small pustules surrounded by necrotic areas (IT=2), what coincided with very low AUDPC, resembling the responses identified earlier (Sillero *et al.* 2000).

The thirty nine less susceptible accessions were further screened in multi-year and multi-location field experiments. After a HA-GGE, these year/locations were grouped in mega-environments, which are defined in the next paragraphs. A wide range of variation was obtained, as shown by average DS values across locations (Tables 2 and 3). In Table 2, groups of accessions were defined according to their average severity and their stability showed in Figure 4; in Table 3 the groups of accessions statistically different were defined with Tukey tests. Disease reaction of each entry was not always stable through all environments, as seen by its ranks in each mega-environment (Table 4) rather than its raw severity values, since disease level varied between locations. This is also confirmed when the frequency distribution for standardized disease severity of the selected accessions for each year and location are observed (Figure 2), where the different patterns showed the genotype x environment interaction. In these figures also the different positions of the check lines confirmed the indicated interaction. With these results, genotype x environment analysis was performed.

HA-GGE Biplot validation

The combined analysis of variance (ANOVA) showed that DS was significantly affected by environments (E) and genotypes (G), which explained respectively 30 and 27% of the treatment sum of squares (Table 5). Genotype x environment interaction (GE) significantly explained 43% of the total variation (Table 5). Disease reaction and

stability of genotypes were visualized graphically through HA-GGE biplot (Figure 3). The partitioning of G+GE through HA-GGE biplot analysis showed that the first two principal components were significant factors that explained 66% of total G+GE sum of squares, suggesting that a biplot of PC1 and PC2 adequately approximates the environment-standardized data (Table 5), and the (G + GE) / (E + G + GE) ratio was much higher than 10% (70%).

Mega-environment identification

To conduct test environment evaluation, it is essential to first conduct a mega-environment analysis, that is, to investigate whether the covered growing region can be divided into mega-environments, because test environment evaluation as well as genotype evaluation becomes meaningful only when conducted within mega-environments (Yan *et al.* 2007). The cosine of the angle between two environmental vectors provided an estimate of their correlation coefficient (Yan and Holland 2010), thus, Figure 3 shows a clear difference between the environments comprising the locations of Egypt, Tunisia and Spain and the rest of locations/environments (United Kingdom (UK) and Austria locations).

Judging from bootstrap confidence intervals for the two first PC's environment scores of the biplot, data showed no overlapping of the 95% confidence intervals between UK or Austria environments and those from Egypt, Tunisia and Spain (data not shown). Figure 3 reveals that the covered growing region can be divided into three significantly different mega-environments (ME), one comprising the locations of Córdoba (Spain), Oued Meliz (Tunisia) and Kafr El-Sheik (Egypt) (ME1) that we named Mediterranean, a second mega-environment comprising Wolverhampton (United Kingdom) (ME2) that could be named Oceanic, and another third comprising the

location of Gleisdorf (Austria) (ME3) that we named Continental. Similar mega-environments were earlier identified in winter and spring faba bean trials (Flores *et al.* 2012; 2013).

ME1: Mediterranean mega-environment

The combined ANOVA analysis for DS of 42 faba bean accessions (the 39 selected accessions and 3 checks lines) at 7 environments showed that DS was significantly affected by environment, which explained 38% of the total treatment (G + E + GE interactions) variation G and GE interaction were significant and accounted for 38 and 24%, respectively (Table 5).

HA-GGE biplot for ME1 was similarly constructed that for total environments (Figure 4). When fitting the HA-GGE model, the first two PCs explained 75% of GGE variation (Table 5) was higher than 60%; additionally, the (G + GE) / (E + G + GE) ratio was much higher than 10% (62%). These two conditions fulfilled suggest that a biplot of PC1 and PC2 adequately approximates the environment- standardized data (Yang *et al.* 2009).

One of the crucial factors for the success of a plant breeding program is to identify suitable breeding and testing locations. For a location to be suitable, it must be discriminating so that the genetic differences among genotypes can be easily observed, representative of the average environment so the selected genotypes at one place show the same effect in another place and repeatability so that selected genotypes show similar performance each year.

Córdoba and Kafr El-Sheik showed as the most useful location for selecting superior DS genotypes within the ME1 according to the biplot (Figure 4) with long projections over the TEA_a (Target Environment Axis abscissa), long vectors and small

angles (aprox. 30º to 45º or smaller) with the average axis indicating their usefulness for genotype discrimination and a high representativeness of the ME (Figure 4). The small angle between years in Kafr El-Sheik and Córdoba (no higher than 30º) locations reflected its repeatability, more than for Oued Meliz (angle higher than 30º), and therefore, Kafr El-Sheik and Córdoba could be considered as a Type I locations according Yan *et al.* (2011), ideal for selecting superior genotypes within ME1. Indeed, the 95% confidence interval graph showed no significant differences between these three locations.

An ideal genotype should have both low DS mean performance and high stability within a ME. These characteristic may be inferred from the projection of each line over the $TEA_{a,}$ which indicates the mean performance for a specific traits across all environments and over the TEA_o (Target Environment Axis ordinate), which indicates the stability, thus, the best genotype would be that with the lowest severity (higher negative projection on TEA_a) and the highest stability, i.e., projection on TEA_o close to 150 (Yan 1999).

A perpendicular to TEA_a leaving on its left those genotypes with a severity lower than 15.5% in all environments was drawn (Figure 4). Five different groups of genotypes can be distinguished, according to their average performance and their stability (Figure 4). Judging from bootstrap confidence intervals for the genotype scores of all the groups, data showed no overlapping of the 95% confidence intervals between groups. Groups 1, 2 and 4 are made up of those genotypes which show a proportional response across all environments, that is, the most stable ones, but, these groups show different principal effects. Thus, Group 1 has the lowest average severities (from 2.5 to 4.0%, Table 2), Group 2 has about 8% average severity and Group 4 has average severity range from 11.0 to 15.0%. Groups 3 and 5 comprise accessions with a moderate

negative interaction with Córdoba location, and a positive interaction with the rest of environments. Both these groups show similar principal effects what groups 2 and 4, respectively. Therefore, accessions 300 and 311 stand out for its consistent low severity across all environments.

