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**Summary Text for the Table of Contents.** Mediterranean environments are of most interest in the current context of global climate change. In our work we have tested adaptation of 9 pea cultivars in South European and North African locations, characterized by different agro climatic conditions within the Mediterranean climate. Our results highlighted the potential interest of genotypes HR1 and Desso in breeding programs and further studies of drought tolerance.

**Assessment of field pea (*Pisum sativum* L.) grain yield, aerial biomass and flowering date stability in Mediterranean environments**

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**Abstract:** Mediterranean environments are of most interest to study pea adaptability to terminal drought conditions especially in the current context of global climate change. In our work we have tested 9 pea cultivars in five South European and North African locations, characterized by different agro climatic conditions within the Mediterranean climate. Data were processed through the additive main effects and multiplicative interaction (AMMI) analysis. Grain yield, aboveground biomass and flowering date were assessed within each Mega Environment (ME) with parametric and non-parametric methods, establishing ranks for the genotypes within each condition attending to their stability parameters and mean values. The field analysis revealed HR1 as a wide adapted genotype, whereas others such as Desso showed the best adaptation in South Mediterranean areas. Our results also highlighted the potential interest of these genotypes and others (i.e. Messire and ZP108) in breeding programs and further studies on drought tolerance.

**Additional Keywords:** dry pea, phenology, Genotype  $\times$  Environment interaction, drought tolerance

## Introduction

Dry pea (*Pisum sativum* L.) is the most widely grown temperate grain legume in Europe and the second-most in the world (FAOSTAT 2013), being a versatile and inexpensive protein source for animal feeding with a widely acknowledged beneficial role in cropping systems (Drinkwater et al., 1998; Badgley et al., 2006; Nemecek et al. 2008). In spite of these advantages, dry pea acreage shows a constantly decreasing trend at a world level from 10.3 million ha in 1962 to 6.3 ha in 2012 (Rubiales and Mikic 2015).

Yield variability and instability are the major problems for pea both within and between sites and seasons due to a poor adaptability and a low tolerance to biotic and abiotic stress (Moot et al. 1995; Jacobsen et al. 2012). Significant efforts have been made in pea breeding for adaptability to continental and oceanic conditions (Cousin 1997). In contrast, little efforts have been paid to improve adaptability to Mediterranean environments with mild winters and dry springs, where spring pea types are autumn sown and challenged by different stresses (Rubiales et al. 2009). As a result, pea cultivation is particularly low in the Mediterranean Basin and recently released pea cultivars are poorly adapted to Mediterranean environments (Rubiales 2015). This is somehow surprising as the East Mediterranean and the West Asia are the primary centre of diversity for pea, where wild forms, such as *P. fulvum* Sm. and *P. sativum* subsp. *elatius* (M. Bieb.) Asch. and Graebn can still be found growing today (Rubiales et al. 2011; Čupina et al. 2011) and where pea once played a very important role in human diets, as witnessed by the first attested ancient DNA extraction from any legume species (Jovanović et al. 2011; Medović et al. 2011).

In field conditions, the development of pea genotypes producing high and stable seed yield is most decisive (Cousin, 1997; Rubiales et al. 2011; Smýkal et al. 2012), albeit high biomass and a short phenological cycle are also generally desired characters (Hebblethwaite et al. 2013). A good approach to study grain yield, aerial biomass and flowering date under field conditions is given by the use of statistic models. First of all, in order to check the stability of the cultivars is essential to analyze genotype x environment interactions (GEIs) in field trials through time and space. This is of great importance in breeding programs, since large GEIs bring about discrepancies between expected and observed responses to selection due to a higher estimation of genetic variances (Haussmann et al. 2001; Pinnschmidt et al. 2002; Brancourt-

Hulmel *et al.* 2003; Zinsou *et al.* 2005). GEIs complicate predictions on the accessions behaviour in situations where they have not been tested before (Dixon *et al.* 2002).

Several statistics techniques have been proposed to investigate GEIs, ranging from univariate parametric models to multivariate ones. Joint regression is the most popular univariate method, providing information on both stability and adaptation through simple calculation and application (Mohammadi *et al.* 2013). Among the multivariate methods, the additive main effects and multiplicative interaction (AMMI) analysis encloses the additive main effects of genotype and environment as well as the multiplicative effect of GE interaction, being more explicative than univariate techniques. In fact, the first Interaction Principal Component Analysis (IPCA1) normally accounts for a higher GE sum of squares compared to regression (Gauch and Kang 1996; Sabaghnia *et al.* 2013). Furthermore, an advantage of AMMI models for breeders is that they allow making recommendations on the best performing genotype identified in each mega-environment (ME) (Gauch 2006; Zobel *et al.* 1994). AMMI analyses are thus required for effective yield stability analysis and description of adaptation patterns (Gauch 1992; Zobel 1994; Gauch *et al.* 2008).

The aim of this work was to assess yield stability and some agronomically interesting traits in field pea cultivars in South Mediterranean and North African environments, in order to identify (i) broadly adapted cultivars offering stable performance across different locations and (ii) cultivars that performed well under specific sites.

## **Materials and methods**

### *Plant material and growth conditions*

Nine pea cultivars were subjected to this study; Ballet, Desso, Frisson, HR1, Kebby, Messire, Polar, Solara and ZP108. These cultivars, all semi-dwarf, were selected on the basis of previous studies according to their possible tolerance or susceptibility to water stress in controlled conditions (Grzesiak 1997; Sánchez *et al.* 1998; Iglesias-García *et al.* 2012a, b). Further information on the genotypes characteristics is given on Table 1.

**Table 1.** Pea genotypes included in the study with some known characteristics.

<b>Genotype</b>	<b>Characteristics</b>					
	<b>Sort</b>	<b>Origin</b>	<b>Flower Colour</b>	<b>Leaves</b>	<b>Grain size</b>	<b>Grain type</b>
Ballet	Cultivar	France	White	Leafless	Big	Round, white
Desso	Cultivar	France	White	Conventional	Small	Round, white
Frisson	Cultivar	France	White	Conventional	Small	Round, white
Kebby	Cultivar	France	White	Conventional	Big	Rough, green
Messire	Cultivar	France	White	Conventional	Big	Round, white
Polar	Cultivar	France	White	Conventional	Big	Rough, green
HR1	Breeding line	France	White	Semi-leafless	Big	Rough, white
Solara	Cultivar	The Netherlands	White	Semi-leafless	Big	Round, white
ZP108	Breeding line	Spain	White	Semi-leafless	Small	Round, white

*Field-based evaluation*

An environment was defined as the combination of one year and one location (Table 2). The Tunisian trials were conducted in a single location (Beja) during three consecutive cropping seasons (from 2009 until 2012). The Spanish trials were performed in three contrasting locations, namely Córdoba, Escacena and Villamor de Cadozos during 2010-2011 and 2011-2012. The Serbian trial was located in Novi Sad during 2012.

