



Article

# Adaptation of Grass Pea (*Lathyrus sativus*) to Mediterranean Environments

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**Abstract:** Grass pea (*Lathyrus sativus*) is an annual legume crop widely cultivated in South Asia and Sub-Saharan Africa, but in regression in Mediterranean region. Its rusticity and nutritious value is calling back attention for its reintroduction into Mediterranean rain-fed farming systems. We studied the adaptation of a range of breeding lines in multi-environment field testing in Spain and Egypt, showing wide variation for grain yield. Broomrape (*Orobanche crenata*) infection appeared as the major limiting factor in both countries. Level of broomrape infection was highly influenced by environmental conditions, being favored by moderate temperatures at crop flowering and rain and humidity after flowering. The additive main effects and multiplicative interaction (AMMI) analysis was applied to understand the interaction between genotype (G) and environment (E) on grain yield and on broomrape infection. AMMI analyses revealed significant G and E effects as well as G\*E interaction with respect to both traits. The AMMI analysis of variance (ANOVA) revealed that both, yield and broomrape infection were dominated by the environment main effect. AMMI1 biplot for grain yield revealed Ls10 and Ls11 as the accession with highest yields, closely followed by Ls16, Ls18 and Ls19. However, these accessions showed also lower stability, being particularly adapted to Delta Nile conditions. On the contrary, accessions Ls12 and Ls14 were more adapted to rain fed Spanish conditions. Accessions Ls7, Ls1 and Ls3 were the most stable over environments for grain yield.

**Keywords:** legumes; yield; broomrape; genotype\*environment interaction

## 1. Introduction

Grass pea (*Lathyrus sativus* L.) is an annual cool season legume grown for human food but also for animal feed and as forage since ancient times in many of the harshest agro-environments of the world [1]. It is still grown in 1.5 Mha, mainly in South Asia and Sub-Saharan Africa, but is in regression in Mediterranean environments. As world demand for legume protein increases, the rusticity of grass pea recalls its potential for cropping systems in marginal environments as those in Mediterranean cropping systems [2–6]. This called for a renewed interest to gather and characterize the adaptation of landraces and to submit them to breeding in order to exploit the potential of the species [7–11].

However, grass pea suffers from a reputation of being toxic, because under certain circumstances its overconsumption has caused neurolathyrism, a neurodegenerative disease in humans and domestic animals, due to its content of the neuroexcitatory  $\beta$ -N-oxalyl-L- $\alpha$ , $\beta$ -diaminopropionic acid ( $\beta$ -ODAP) [6,12]. This directed most efforts in breeding to the development of cultivars with a low  $\beta$ -ODAP [1,13]. However, the long-term results of these efforts are often questioned [3,4,6,12]. The  $\beta$ -ODAP content is highly influenced by climatic and edaphic conditions and has a strong

genotype  $\times$  environment effect [3,14,15]. It is today understood that the occurrence of the disease can be prevented by addition of sufficient cereals and fruits and vegetables in the diet [6,16]. Moreover, seeds can be partly detoxified by various food processing methods [4,17]. Also,  $\beta$ -ODAP is today looked as a multifunctional plant metabolite with a number of therapeutic potentials [6,12]. All this made breeders reconsider yield stability as a higher concern. Therefore, the objective of this research was to evaluate the performance and stability of yield among grass pea accessions in Mediterranean cropping systems. Yield stability is a complex trait. Its expression depends on factors such as the genotype (G), the environment (E) and G $\times$ E interaction. G $\times$ E is the most common feature resulting from the differential expression of G over the E which complicate the selection of a genotype for a target trait. This is of most relevant in breeding programs, as large G $\times$ E brings about discrepancies between expected and observed responses to selection due to a higher estimation of genetic variances. Under such conditions, stability analysis provides the best solution for the relative performance of G over the E. Genotype-by-Environment (G $\times$ E) interactions have already been reported for grain yield of grass pea [8–10], thus hampering proper selection. Here we use the multivariate Additive Main Effect and Multiplicative Interaction (AMMI) model [18,19] to give the correct picture of G $\times$ E interaction on G. The AMMI model uses analysis of variance (ANOVA) and principal component analysis (PCA) to achieve a better understanding of G $\times$ E interaction, its causes and consequences.