ME2: Oceanic mega-environment

Data collected at Wolverhampton (United Kingdom, ME1) were subjected to analysis of variance (ANOVA) and the means were separated by the Tukey test at P ≤0.05 (Table 5). ANOVA (data not shown) reported significant differences (P<0.0001) between 42 faba bean accessions for DS. These results are presented in Tables 3 and 4, where severity values and ranking position for severity of each line are given. Five different groups of accessions were distinguished, according to their average performance. Several accessions with moderate level of severity could be interesting in breeding programs (Table 3), but group 5 was made up of the seventeen accessions showing the lowest average severities: lines from1067 to 330 in Table 3.

ME3: Continental mega-environment

Similarly data collected at Gleisdorf (Austria, ME3) were subjected to analysis of variance (ANOVA) and means separated by the Tukey test at P ≤0.05 (Table 5). ANOVA (data not shown) showed significant differences (P<0.0001) between 42 faba bean genotypes for DS. The severity values and ranking position for severity of each line are given in Tables 3 and 4, where, five different groups of genotypes were distinguished. Promising accessions with moderate DS are shown in Table 3, where the eleven genotypes which show the lowest average severities are included in Group 5: lines from 239 to 2N34 in Table 3.

Discussion

Several sources of resistance to *U. viciae-fabae* have been reported in germplasm, but little resistance is so far available in faba bean cultivars (Sillero *et al.* 2010 for a review). Also, the possible physiologic specialisation in *U. viciae-fabae* reinforced the need for strategies to prolong the durability. In the present study we report additional sources of resistance and their stability in a range of environments.

In the different years and locations, DS was significantly affected by environments (E) and genotypes (G) showing high GE interactions, therefore performance and stability of genotypes were determined through HA-GGE biplot. The presence of GE interaction complicates the selection process as GE interaction reduces the usefulness of genotypes by confounding their DS performance through minimizing the association between genotypic and phenotypic values (Comstock and Moll 1963).

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 fulfilled in the present study for the HA-GGE biplot, where the PC1 + PC2 sum was 66% (Figure 3), and the (G + GE) / (E + G + GE) ratio was much higher than 10% (70%), which confirmed the suitability of the methodology used. An ideal genotype should have both low DS mean performance as well as high stability within a mega-environment (ME). HA-GGE biplot allowed to discern the average performance (higher negative projection on TEA_a) and stability (projection on TEA_o). Thus, the best genotype would be that with the lowest severity (higher negative projection on TEA_a) and the highest stability, i.e., projection on TEA_o close to 0 (Yan 1999). In the HA-GGE biplots, the vector length of an environment will be proportional

to the square root of the heritability in the environment (√H) and therefore indicative of it discrimination power (Yan and Holland 2010; Yan *et al.* 2011). The cosine of the angle between an environment with TEA_a (average environment) or between two environments indicates the genetic correlation (r) between them and is an indicative of the representativeness and repeatability respectively and the projection of the vector onto the TEA_a should approximate r√H which is an overall measure of the usefulness of an environment (Allen *et al.* 1978; Flores *et al.* 2013). In the present work, three clearly distinct mega-environments were identified from HA-GGE biplot: the Mediterranean comprising Córdoba (Spain), Oued Meliz (Tunisia) and Kafr El-Sheik (Egypt) (ME1); the Oceanic, comprising the location of Wolverhampton (United Kingdom) (ME2); and the Continental, comprising the location of Gleisdorf (Austria) (ME3). Whereas in ME1 the crop was sown in autumn, in ME2 and ME3 it was sown in spring.

The environments that are both discriminating and representative are preferred for selecting widely adaptive genotypes (Yan and Tinker 2006). Our results showed that Córdoba and Kafr El-Sheik were the most useful locations for selecting resistant genotypes within the Mediterranean environment.

The susceptible check 176 displayed high average severity in the whole set, according to previous research (Sillero *et al.* 2000), but it was not the most susceptible one in all the environments in this study. Accessions 300 and 311 were the most resistant and stable lines across the Mediterranean environment, followed by accessions 720, 1022, 1272, 1320 and BPL261, being all them very stable in this environment. We identified two groups with highly resistant accessions (groups 5, Table 3) both in the Oceanic and the Continental environments, being accessions 313, 452, 481 and 1196 part of the most resistant group in both MEs. Only two of these accessions (313 and 251196) showed moderate resistance also in the Mediterranean environment. This

contrasting performance of such accessions is further supported by the opposite reactions of the checks BPL261 and Baraca in ME2 and ME3. Whereas the resistant check BPL261 showed moderate reaction in ME2 (Group 3, Table 3) it was highly susceptible in ME 3 (Group 1, Table 3). On the contrary, the susceptible check Baraca was included in one of the most resistant group in ME2 (Group 4, Table 3). These reactions suggested differential virulence in rust populations.

Four accessions with low infection type in the first field screening (303, 311, 1196 and 1271) were studied under controlled conditions (unpublished data). This resistance was associated with late-acting hypersensitive response and a small colony size, in spite of long latency period, similarly to previous reactions reported in faba bean (Sillero and Rubiales 2002). These accessions together with genotypes 300, 313 and 720 have been grouped as moderately to highly resistant in the three defined MEs (Table 4) and stand out for their response, both in terms of reduced disease severity and high stability, which make them good candidates for faba bean rust resistance. However, the less-stable resistant genotypes, this is those accessions which are resistant only in determined MEs, can be used in those areas where their resistance has been confirmed.