At each location, a randomized complete block design with three replications was used. Each replicate was an independent plot consisting in three 1 m-long rows bordered by lentils. Within each plot, the rows were separated from each other by 30 cm. According to local practice, sowing took place between the end of November and the beginning of January in all locations except for Novi Sad, where sowing was done at the beginning of March. The sowing density was around 30 seeds per m<sup>2</sup>. No herbicides were applied over the trials and only hand weeding was carried out when necessary.

**Table 2.** Description of the environments (combination of location and season) of the trials for the multi-environment study. Climatic data corresponding to each growing season are provided.

Environments	Location	Soil type*	Soil pH	Latitude	Longitude	Altitude (mASL)	Growing season	Average T <sub>max</sub> (°C)	Average T <sub>min</sub> (°C)	Rain (mm)
Bej09	Beja, Tunisia	Rendric leptosol	7.2 - 7.5	36° 44' N	9° 13' E	164	2009-10	23	10,5	495
Bej10				36° 44' N	9° 13' E	164	2010-11	22,6	10,2	592
Bej11				36° 44' N	9° 13' E	164	2011-12	22,7	10,7	901
Cor10	Córdoba, Spain	Cambisol	6.5-7	37° 50' N	4° 50' W	90	2010-11	19,1	8,3	239
Cor11				37° 50' N	4° 50' W	90	2011-12	19,8	5,6	741
Esc10	Escacena, Spain	Fluvisol	7-7.5	37° 25' N	6° 15' W	88	2010-11	19,7	10,0	189
Esc11				37° 25' N	6° 15' W	88	2011-12	20,0	9,1	668
Nov12	Novi Sad, Serbia	Cambisol	7,9	45° 33' N	19° 50'E	87	2012	19,4	7,5	157
Vil10	Villamor, Spain	Regosol	4.5-6	41° 19' N	6° 6' W	777	2010-11	18,1	2,8	273
Vil11			4.5-6	41° 19' N	6° 6' W	777	2011-12	15,7	3,0	132

\* According to FAO/IIASA/ISRIC/ISSCAS/JRC, 2012.



At harvesting time, whole-plants and seeds were weighted together and separately to obtain the total aboveground biomass as well as the yield per plot. Grain and aerial biomass values were referred to the number of plants (grams per plant) in the plot, as well as the size of the experimental plot and their values were calculated as grams per square meter, obtaining an estimation of kilograms production per hectare. Date of flowering was processed as the number of days from the sowing until 50% anthesis.

#### *Statistical analysis*

Data for each trait (grain yield, biomass and flowering) were submitted to a combined analysis of variance (ANOVA) with genotype and location-year (environment) as fixed factors using SAS® 9.3 (SAS Institute Inc.). F-ratios were used to test effects for randomized complete block experimental design (McIntosh 1983).

Prior to each ANOVA, tests for normality and equality of variance were conducted for each dependent variable. To detect correlation among traits, Pearson correlations were calculated.

#### *AMMI analysis*

A simple protocol for using AMMI effectively was used here according to Gauch (2013), this protocol includes four steps: 1) analysis of variance, 2) model diagnosis, 3) mega-environment delineation, and 4) agricultural recommendations.

1) Three numbers from the AOV provide a preliminary indication whether AMMI analysis will be worthwhile: the sum of squares (SS) for genotypes (G), GE signal ( $GE_s$ ), and GE noise ( $GE_N$ ). AMMI analysis is appropriate for datasets having substantial G and substantial  $GE_s$  (Gauch 2013).

To estimate the SS for  $GE_N$  we multiplied the error mean square from replication by the number of degrees of freedom (df) for GE. Also we obtained  $GE_s$  by subtracting  $GE_N$  from GE. According to the high SS values of G and  $GE_s$ , the AMMI model was employed to study the GEI of pea yield, biomass and flowering.

2) AMMI constitutes a model family, not a single model. Consequently, model diagnosis was required to determine which member of this model family was the best for a given dataset and research purpose (Gauch, 2013). Piepho (1995) showed that the  $F$  test proposed by Cornelius *et al.* (1992) was more robust than that proposed by Gollob (1968); the statistic was calculated as:

$$F_{R,n} = (SS_{GEI} - \sum_{k=1}^n \lambda_k^2) / (f_2 MS_{mean\ error})$$

Where  $f_2 = (g - 1 - n)(e - 1 - n)$  and  $n$  is the number of multiplicative terms in the model. The  $F_{R,n}$  statistic, with null hypothesis that there are no more than  $n$  terms determining the interaction, has an approximate  $F$  distribution with  $f_2$  degrees of freedom and  $DF_{mean\ error}$ . Once the estimated yields for each AMMI model were calculated, mean values were compared according to Tukey's method.

3) Mega-environments can be displayed by both tables and graphs (Gauch 2013). In our work, we have obtained a ranking table which showed the ranks for the best several genotypes in each environment (Table 3).

In order to characterize testing sites in terms of environmental factors which would possibly support the AMMI analysis results, a correlation matrix-based principal component analysis (PCA) was performed on the environment x covariate two-way table, based on growing season absolute maximum and minimum temperatures, altitude and rainfall (Table 2). Climatic data were obtained from trial responsible when available, or from local government websites. In the case of rainfall, it was calculated for the growing season from the date of sowing until the collection. A biplot of the first two principal component (PC) axes was used to visualise the environmental factors characterising the different testing sites and help interpret the interaction patterns observed in the AMMI analysis.

4) A major purpose of yield-trial research is selection of the best genotypes to be used in a breeding program or recommendation of the best cultivars for a growing region. Selection or recommendation of the best genotypes must inevitably be done in the context of a given mega-environment scheme (Yan *et al.* 2007; Gauch 2013).