## 2. Materials and Methods

### 2.1. Plant Material and Experimental Design

Performance of 19 grass pea breeding accession was studied at 7 location–year environments (Table 1). At each location, a randomized complete block design with three replications was used. The experimental unit consisted of 1 m long rows, one per accession, separated 35 cm, 10 plants per row. Sowing took place by middle December each season. Following local practice, Spanish plots were rain-fed, whereas those of Egypt were flood irrigated (4 irrigations of 40 mm each). Weeds were controlled by hand weeding. Number of emerged broomrape plants per row were recorded and referred as number of broomrapes per grass pea plant. Attention was paid to record presence and to quantify naturally occurring pests and disease. The harvest of the plants took place by late April, May, depending on the environment.

**Table 1.** Description of the environments (combination of location and season) of the trials of the multi-environment study. Summary climatic data corresponding to each growing season are provided (more detailed data used in Figures 3 and 4 are not listed here).

Environm	Location	Soil Type	Soil pH	Latitude	Longit.	Altit. (m)	Season	AvT <sub>max</sub> (°C)	AvT <sub>min</sub> (°C)	Rain (mm)
CAMP08	Campillo, Spain	Vertisol	7.5–8	37°20' N	4°51' W	461	2007–08	18.8	7.8	264
TOM09	Tomejil, Spain	Vertisol	7–7.5	37°30' N	5°57' W	12	2008–09	22.6	7.5	219
CORD09	Córdoba, Spain	Fluvisol	6.5–7	37°50' N	4°50' W	90	2008–09	21.7	7.9	280
ESC08	Escacena, Spain	Fluvisol	7–7.5	37°25' N	6°15' W	88	2007–08	20.7	10.1	391
ESC09	Escacena, Spain	Fluvisol	7–7.5	37°25' N	6°15' W	88	2008–09	21.4	9.4	252
KAFR08	El-Sheik, Egypt Kafr	Entisol	7.5–8	30°47' N	30°59' E	0	2007–08	23.9	5.4	276 <sup>B</sup>
KAFR09	El-Sheik, Egypt	Entisol	7.5–8	30°47' N	30°59' E	0	2008–09	23.4	8.3	193 <sup>B</sup>

<sup>B</sup> this includes the irrigations performed at KAFR08 and 09, consisting on 4 flooding irrigations of 40 mm.

## 2.2. Statistical Analysis

Data for each trait (grain yield and number of broomrapes per plant) were submitted to a combined analysis of variance (ANOVA) with genotype and location-year (environment) as fixed factors using SAS<sup>®</sup> 9.3 (SAS Institute Inc., Cary, NC, USA). F-ratios were used to test effects for randomized complete block experimental design [20]. Prior to each ANOVA, tests for normality and equality of variance were conducted for each dependent variable. ANOVA analysis to quantify the contribution of genotype, environment and their interaction (G\*E) was performed according to the statistical model:

$$y_{egk} = \mu + E_e + G_g + G^*E_{eg} + B_{k(e)} + \varepsilon_{egk}$$

where  $y_{egk}$  was the yield or the number of broomrapes per plant of the  $k$  block of the  $g$  genotype in the  $e$  environment;  $\mu$  the overall mean of the group of genotypes;  $E_e$  the effect of the  $e$  environment,  $G_g$  the effect contributed by the  $g$  genotype;  $G^*E_{eg}$  the effect interaction between the  $g$  genotype and the  $e$  environment;  $B_{k(e)}$  is the replication effect for  $k$  at environment  $e$  and  $\varepsilon_{egk}$  was the residual error effect.