In conclusion, stability of resistance to faba bean rust is crucial for the success of a faba bean breeding program. The present work allowed the identification of new sources of resistance to *U. viciae-fabae* stable in different mega environments, being highly promising to be included in international faba bean breeding programs. The suggested existence of differences in virulence in rust populations reinforces the urgent need to standardise differential sets for race identification and to monitor evolution of virulence both in temporal and geographical terms.

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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, maximum temperature; Min T, minimum temperature) are provided for the whole growing season.

Environment	Location	Season	Latitude	Longitude	Altitude	Max. T (ºC) Absolute	Min. T (ºC) Absolute	Max. T (ºC) Average[1]	Min. T (ºC) Average[1]	Rain (mm)
CORD02	Córdoba, Spain	2002/2003	37º51' N	4º47' W	117 m	38,5	-2,0	20,8	8,8	504
CORD03	Córdoba, Spain	2002/2003	37º51' N	4º47' W	117 m	35,3	-2,5	19,5	8,0	484
CORD04	Córdoba, Spain	2004/2005	37º51' N	4º47' W	117 m	38,0	-8,0	21,1	6,7	201
TUN04	Oued Meliz, Tunisia	2004/2005	36°28' N	8°29' E	179 m	37,2	-0,5	22,8	10,3	650
EGYP04	Kafr El-Sheik, Egypt	2004/2005	31º05' N	30º56' E	12 m	34,0	2,0	22,4	7,9	122
CORD05	Córdoba, Spain	2005/2006	37º51' N	4º47' W	117 m	38,5	-2,0	20,1	8,1	353
TUN05	Oued Meliz, Tunisia	2005/2006	36°28' N	8°29' E	179 m	39,7	-1,3	22,3	10,1	339
EGYP05	Kafr El-Sheik, Egypt	2005/2006	31º05' N	30º56' E	12 m	34,0	1,0	22,5	7,1	62
AUST05	Gleisdorf, Austria	2005/2006	45º06' N	15º42' E	361 m	32,8	-4,1	N.A.	N.A.	426
UK05	Wolverhampton, UK	2005/2006	52°35' N	2°10' W	113 m	25,7	-2,4	N.A.	N.A.	125

5[1] N.A., data not available

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Table 2. Disease severity (DS, %) of 39 different accessions together with two susceptible (176 and cv. Baraca) and a partially resistant (BPL261) checks at the Mediterranean mega-environment (ME1): Córdoba, Spain, Kafr El-Sheik, Egypt and Oued Meliz, Tunisia (growing seasons 2003/04, 2004/05 and 2005/06)[1].

Accession	DS average ME2	Group[2]	Córdoba, Spain			Kafr El-Sheik, Egypt		Oued Meliz, Tunisia	
			COR03	COR04	COR05	EGIP04	EGIP05	TUN04	TUN05
311	2.5	1	8.3	1.0	0.7	5.0	1.7	0.7	0.0
300	3.9	1	5.0	2.3	0.7	10.0	2.3	0.0	6.7
720	8.3	2	8.3	6.7	11.7	11.7	18.3	1.7	0.0
1272	8.6	2	8.3	6.7	15.0	10.0	18.3	2.0	0.0
303	5.5	3	5.0	1.0	3.7	10.0	13.3	0.7	5.0
2N34	6.2	3	3.7	6.7	0.8	11.7	16.7	3.0	1.0
2N52	6.3	3	5.3	5.3	5.3	9.0	8.3	1.0	10.0
1273	7.5	3	6.7	6.7	5.0	10.0	23.3	1.0	0.0
313	9.3	3	3.7	8.3	10.0	8.3	26.7	3.3	5.0
315	10.8	3	15.0	4.0	2.3	26.7	20.0	2.3	5.0
1271	11.0	3	13.3	5.3	12.0	20.0	22.5	4.0	0.0
1022	11.1	4	20.0	20.0	11.7	10.0	10.0	1.0	5.0
1320	12.3	4	18.3	20.0	13.3	6.3	21.7	1.3	5.0
BPL261	13.9	4	8.3	11.7	36.7	10.0	18.7	2.0	10.0
312	14.4	5	6.7	5.0	23.3	7.3	51.7	1.7	5.0
1196	15.4	5	5.0	6.7	18.3	33.3	43.3	1.3	0.0
1164	17.1		15.0	23.3	46.7	8.3	13.3	1.3	11.7
257	17.3		25.0	26.7	26.7	10.0	21.7	6.0	5.0
1155	18.8		18.3	25.0	30.0	21.7	25.0	1.3	10.0
330	19.2		10.0	33.3	26.7	18.3	30.0	6.3	10.0
316	19.5		30.0	25.0	18.3	23.3	28.3	1.3	10.0
1265	19.6		20.0	33.3	33.3	31.7	18.3	0.7	0.0
1108	20.5		18.3	23.3	30.0	10.0	40.0	2.0	20.0
1292	20.6		20.0	18.3	46.7	16.7	41.7	0.7	0.0
1054	21.5		25.0	33.3	40.0	20.0	30.0	2.3	0.0
314	23.2		36.7	43.3	26.7	13.3	31.7	6.0	5.0
129	24.6		30.0	43.3	36.7	11.7	30.0	4.0	16.7
113	25.6		26.7	40.0	20.0	55.0	23.3	2.3	11.7
239	26.2		26.7	53.3	56.7	18.3	5.0	8.7	15.0
285	27.2		20.0	36.7	23.3	23.3	41.7	5.3	40.0
1063	27.5		23.3	40.0	46.7	26.7	31.7	7.3	16.7
1009	29.1		26.7	30.0	46.7	33.3	43.3	3.7	20.0
543	29.7		31.7	33.3	50.0	16.7	50.0	3.0	23.3
1067	32.0		16.7	46.7	46.7	51.7	48.3	7.0	6.7
320	33.6		30.0	33.3	36.7	58.3	55.0	6.7	15.0
481	35.0		33.3	53.3	46.7	50.0	36.7	6.7	18.3
Baraca	35.2		63.3	43.3	55.0	21.7	38.3	7.7	17.0
1220	35.3		36.7	36.7	38.3	71.7	38.3	7.0	18.3
452	35.5		26.7	40.0	46.7	46.7	63.3	3.7	21.7
417	36.0		26.7	43.3	40.0	56.7	48.3	8.3	28.3
236	38.6		30.0	50.0	50.0	55.0	35.0	11.7	38.3
176	39.0		56.7	50.0	50.0	46.7	53.3	5.0	11.7

[1] Environments defined in Table 1.