To describe stability and make recommendations of the best genotypes within each mega environment, we calculated the mean value of each variable as well as four stability parameters derived from the most specific AMMI analysis (AMMI5 in our case): **SIPC5**, which is the sum of the absolute value of the first five PCA scores; **EV5**, which is the average of the squared eigenvector values (Zobel 1994); the Euclidean distance from the origin of significant interaction IPCA axes, parameter **D5** in our work, described by Annicchiarico (1997); **MASV5** or the “Modified AMMI’s Stability Value”, a new parameter introduced for an effective interpretation of GE interactions via AMMI model which was calculated using ASV for AMMI2 by Purchase (1997), but in our case included all principal component from the most accurate model for our dataset (AMMI5). The lower these statistics were the more stable was the genotype to environments.

#### *Univariate analysis*

Selected parametric methods from univariate analysis were also applied in the present work in order to estimate the range of data and the uniformity of variance. In both cases, lower values were indicators of a greater stability:

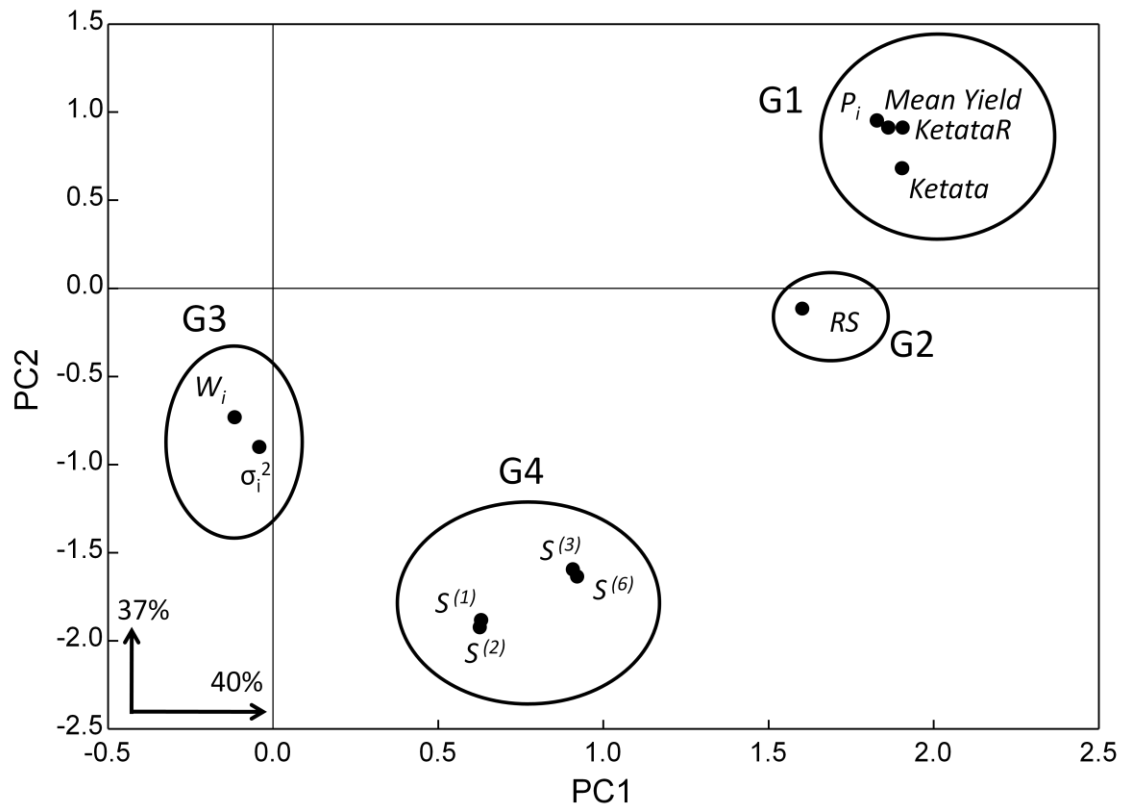
- Mean value ( $M$ ) of each trait: a genotype was classified as the most favourable if its mean value was relatively consistent in all the environments (Ketata *et al.* 1989; Flores *et al.* 1998).
- Superiority index ( $P_i$ ): defined by Lin and Binns (1988) as the distance mean square between the cultivar’s response and the maximum response over locations.
- Ketata’s rank and graph: each mean value of a trait for a genotype was compared with or plotted against standard deviation of yields for all genotypes (Ketata *et al.* 1989).
- Wricke's ecovalence ( $W_i^2$ ): represents the proportion of  $G \times E$  variance attributed to each genotype (Wricke 1962), obtained from  $G \times E$  squared and summed across all environments.
- Stability variance ( $\sigma_i^2$ ): proposed by Shukla (1972), which partitioned  $G \times E$  and an error term, and assigned it to individual genotypes.

Furthermore, we used non-parametric statistics, which were not affected by data distribution as they were based on ranks instead of values.

- Kang's rank-sum (*RS*): non-parametric stability parameter where a weight of one was assigned to both yield and stability statistics to identify high-yielding and stable genotypes, using both yield and Shukla's (1972) stability variance as selection criteria.
- $S_i^{(1)}$ ,  $S_i^{(2)}$ ,  $S_i^{(3)}$  and  $S_i^{(6)}$ : non parametric statistics (Huehn 1979; Nassar and Huehn 1987) combining mean yield and stability (Becker and Leon 1988).  $S^{(1)}$  and  $S^{(2)}$  are based on ranks of genotypes across environments and give equal weight to each environment, whereas  $S^{(3)}$  and  $S^{(6)}$  combine yield and stability based on variable's ranks of the genotypes in each environment (Nassar and Huehn 1987). A zero variance is an indicator of maximum stability

In order to determine which of these 9 tools could be most useful in our analysis, a principal components biplot was made for each trait considered. Given that we obtained similar grouping in all cases, four groups of parameters were determined: in the first group were included **Mean**,  **$P_i$**  and Ketata rankings and graphs, named in our work **Ketata R** and **Ketata**, respectively; in the second group was only **RS**, whereas the third group enclosed  **$W_i$**  and  **$\sigma_i^2$** ; the last group included  **$S_i^{(1)}$** ,  **$S_i^{(2)}$** ,  **$S_i^{(3)}$**  and  **$S_i^{(6)}$** . An example of the aggregations of these parameters in the PCA biplot for yield (77% of information extracted) is shown in Supplementary figure 1. We selected one parameter from each group consequently, in order to simplify data interpretation: *Mean*, *RS*,  *$W_i$*  and  *$S_i^{(3)}$* .