## 2.3. AMMI Analysis

AMMI statistical model structure and computational methods were used in the current study as previously described [21–23]. The AMMI method combines the traditional analysis of variance for the main effects of G and E as well as the principal component analysis (PCA) with multiplicative parameters which was used for the partition of the G\*E interaction into several components [21]. Analyses were made with the SAS<sup>®</sup> 9.4 (SAS Institute Inc., Cary, NC, USA) [24] to graph AMMI biplots.

The AMMI model [21] is given by:

$$y_{ge} = \mu + \alpha_g + \beta_e + \sum_{n=1}^N \lambda_n \gamma_{gn} \delta_{en} + \varepsilon_{ge}$$

where  $Y_{ge}$  is either the yield or the number of broomrapes per plant of the  $g$ -th genotype in the  $e$ -th environment;  $\mu$  is the grand mean;  $\alpha_g$  and  $\beta_e$  are the genotype and environment deviations from the grand mean, respectively;  $\lambda_n$  is the eigenvalue of the PCA axis  $n$ ;  $\gamma_{gn}$  and  $\delta_{en}$  are the genotype and environment principal component scores for axis  $n$ ;  $N$  is the number of principal components retained in the model; and  $\varepsilon_{ge}$  is the residual term.

A simple protocol for using AMMI effectively was used here according to Gauch [22]. This protocol includes four steps: (1) analysis of variance, (2) model diagnosis, (3) mega-environment delineation, and (4) agricultural recommendations.

(1) Analysis of variance: the sums of squares (SS) for genotypes (G), G\*E signal ( $GE_s$ ), and G\*E noise ( $GE_N$ ) provide a preliminary indication whether AMMI analysis will be worthwhile. AMMI analysis is appropriate for datasets having substantial G and substantial  $GE_s$  [22]. To estimate the SS for  $GE_N$  we multiplied the error mean square from replication by the number of degrees of freedom (df) for G\*E. Also we obtained  $GE_s$  by subtracting  $GE_N$  from G\*E. According to the high SS values of G and  $GE_s$ , the AMMI model was employed to study the GEI of grass pea yield and number of broomrapes per plant.

(2) Model diagnosis: 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 [22]. Malik et al. [25] proposed resampling-based methods for multi-environment trial data with replicates, which are free from distributional assumptions. The R code for the proposed method [25] was used. The  $p$  values of the resampling-based tests were derived using  $B = 5000$  bootstrap samples.

(3) Mega-environment delineation: we obtained a ranking table showing the ranks for the accessions in each environment. Mega-environments can be displayed by this table [22]. In order to characterize testing sites in terms of environmental factors which would possibly support

the AMMI analysis results, a Non-Metric Multidimensional Scaling (NMDS) ordination [26] was performed. Climate data for each location [maximum, minimum and average temperature, maximum, minimum and average humidity, evapotranspiration and accumulated rain during pre-flowering, at flowering and post-flowering period, also during the growing season] were obtained from the Red Andaluza de Estaciones Meteorológicas (<https://www.juntadeandalucia.es/agriculturaypesca/ifapa/riaweb/web/estaciones>) for Spanish locations, and from Metrological Station, Rice Research and Training Center, ARC, Sakha, for Egyptian ones.

(4) Drawing recommendations on best accessions: the goal of yield-trial research is the identification of the best performing genotypes [22]. For this purpose, we used AMMI1 biplot graphs, in which the displacements along the abscissa indicate differences in main (additive) effects whereas; displacements along the ordinate indicate differences in the interaction effects. This was complemented with the rankings of the best accessions per environment in terms of yield and broomrape infection estimated by AMMI1.

### 3. Results

Results showed the impact of the environment on the yield and broomrape infection on grass pea accessions. Grain yields of the accessions for each environment are shown in Table 2. Global average for grain yield over environments and accessions was 1883 kg ha<sup>-1</sup>. Average yield over environments was highest for Ls10 (2762 kg ha<sup>-1</sup>) and lowest for Ls12 (1368 kg ha<sup>-1</sup>). Average yield over accessions was highest at TOM09 (4103 kg ha<sup>-1</sup>) and lowest at CAMP09 (407 kg ha<sup>-1</sup>).