[2] Group of accessions defined in Figure 4, according to their average severity and their stability.

Table 3. Disease severity (DS, %) of 39 different accessions together with two susceptible (176 and cv. Baraca) and a partially resistant (BPL261) checks at the Oceanic mega-environment (ME2, Wolverhampton-United Kingdom) and the Continental mega environment (ME3, Gleisdorf (Austria)[1].

Accessions	DS ME2		Group[2]	Accessions	DS ME3		Group[2]
1155	58.3	a	1	176	52.0	a	1
257	50.0	ab	1	543	47.0	a	1
285	41.7	bc	2	312	46.7	ab	1
236	40.0	bcd	2	BPL261	44.3	ab	1
1009	40.0	bcd	2	236	44.0	ab	1
1273	40.0	bcd	2	316	44.0	ab	1
316	30.0	cde	3	1265	40.3	abc	2
1022	30.0	cde	3	1108	39.7	abc	2
1054	30.0	cde	3	Baraca	39.0	abcd	2
1108	30.0	cde	3	314	36.3	abcde	2
176	29.7	cde	3	113	30.7	bcdef	2
1292	29.7	cde	3	1067	30.7	bcdef	2
BPL261	29.3	cdef	3	330	27.3	cdefg	2
417	26.3	cdefg	3	1054	27.0	cdefg	2
312	23.3	defg	4	320	23.3	defg	3
1220	23.3	defg	4	1164	22.7	efg	3
1320	23.3	defg	4	129	22.3	efg	3
239	21.7	efg	4	1063	22.3	efg	3
1063	20.0	efg	4	1292	22.3	efg	3
2N34	20.0	efg	4	1272	22.0	efg	3
2N52	20.0	efg	4	2N52	19.3	fg	3
720	16.7	efg	4	315	17.7	fg	3
300	16.7	efg	4	1155	15.0	fgh	4
113	11.7	fg	4	300	14.0	gh	4
Baraca	11.7	fg	4	303	13.0	gh	4
1067	11.0	g	5	311	13.0	gh	4
1164	11.0	g	5	720	13.0	gh	4
129	10.3	g	5	1273	13.0	gh	4
303	10.3	g	5	1271	12.7	gh	4
313	10.3	g	5	257	12.3	gh	4
311	10.0	g	5	1022	12.0	gh	4
315	10.0	g	5	239	0.0	h	5
452	10.0	g	5	285	0.0	h	5
481	10.0	g	5	313	0.0	h	5
1265	10.0	g	5	417	0.0	h	5
314	9.7	g	5	452	0.0	h	5
320	9.7	g	5	481	0.0	h	5
1196	9.7	g	5	1009	0.0	h	5
543	9.0	g	5	1196	0.0	h	5
1271	9.0	g	5	1220	0.0	h	5
1272	9.0	g	5	1320	0.0	h	5
330	8.7	g	5	2N34	0.0	h	5

[1] Environments defined in Table 1.

[2] Different letters indicate a statistically significant difference at $P < 0.05$ within the column (Tukey test), which allowed to identify different groups of accessions.

Table 4. Averages values and ranking position for disease severity (DS) of 39 different accessions and three check lines (BPL261, 176, cv. Baraca) at the Mediterranean (ME1), the Oceanic (ME2) and the Continental (ME3) mega-environments.

Accession	DS average ME1	Ranking ME1	DS ME2	Ranking ME2	DS ME3	Ranking ME3
113	25.6	28	11.7	18	30.7	31
129	24.6	27	10.3	13	22.3	24
176	39.0	42	29.7	31	52.0	42
236	38.6	41	40.0	37	44.0	37
239	26.2	29	21.7	25	0.0	1
257	17.3	18	50.0	41	12.3	13
285	27.2	30	41.7	40	0.0	1
300	3.9	2	16.7	20	14.0	19
303	5.5	5	10.3	13	13.0	15
311	2.5	1	10.0	8	13.0	15
312	14.4	15	23.3	26	46.7	40
313	9.3	9	10.3	13	0.0	1
314	23.2	26	9.7	5	36.3	33
315	10.8	10	10.0	8	17.7	21
316	19.5	21	30.0	33	44.0	37
320	33.6	35	9.7	5	23.3	28
330	19.2	20	8.7	1	27.3	30
417	36.0	40	26.3	29	0.0	1
452	35.5	39	10.0	8	0.0	1
481	35.0	36	10.0	8	0.0	1
543	29.7	33	9.0	2	47.0	41
720	8.3	3	16.7	20	13.0	15
1009	29.1	32	40.0	37	0.0	1
1022	11.1	12	30.0	33	12.0	12
1054	21.5	25	30.0	33	27.0	29
1063	27.5	31	20.0	22	22.3	24
1067	32.0	34	11.0	16	30.7	31
1108	20.5	23	30.0	33	39.7	35
1155	18.8	19	58.3	42	15.0	20
1164	17.1	17	11.0	16	22.7	27
1196	15.4	16	9.7	5	0.0	1
1220	35.3	38	23.3	26	0.0	1
1265	19.6	22	10.0	8	40.3	36
1271	11.7	11	9.0	2	12.7	14
1272	8.6	4	9.0	2	22.0	23
1273	7.5	8	40.0	37	13.0	15
1292	20.6	24	29.7	31	22.3	24
1320	12.3	13	23.3	26	0.0	1
2N34	6.2	6	20.0	22	0.0	1
2N52	6.3	7	20.0	22	19.3	22
Baraca	35.2	37	11.7	18	39.0	34
BPL261	13.9	14	29.3	30	44.3	39

5

Table 5. Genotype (G), environment (E) and genotype by environment interaction (GE) terms for DS faba bean performance trials, 2003 to 2005 years (arcsin square root transformation).