**Supplementary figure 1.** Principal component analysis (PCA) biplot of AMMI parameters for yield (77% of the information extracted).



## Results and discussion

### Model diagnosis

The AOV results for grain yield, aerial biomass and flowering date are shown in supplementary Tables 1, 2 and 3, respectively, in which the degrees of freedom (DF) for the eight incremental principal components (IPCs) were assigned according to the method of Gollob (1968). In all cases, the  $GE_s$  was more than once as large as the SS of the genotype, so that AMMI analysis was quite likely to be used worthwhile.

**Supplementary Table 1.** Analysis of variance for the AMMI model of grain yield (t/ha) for an international pea trial.

Source of variation	DF	SS	<sup>a</sup> DF <sub>C</sub>	<sup>b</sup> F <sub>C</sub>	p-value
<b>Model</b>	109	800.58***			
<b>Environment (E)</b>	9	573.40***			
<b>Replication within E</b>	20	0.79			
<b>Genotype (G)</b>	8	55.88***			
<b>E x G</b>	72	179.74***			
<b>IPCA1</b>	16	61.50 ***	56	39.50	0.0000
<b>IPCA2</b>	14	50.48 ***	42	30.18	0.0000
<b>IPCA3</b>	12	43.30 ***	30	15.24	0.0000
<b>IPCA4</b>	10	13.66 ***	20	10.08	0.0000
<b>IPCA5</b>	8	5.99 ***	12	7.46	0.0000
<b>IPCA6</b>	6	2.95***	6	5.71	0.0000
<b>IPCA7</b>	4	1.78	2	0.41	0.6636
<b>IPCA8</b>	2	0.04	0		
<b>Error</b>	151	8.07			
<b>Total variation</b>	260	808.65			

<sup>a</sup>DF<sub>C</sub>: Degrees of Freedom calculated according to the FR-test proposed by Cornelius et al., (1992).<sup>b</sup>F<sub>C</sub>: F-value (Cornelius et al., 1992).

**Supplementary Table 2.** Analysis of variance for the AMMI model of biomass (t/ha) for an international pea trial.

Source of variation	DF	SS	<sup>a</sup> DF <sub>C</sub>	<sup>b</sup> F <sub>C</sub>	p-value
<b>Model</b>	109	9921.49***			
<b>Environment (E)</b>	9	7411.52***			
<b>Replication within E</b>	20	35.90			
<b>Genotype (G)</b>	8	556.63***			
<b>E x G</b>	72	2058.81***			
<b>IPCA1</b>	16	1010.27***	56	10.95	0.0000
<b>IPCA2</b>	14	361.47***	42	9.56	0.0000
<b>IPCA3</b>	12	320.00***	30	7.15	0.0000
<b>IPCA4</b>	10	227.06***	20	4.09	0.0000
<b>IPCA5</b>	8	92.08***	12	2.33	0.001
<b>IPCA6</b>	6	30.68	6	1.68	0.1294
<b>IPCA7</b>	4	14.35	2	0.85	0.4303
<b>IPCA8</b>	2	2.90	0		
<b>Error</b>	149	254.89			
<b>Total variation</b>	258	10176.38			

<sup>a</sup>DF<sub>C</sub>: Degrees of Freedom calculated according to the FR-test proposed by Cornelius et al., (1992).<sup>b</sup>F<sub>C</sub>: F-value (Cornelius et al., 1992).

**Supplementary Table 3.** Analysis of variance for the AMMI model of flowering for an international pea trial.

Source of variation	DF	SS	<sup>a</sup> DF <sub>C</sub>	<sup>b</sup> F <sub>C</sub>	p-value
<b>Model</b>	109.00	123958.92***			
<b>Environment (E)</b>	9.00	108381.51***			
<b>Replication within E</b>	20.00	81.70			
<b>Genotype (G)</b>	8.00	6201.99***			
<b>E x G</b>	72.00	9293.72***			
<b>IPCA1</b>	16.00	5493.64***	56	20.30	0.0000
<b>IPCA2</b>	14.00	2158.67***	42	11.69	0.0000
<b>IPCA3</b>	12.00	878.03***	30	7.61	0.0000
<b>IPCA4</b>	10.00	426.28***	20	5.04	0.0000
<b>IPCA5</b>	8.00	254.03***	12	2.07	0.01
<b>IPCA6</b>	6	60.79	6	1.11	0.3585
<b>IPCA7</b>	4	20.66	2	0.24	0.7853
<b>IPCA8</b>	2	1.62	0		
<b>Error</b>	160.00	534.96			
<b>Total variation</b>	269.00	124493.89			

<sup>a</sup>DF<sub>C</sub>: Degrees of Freedom calculated according to the FR-test proposed by Cornelius et al., (1992).<sup>b</sup>F<sub>C</sub>: F-value (Cornelius et al., 1992).



While the selection of axes is a liberal criterion (Piepho 1995), an alternative would be to apply the F test to the AMMI residuals, which Piepho (1995) defends as one of the more robust methods. Based on this criterion, the AMMI model was selected from the IPCA in which the AMMI residue became non significant.

From Supplementary Table 1, the model diagnosis for optimizing predictive accuracy of grain yield was AMMI6, as the IPCA7 residual was not significant. In the case of aerial biomass and flowering date, AMMI5 was the most precise model, because the IPCA6 was found to be non significant (supplementary tables 2 and 3, respectively).

#### *MEs delineation and description*

In the next step of our analysis, we employed the most precise AMMI model to determine if there were MEs (Gauch 2013).

Combined analysis of variance for grain yield, aerial biomass and flowering date showed that main effects for genotypes and environments, as well as GEI were significant at  $P < 0.001$ . The significance of the GEI effect suggested that there were differences in responses of genotypes to environments, and hence sensitivity and instability. Genotypic rank differences over environments, confirmed by Tukey's test (Table 3), showed the existence of crossover GEIs (Crossa 1990), which enforced the necessity to assess the response of the genotypes to environmental variation.