Data set	Source of variation	df[a]	Mean Squares	Percentage respect (E+G+GE) sum of squares	% of PC1 + PC2[b]
Total	E	8	2.2558***	30	51+15
	G	41	0.3958***	27	
	GE	328	0.0769***	43	
ME1	E	6	2.9544***	38	64+11
	G	41	0.4362***	38	
	GE	246	0.0471***	24	

5[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 0.001 level of probability.

10

FIGURES CAPTIONS:

Figure 1: Frequency distribution for disease severity (DS%, figure A) and for AUDPC values (figure B) of 484 accessions of *Vicia faba* evaluated under field conditions in Córdoba (Spain) during the season 2002/03. Positions of the partially resistant (BPL261) and susceptible (Baraca and 176) checks are indicated.

Figure 2: Frequency distribution for standardised disease severity of 39 faba bean accessions in the 9 environments (defined in Table 1) where they were evaluated. Positions of the checks BPL261, Baraca and 176 are indicated to favour comparisons.

Figure 3. HA-GGE biplot based on DS of 39 selected faba bean accessions together with two susceptible (176 and cv. Baraca) and a partially resistant (BPL261) checks in 9 environments (combination season-location).

Figure 4. HA-GGE biplot based on DS of 39 selected faba bean accessions together with two susceptible (176 and cv. Baraca) and a partially resistant (BPL261) checks in 7 environments (combination season-location) for Mediterranean Mega-environment (ME1).

Figure 1: Frequency distribution for disease severity (DS%, figure A) and for AUDPC values (figure B) of 484 accessions of *Vicia faba* evaluated under field conditions in Córdoba (Spain) during the season 2002/03. Positions of the partially resistant (BPL261) and susceptible (Baraca and 176) checks are indicated.