According to the AMMI analysis, genotype 3 (Frisson), 6 (Messire) and 8 (Solara) were ranked winner for grain yield and biomass in most locations, whereas genotypes 4 (HR1) and 9 (ZP108) were the winners for these traits only in Vill10 and Vill11, although means were similar for nearly all genotypes in the first and second positions of the rank regarding Tukey's test. Different cultivars showed earlier date of flowering, being 5 (Kebby) and 7 (Polar) in the first rank positions for most environments, while Desso and Frisson (genotypes 2 and 3, respectively) highlighted in Vill10 and Vill11. These distinctions helped to describe two different ME, which could be interpreted in agricultural terms as the result of a temperature gradient.

**Table 3.** Ranking tables showing the top 5 genotypes for grain yield, aerial biomass and flowering, according to AMMI6 in the case of grain yield and AMMI5 for biomass and flowering date. Once the estimated yields for each AMMI model were calculated, mean values were compared according to Tukey's method. Similar means are highlighted in bold.

Environment	AMMI6 Rank for grain yield					AMMI5 Rank for aerial biomass					AMMI5 Rank for flowering date				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Beja09	<b>Messire<sup>a</sup></b>	<b>Solara<sup>a</sup></b>	Ballet <sup>b</sup>	Desso <sup>b</sup>	Frisson <sup>b</sup>	<b>Messire<sup>a</sup></b>	Solara <sup>ab</sup>	Frisson <sup>b</sup>	Desso <sup>b</sup>	Ballet <sup>b</sup>	<b>Polar<sup>a</sup></b>	<b>Kebby<sup>a</sup></b>	Messire <sup>b</sup>	Frisson <sup>b</sup>	Solara <sup>b</sup>
Beja10	<b>Messire<sup>a</sup></b>	<b>Frisson<sup>a</sup></b>	<b>ZP108<sup>a</sup></b>	<b>Solara<sup>a</sup></b>	HR1 <sup>ab</sup>	<b>Messire<sup>a</sup></b>	Frisson <sup>ab</sup>	ZP108 <sup>b</sup>	Ballet <sup>bc</sup>	Solara <sup>bc</sup>	<b>Kebby<sup>a</sup></b>	<b>Polar<sup>a</sup></b>	Frisson <sup>b</sup>	Messire <sup>b</sup>	Solara <sup>b</sup>
Beja11	<b>Frisson<sup>a</sup></b>	<b>Polar<sup>a</sup></b>	<b>Messire<sup>a</sup></b>	ZP108 <sup>b</sup>	Solara <sup>b</sup>	<b>Frisson<sup>a</sup></b>	Messire <sup>b</sup>	Kebby <sup>bc</sup>	Ballet <sup>bc</sup>	ZP108 <sup>cd</sup>	<b>Polar<sup>a</sup></b>	<b>Kebby<sup>a</sup></b>	ZP108 <sup>b</sup>	Frisson <sup>b</sup>	Messire <sup>b</sup>
Cord08	<b>Messire<sup>a</sup></b>	<b>HR1<sup>a</sup></b>	Frisson <sup>ab</sup>	Desso <sup>ab</sup>	Ballet <sup>ab</sup>	<b>Frisson<sup>a</sup></b>	<b>Messire<sup>a</sup></b>	Desso <sup>b</sup>	HR1 <sup>bc</sup>	Polar <sup>c</sup>	<b>Kebby<sup>a</sup></b>	<b>Polar<sup>a</sup></b>	Frisson <sup>b</sup>	Solar <sup>ab</sup>	Desso <sup>b</sup>
Cord11	<b>Messire<sup>a</sup></b>	<b>Solara<sup>a</sup></b>	<b>Desso<sup>a</sup></b>	<b>Ballet<sup>a</sup></b>	ZP108 <sup>ab</sup>	<b>Messire<sup>a</sup></b>	<b>Kebby<sup>a</sup></b>	Solara <sup>ab</sup>	Desso <sup>bc</sup>	ZP108 <sup>cd</sup>	<b>Kebby<sup>a</sup></b>	<b>Polar<sup>a</sup></b>	Solara <sup>ab</sup>	Ballet <sup>b</sup>	Frisson <sup>b</sup>
Esc10	<b>Solara<sup>a</sup></b>	<b>HR1<sup>a</sup></b>	Desso <sup>b</sup>	Polar <sup>b</sup>	ZP108 <sup>b</sup>	<b>Messire<sup>a</sup></b>	<b>Solara<sup>a</sup></b>	<b>Desso<sup>a</sup></b>	Frisson <sup>b</sup>	ZP108 <sup>b</sup>	<b>Polar<sup>a</sup></b>	<b>Kebby<sup>a</sup></b>	Frisson <sup>ab</sup>	Solar <sup>ab</sup>	HR1 <sup>b</sup>
Esc11	<b>Solara<sup>a</sup></b>	<b>Polar<sup>a</sup></b>	<b>HR1<sup>a</sup></b>	<b>ZP108<sup>a</sup></b>	Desso <sup>ab</sup>	<b>Messire<sup>a</sup></b>	<b>Solara<sup>a</sup></b>	<b>Desso<sup>a</sup></b>	Polar <sup>b</sup>	HR1 <sup>b</sup>	<b>Kebby<sup>a</sup></b>	Polar <sup>ab</sup>	Solara <sup>ab</sup>	Ballet <sup>ab</sup>	HR1 <sup>bc</sup>
Nova12	<b>Polar<sup>a</sup></b>	<b>Ballet<sup>a</sup></b>	<b>Frisson<sup>a</sup></b>	<b>Solara<sup>a</sup></b>	Messire <sup>ab</sup>	<b>Messire<sup>a</sup></b>	Polar <sup>ab</sup>	Solara <sup>b</sup>	Desso <sup>bc</sup>	HR1 <sup>bc</sup>	<b>Polar<sup>a</sup></b>	<b>Kebby<sup>a</sup></b>	Solar <sup>ab</sup>	Frisson <sup>b</sup>	Ballet <sup>bc</sup>
Vill10	<b>HR1<sup>a</sup></b>	<b>ZP108<sup>a</sup></b>	Messire <sup>b</sup>	Frisson <sup>b</sup>	Kebby <sup>b</sup>	<b>HR1<sup>a</sup></b>	<b>ZP108<sup>a</sup></b>	Polar <sup>b</sup>	Messire <sup>bc</sup>	Kebby <sup>bc</sup>	<b>Desso<sup>a</sup></b>	<b>Frisson<sup>a</sup></b>	HR1 <sup>b</sup>	Kebby <sup>b</sup>	Messire <sup>b</sup>
Vill11	<b>HR1<sup>a</sup></b>	<b>ZP108<sup>a</sup></b>	Frisson <sup>b</sup>	Messire <sup>b</sup>	Kebby <sup>b</sup>	<b>ZP108<sup>a</sup></b>	<b>HR1<sup>a</sup></b>	Frisson <sup>b</sup>	Messire <sup>b</sup>	Kebby <sup>bc</sup>	<b>Desso<sup>a</sup></b>	Frisson <sup>ab</sup>	Kebby <sup>b</sup>	Messire <sup>b</sup>	Solara <sup>b</sup>

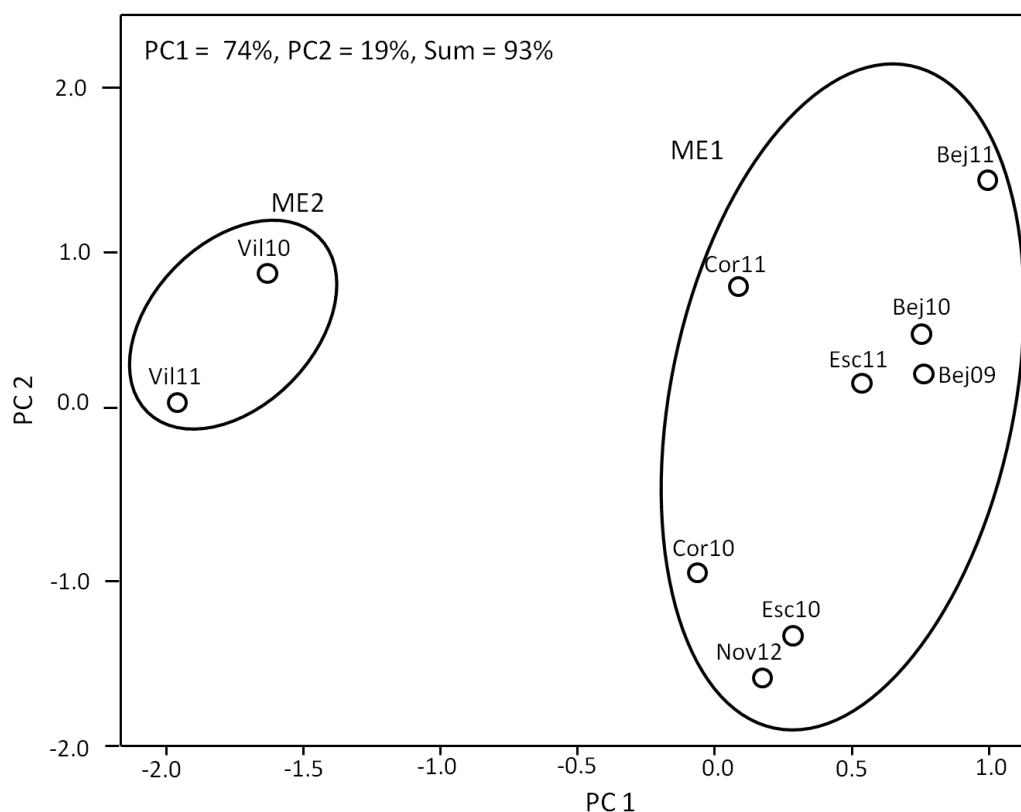
A PC analysis was made to check if the differences could be due to the effect of some known environmental variables, supporting AMMI results.

#### *PCA of environmental variables*

Environmental PC1 for grain yield was positive and significantly correlated to the growing season minimum and maximum temperatures ( $r^2 = 0.78$ ,  $P < 0.01$  and  $r^2 = 0.69$ ,  $P < 0.01$ ). Also, PC1 was negative significantly correlated to the altitude ( $r^2 = -0.78$ ,  $P < 0.01$ ), being a selective component for mutually exclusive genotypes. Therefore, the  $G \times E$  interaction pull was proportional to minimum and maximum temperatures of the growing season.

Regarding the aerial biomass, the positive correlated selective component was the altitude, ( $r^2 = 0.94$ ,  $P < 0.01$ ). On the other hand, the  $T_{\min}$  ( $r^2 = -0.82$ ,  $P < 0.01$ ) and soil pH ( $r^2 = -0.89$ ,  $P < 0.01$ ) showed a negative significant correlation coefficient. Surprisingly, there was no correlation between flowering and rain or temperature, albeit the altitude seemed to have a high significant and positive correlation coefficient ( $r^2 = 0.7306$   $P < 0.05$ ). This could be explained as a contradictory effect of temperature: although low temperatures promoted flowering, if they continue after floral initiation node formation could be delayed. The altitude, photoperiod or sowing date would be much more important to determine flowering date than other factors (Roche *et al.* 1999).

Separation between Villamor and the rest of the environments by altitude, pH, average  $T_{\max}$ , average  $T_{\min}$  and rain could also be observed in the plot for the two first principal components of climatic and edafic variables (Figure 1), as well as the conjunction of Beja, Córdoba, Escacena and Novi Sad locations in one ME.



**Figure 1.** Principal Component Analysis (PCA) for climatic and edaphic variables.

Therefore, we considered two different regions attending to the Mediterranean climate stratification proposed by Metzger (2005). The first ME (ME1) consisted of Beja, Córdoba, Escacena and Novi Sad locations, which would be enclosed within the South Mediterranean area. Although Serbia is a North Mediterranean country (Metzger 2005), this grouping is probably due to the spring sown of the Novi Sad trial, which was thus subjected to warmer climate conditions. The second region would be located in the North Mediterranean area and represented by Villamor location, which formed another ME (ME2). Despite the ME2 was only formed by two environments, we considered it as a simple mega-environment, as it was enough to identify the best cultivars that could be recommended (Yan and Kang 2002). ME1 and ME2 were in all cases similar to the MEs identified by ranking table. As we commented previously, selection and recommendation of the best genotypes must inevitably be done in the context of each ME scheme (Yan *et al.* 2007; Gauch 2013). All these considerations made, we proceeded with a deeper analysis of each parameter within ME1 and ME2.