FIGURE 1.A

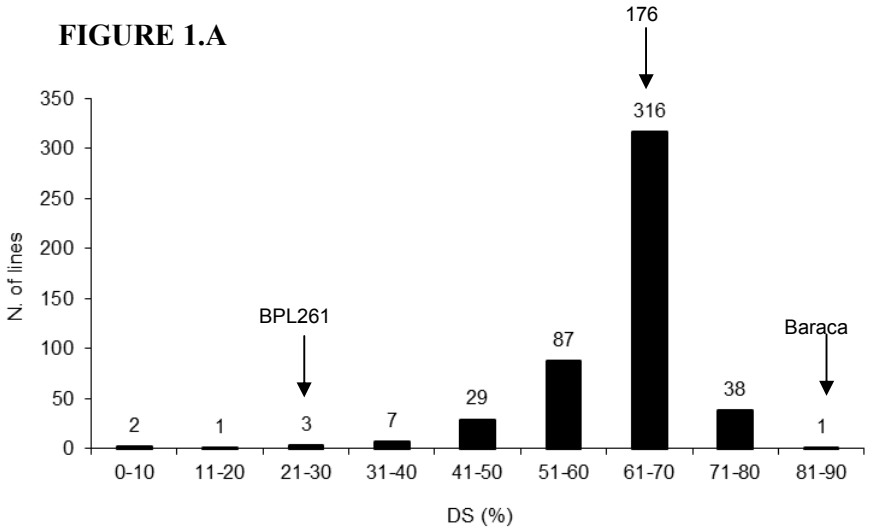


FIGURE 1.B

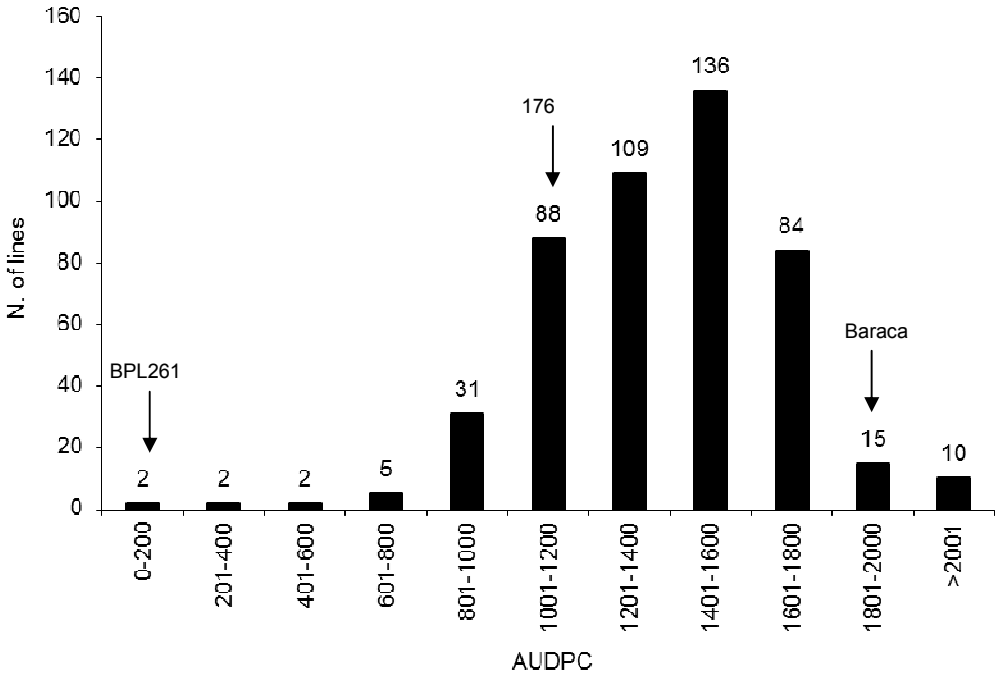
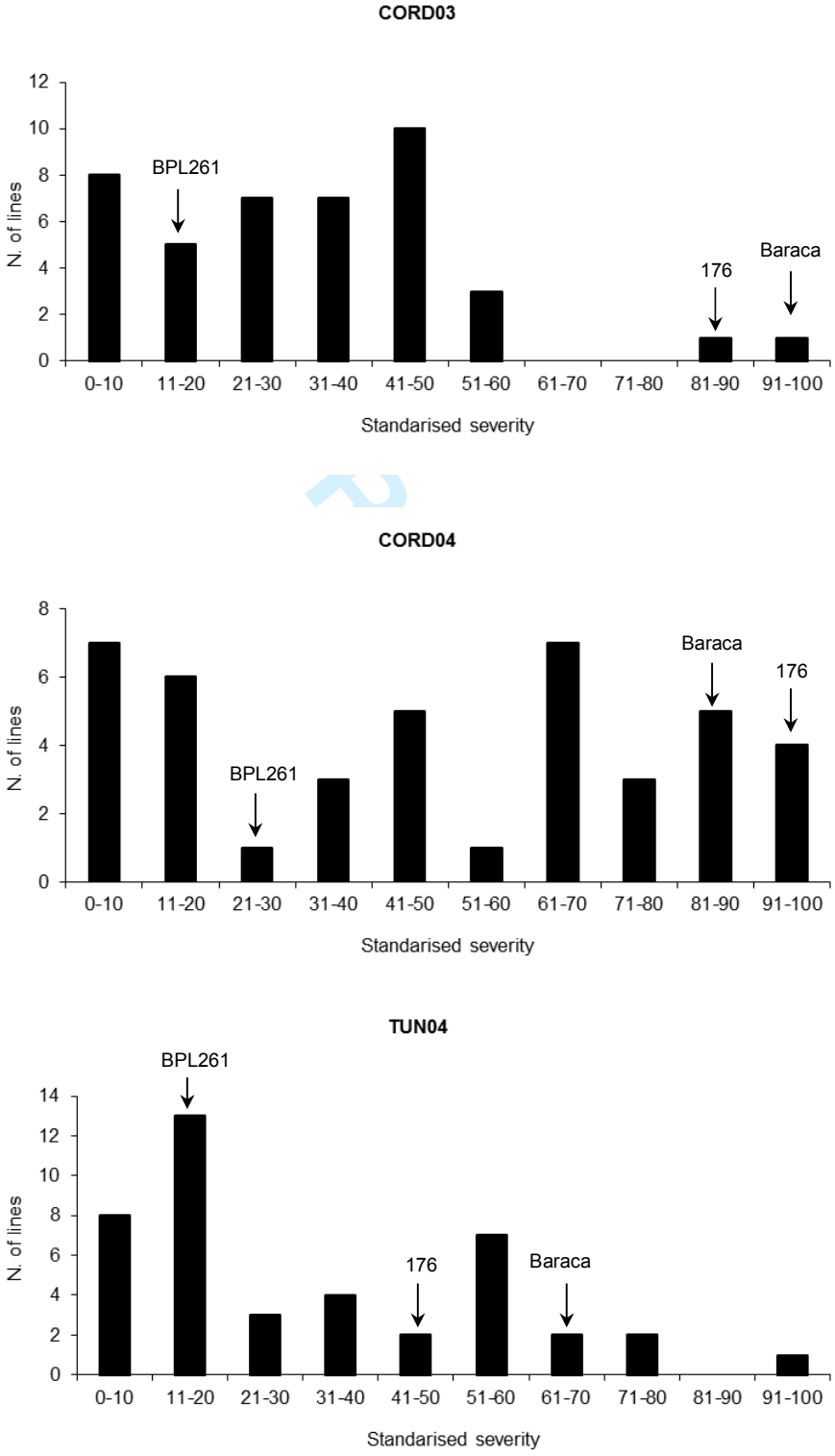
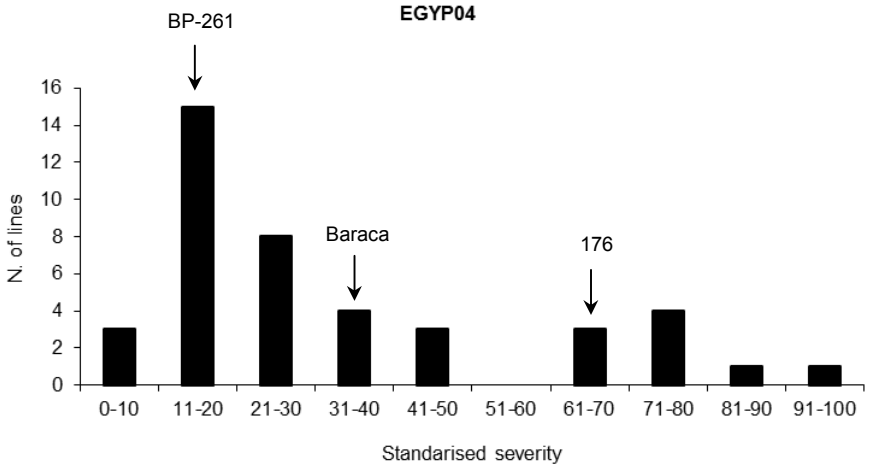
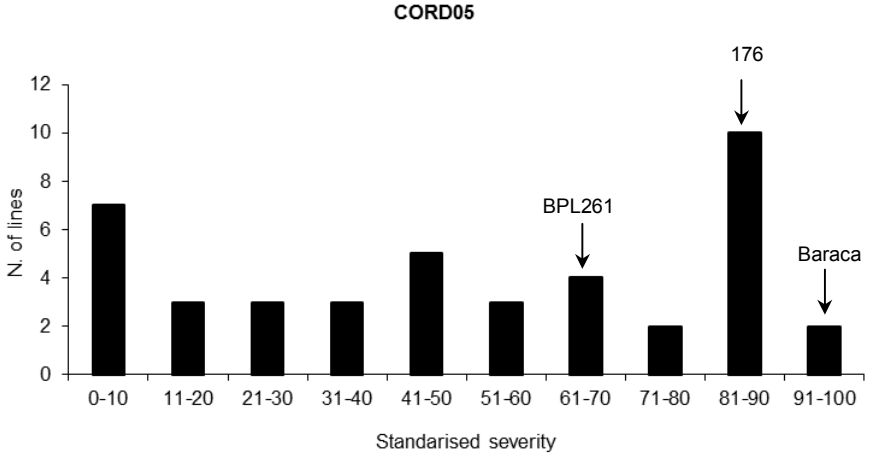