*Grain yield, aerial biomass and flowering date in South Mediterranean Environments*

A new ANOVA test was made in order to analyze the variations within South Mediterranean locations (data not shown). As a result, the most accurate AMMI model for grain yield, aerial biomass and flowering was the AMMI5 which was used to identify the most stable genotypes for each AMMI stability parameter (Table 4).

Solara and Messire were the genotypes with the highest grain yield mean (4178 and 3953 kg/ha, respectively), albeit not with a remarkable stability. Desso and HR1 were the most stable according to SIPC5, EV5, D5 and MASV5 statistics values together, which were generally lower if compare with the others, indicating lower genotype interactions across environments, and also showed a good behavior in terms of grain yield (3552 kg/ha for Desso and 3548 kg/ha for HR1). On the other hand, Ballet and Kebby would be unstable for most parameters (Table 4).

**Table 4.** Mean and four stability parameters derived from AMMI5 for South Mediterranean environments (ME1) and grain yield (kg/ha), aerial biomass (kg/ha) and flowering date (days until 50% of flowering).

	Genotype	Mean	<sup>a</sup> SIPC5	<sup>b</sup> EV5	<sup>c</sup> D5	<sup>d</sup> MASV5
Grain yield (kg/ha)	Ballet	2.610	2.433	0.428	3.036	1.981
	Desso	3.552	1.308	0.141	0.935	0.678
	Frisson	3.481	2.423	0.301	2.479	1.801
	HR1	3.548	1.332	0.195	1.845	1.062
	Kebby	2.455	2.395	0.214	2.128	2.307
	Messire	3.953	2.506	0.298	2.459	2.201
	Polar	3.447	1.902	0.191	1.707	2.299
	Solara	4.178	2.215	0.282	2.226	2.525
	ZP108	3.424	2.456	0.229	2.246	2.302
Aerial biomass (kg/ha)	Ballet	10.895	3.106	0.287	6.122	2.842
	Desso	13.200	4.077	0.331	6.641	3.862
	Frisson	13.719	4.456	0.348	7.281	5.045
	HR1	11.204	2.454	0.117	3.049	1.571
	Kebby	9.547	4.279	0.354	8.648	4.121
	Messire	15.267	2.331	0.109	4.645	2.097
	Polar	11.118	3.069	0.232	5.257	2.633
	Solara	13.297	4.292	0.233	5.653	3.273
	ZP108	12.364	4.103	0.269	5.807	2.161
Flowering date (days till 50% of flowering)	Ballet	100.917	3.267	0.073	6.181	2.269
	Desso	102.792	4.037	0.133	6.445	2.083
	Frisson	98.958	5.122	0.192	8.915	3.731
	HR1	102.083	3.794	0.098	7.326	2.554
	Kebby	85.292	5.694	0.883	29.862	9.301
	Messire	102.875	5.065	0.189	8.972	4.597
	Polar	89.875	6.104	0.383	15.738	6.383
	Solara	99.208	2.866	0.082	6.914	2.496
	ZP108	103.417	5.118	0.249	9.881	6.212

<sup>a</sup>SIPC5: sums of the absolute value of the IPC5 scores.<sup>b</sup>EV5: averages of the square eigenvector values.<sup>c</sup>D5: distance from IPC5 point to origin of space.<sup>d</sup>MASV5: modified AMMI5 stability value.

Mean biomass production for genotypes and stability parameters derived from AMMI5 model within ME1 are given in Table 4. According to all stability parameters HR1 and Messire were the most stable genotypes across all the environments whereas Kebby was the genotype which showed a higher influence of the environments in its behaviour. Pea genotypes with a high aerial biomass across environment, such as Messire (15267 kg/ha) are essential forage producers (Hebblethwaite *et al.* 2013), being this also a desirable characteristic for crop rotations, due to higher soil enrichment in nitrogen, as well as for

thermal power generation (Karperstein-Machan and Stuelpnagel 2000; Huang *et al.* 2011). In fact, a high biomass is a trait with an increasing importance for long-term improvement of grain crops (Annicchiarico and Lanucci 2008).

In regions belonging to the Mediterranean area, pea genotypes with a high biomass production and good stability could also be useful for livestock feeding. Interestingly, Messire is also described as a water stress tolerant genotype (Iglesias-García *et al.* 2012a, b; Castillejo *et al.* 2016) which could be of great interest in southern Mediterranean regions, where terminal drought is decisive in the growing season and legumes are often grown after other crops such as corn or rice (Sadras *et al.* 2009).

Given the characteristics of the climatic regions assessed, we considered a shorter phenological cycle, from sowing until 50% flowering, as a valuable trait to be considered within the AMMI model analysis, as it could help the selection for genotypes which would be able to avoid drought through the years (Hebblethwaite *et al.* 2013). However, in our study we could observe a trend towards stability for the genotypes with a moderated phenological development. The shortest phenological cycles belonged to Polar and Kebby, but they were not stable across environments, as indicated by the higher values of their stability parameters (especially D5 for both cases). On the other hand, Ballet and Solara, both with around 100 days from sowing date until 50% of flowering completed, were found to be stable for most parameters assessed within AMMI5. Finally, Messire and Solara showed the highest values for this trait, indicating a flowering delay that was not maintained through the different environments assessed regarding SIPC5, EV5, D5 and MASV5.

#### *Grain yield, aerial biomass and flowering date in the North Mediterranean area*

In the North Mediterranean area defined by Vill10 and Vill11, HR1 and ZP108 were the genotypes with biggest grain yields according to the different univariate models statistics analysed, also showing reduced values for  $W_i^2$  and  $P_i$  together, which was an indicator of a higher stability (Lin and Binns 1988). Non parametric statistics based in rankings,  $S^{(3)}$  and  $RS$ , also pointed towards the stability of grain yield for these genotypes across environments given

by their low values (Kang 1988; Becker and Leon 1988), so that HR1 and ZP108 would be the most favourable pea genotypes for these locations.

HR1 was the genotype with most stable aerial biomass according to parametric and non parametric statistics (Table 5). Also, Messire was found to be stable across environments regarding non parametric parameters.

**Table 5.** Parametric and non parametric stability statistics for grain yield (kg/ha), biomass yield (kg/ha) and flowering date (days till 50% of flowering) of 9 pea genotypes across the two South Mediterranean environments (Vill10 and Vill11).