Figure 2: Frequency distribution for standardised disease severity of 39 faba bean accessions in the 9 environments (defined in Table 1) where they were evaluated. Positions of the checks BPL261, Baraca and 176 are indicated to favour comparisons.




EGYP04
BP-261
Baraca
176
N. of lines
0
2
4
6
8
10
12
14
16
0-10
11-20
21-30
31-40
41-50
51-60
61-70
71-80
81-90
91-100
Standarised severity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176
BPL261
Baraca
N. of lines
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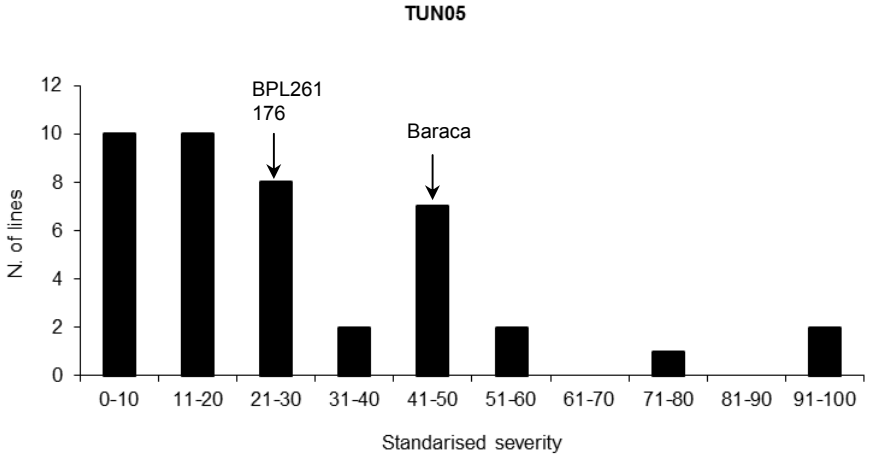
41-50
51-60
61-70
71-80
81-90
91-100
Standarised severity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BPL261
176
Baraca
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2
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41-50
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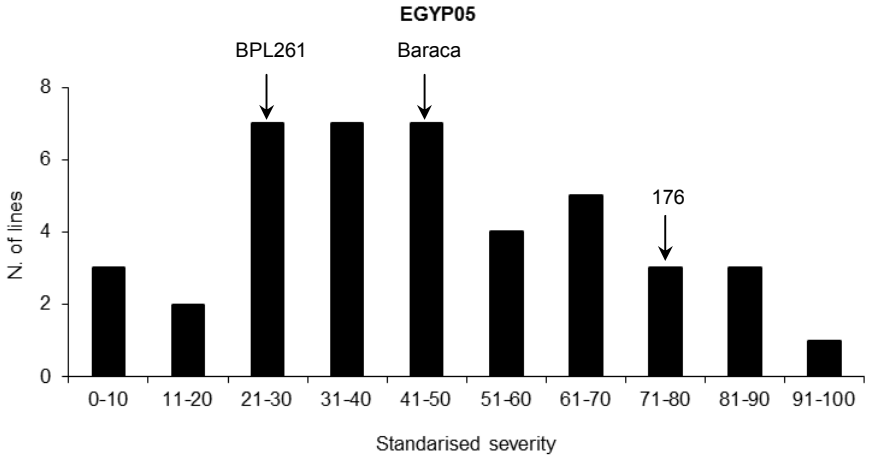
61-70
71-80
81-90
91-100
Standarised severity

5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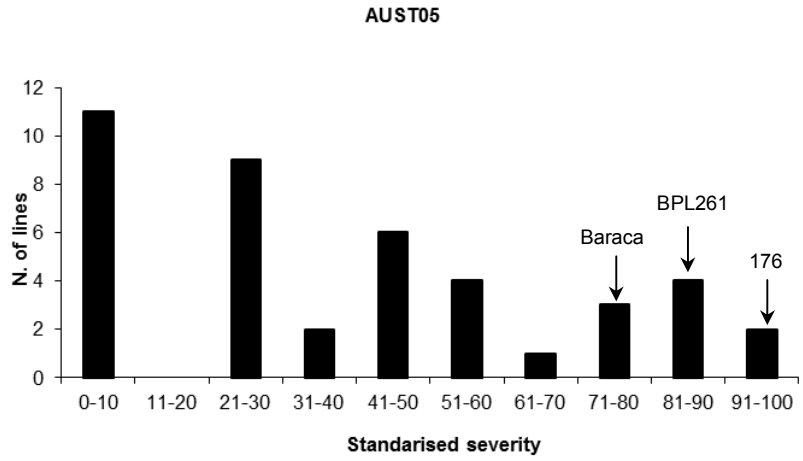
81-90
91-100
Standarised severity


AUST05
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BPL261
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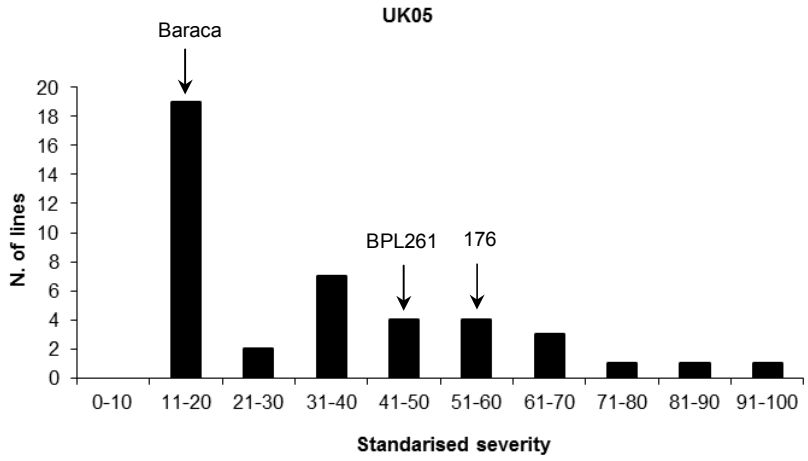
71-80
81-90
91-100
Standarised severity


UK05
Baraca
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8
10
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14
16
18
20
BPL261
176
0-10
11-20
21-30
31-40
41-50
51-60
61-70
71-80
81-90
91-100
Standarised severity

5

Figure 3. HA-GGE biplot based on DS of 39 selected faba bean accessions together with two susceptible (176 and cv. Baraca) and a partially resistant (BPL261) checks in 9 environments (combination season-location).

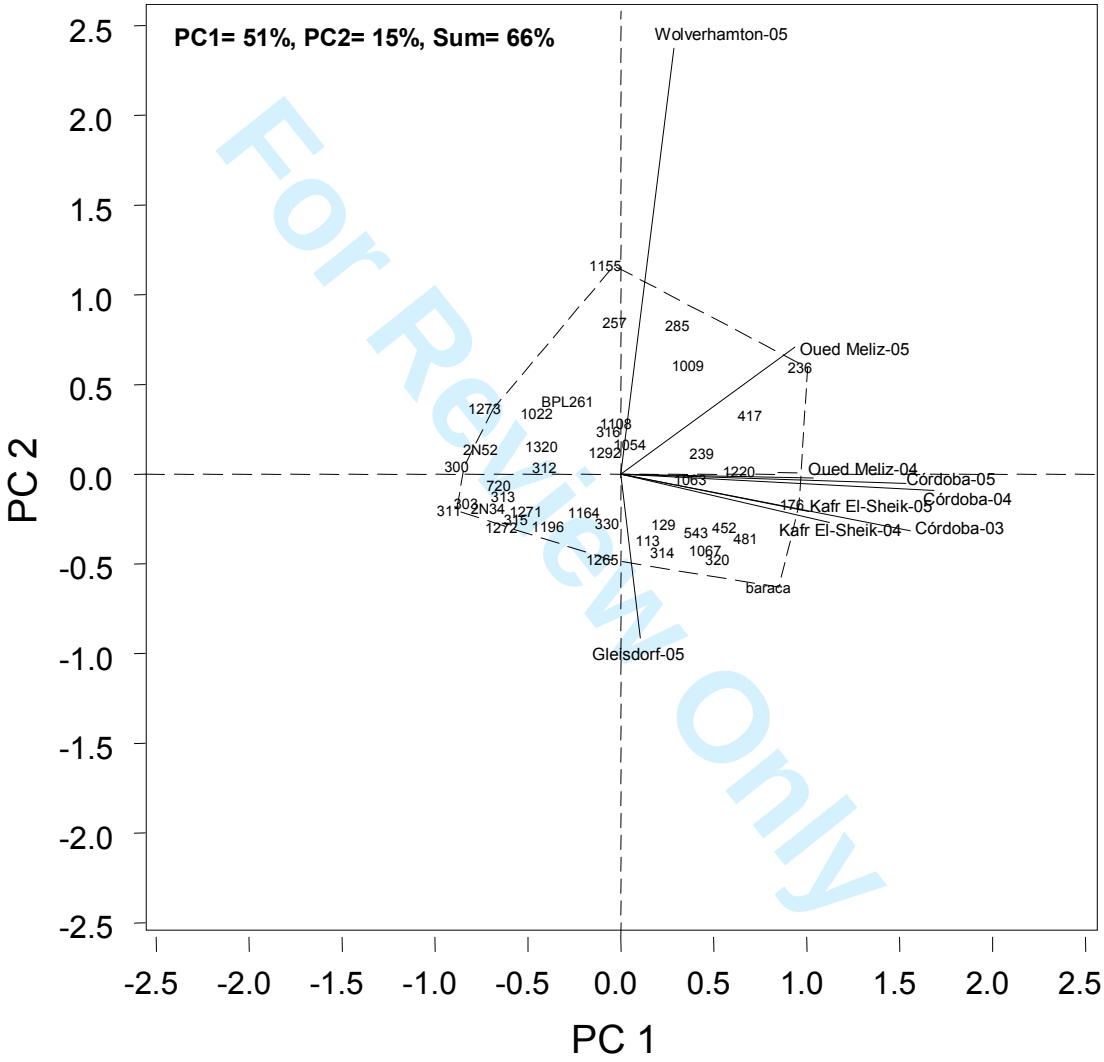

Figure 4. HA-GGE biplot based on DS of 39 selected faba bean accessions together with two susceptible (176 and cv. Baraca) and a partially resistant (BPL261) checks in 7 environments (combination season-location) for Mediterranean Mega-environment (ME1).