	Genotype	Mean	Parametric methods		Non-Parametric methods	
			$^a W_i^2$	$^b P_i$	$^c S^{(1)}$	$^d RS$
Grain yield (Kg/ha)	Ballet	2.702	0.030	9.743	0	11
	Desso	3.066	0.013	6.776	1	8.5
	Frisson	3.992	0.587	1.928	1	12.5
	HR1	4.892	0.045	0.000	0	7
	Kebby	3.549	0.038	3.608	0	10
	Messire	3.990	0.138	1.965	1	10.5
	Polar	2.957	0.002	7.512	1	10.5
	Solara	2.193	0.476	15.377	0	17
	ZP108	4.600	0.017	0.177	0	3
Aerial biomass (Kg/ha)	Ballet	8.701	11.115	283.963	2.5	15.25
	Desso	8.324	0.213	264.024	2	10
	Frisson	10.792	5.492	157.153	4	11
	HR1	17.558	0.226	17.090	1	3.5
	Kebby	10.519	1.686	187.894	0	10
	Messire	11.779	0.482	139.854	1	4.5
	Polar	9.529	7.310	241.078	3	12.5
	Solara	6.027	0.201	378.484	0.5	12.75
	ZP108	18.986	18.511	1.634	1	10.5
Flowering date (days till 50% of flowering)	Ballet	128.333	12.500	37.000	0.5	11.5
	Desso	111.000	234.722	1102.780	0	10
	Frisson	120.000	16.056	296.110	0.5	9.5
	HR1	125.667	1.389	77.440	2	5
	Kebby	125.167	10.889	99.780	1.5	6
	Messire	125.833	14.222	86.220	1	11
	Polar	129.333	12.500	25.000	0.5	12.5
	Solara	128.000	16.056	45.440	0.5	13.5
	ZP108	131.833	0.000	0.000	0.5	11



Longer phenological cycles were observed for genotypes in North Mediterranean locations, in agreement with colder temperatures during the growing seasons. For all genotypes it took more than 110 days between sowing date and 50% of flowering. As it happened in the South Mediterranean locations, a short phenological cycle is related with instability. However, the genotype with the earliest flowering date was Desso, which could be considered moderately stable across environments regarding non parametric statistics. Furthermore, genotypes such as HR1 and ZP108 with intermediate cycle length compared with others were found to be stable regarding both parametric and non parametric methods, which indicated their independence from environments influence. In both Mediterranean and temperate regions, earliness is highly desirable, since, if combined with autumn sowing, the accumulation of precipitations during the winter is much better used during the growing season and assists in overcoming occasional droughts and climatic perturbations (Mikić *et al.* 2011).

To conclude, we must outline the importance of genotypes such as HR1 which seemed to be wide adapted, showing nice yields and stability within different environments. Furthermore, cultivar Desso, which showed a nice yield and endurance in South Mediterranean areas could be an interesting genotype for further drought studies regarding the molecular basis of drought stress tolerance, but also to be considered as a source of genetic variability in breeding programs developed in the context of climate change, due to the current and increasing necessity of legume crops with good development under drought conditions (Graham *et al.* 2003). At the same time, further deeper physiological studies with these genotypes under controlled conditions could be of most interest in order to unravel the mechanisms of drought tolerance and sensitivity residing on the genetic pool within each genotype.

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### Conflicts of interest

The authors declare no conflicts of interest.

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## FIGURE LEGENDS

**Figure 1.** Principal Component Analysis (PCA) for climatic and edaphic variables.

## FIGURES

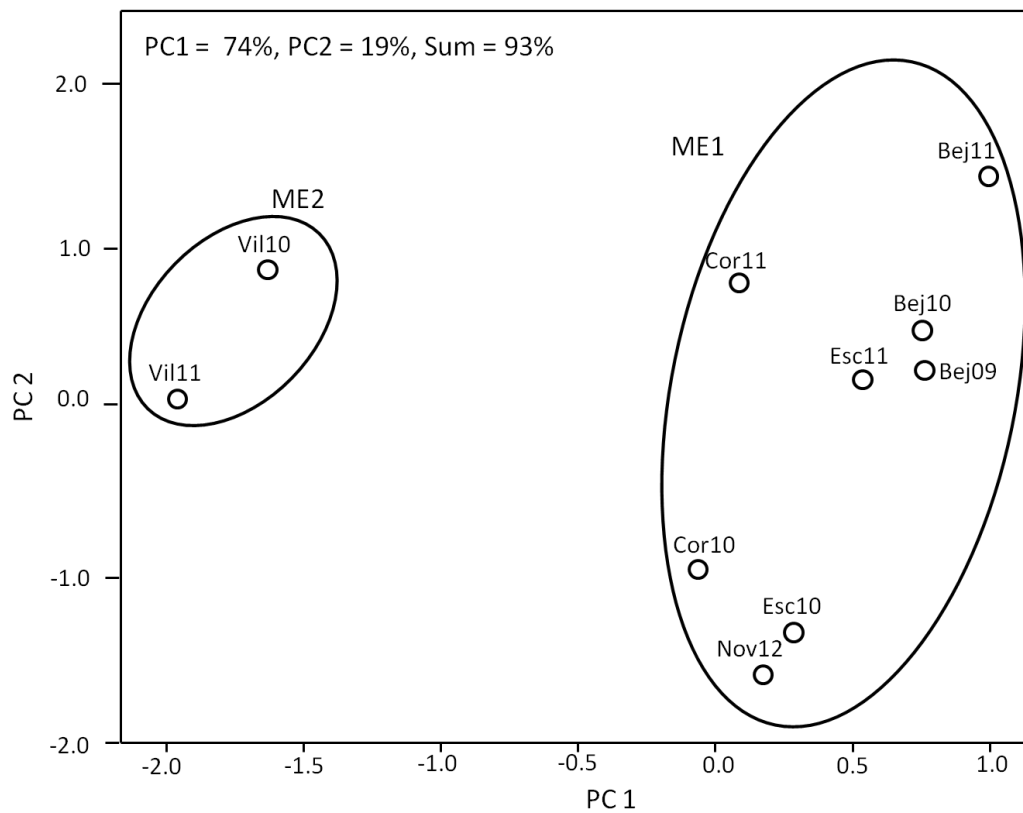


Figure 1