**Table 2.** Mean grain yield (kg ha<sup>-1</sup>) of 19 grass pea accessions grown at 7 location–year environments.

		Grain Yield (kg ha <sup>-1</sup> ) in Different Environments								
Accession	Synom.	SPAIN				EGYPT				Mean
		CAMP08	CORD09	ESC08	ESC09	TOM09	KAFR08	KAFR09		
Ls1	Sel190	533	926	667	1567	2903	2599	2343	1648	
Ls2	Sel288	340	1021	667	1747	4468	2189	2069	1786	
Ls3	Sel289	340	1370	645	1614	3631	2302	1889	1685	
Ls4	Sel290	507	978	440	1786	4398	1903	2267	1754	
Ls5	Sel299	355	900	520	1638	4238	2465	1908	1718	
Ls6	Sel387	467	934	300	1692	4402	1459	1569	1546	
Ls7	Sel390	393	873	457	1620	4220	2737	2717	1860	
Ls8	Sel449	320	1238	889	1984	4724	2237	1995	1912	
Ls9	SelB111	311	942	600	1708	4343	2076	1884	1695	
Ls10	SelB222	140	1556	1534	2343	3963	4999	4800	2762	
Ls11	Sel2177	607	2011	908	1619	4325	4789	4087	2621	
Ls12	Sel2119	207	685	329	1436	4035	1373	1507	1368	
Ls13	SelETH-7	307	985	400	1745	4374	1214	1674	1528	
Ls14	SelETH-15	160	716	334	1472	4146	1604	978	1344	
Ls15	Sel945	440	534	806	1637	2565	2800	2787	1653	
Ls16	Sel1784	613	515	1045	2688	3892	3427	5092	2468	
Ls17	Sel1942	338	840	480	1590	4256	2117	1861	1640	
Ls18	Sel1959	547	1197	1227	1464	4083	4808	3399	2389	
Ls19	Lisa	810	753	703	1988	4985	3988	3567	2399	
<b>Mean</b>		407	999	682	1755	4103	2689	2547	1883	
<b>SE</b>		30	57	52	50	117	208	178	76	

No significant levels of infection of insect pests and fungal diseases were noticed at any site. However, high infection by broomrape infection was recorded in most sites (Table 3). Global average of broomrape infection over accessions and environments was 0.98 broomrapes per grass pea plant. Average infection over accessions was lowest at TOM09 and highest at KAFR08 (0.04 and 1.91 broomrapes per plant, respectively). Average infection over environments was lowest on Ls3 and highest on Ls6 (0.31 and 1.69 broomrapes per plant, respectively).

**Table 3.** Number of broomrapes per plant of 19 grass pea accessions grown at 7 location–year environments.

Accession	CAMP08	CORD09	ESC08	ESC09	TOM09	KAFR08	KAFR09	Mean
Ls1	0.13	0.48	0.37	0.34	0.0	0.50	0.56	0.40
Ls2	0.23	1.15	0.63	0.66	0.0	2.18	0.86	0.95
Ls3	0.10	0.40	0.30	0.47	0.0	0.38	0.19	0.31
Ls4	0.37	1.12	0.50	0.49	0.0	2.52	1.47	1.08
Ls5	0.10	1.02	0.70	0.79	0.0	2.07	1.94	1.10
Ls6	0.33	1.58	0.63	0.68	0.0	3.61	3.30	1.69
Ls7	0.17	0.98	0.50	0.51	0.0	0.99	0.69	0.64
Ls8	0.00	0.80	0.57	0.40	0.0	1.02	1.18	0.66
Ls9	0.03	0.98	0.60	0.59	0.0	1.54	1.51	0.88
Ls10	0.10	1.63	0.50	0.53	0.0	0.99	1.00	0.79
Ls11	0.10	0.83	0.40	0.39	0.03	2.40	1.64	0.96
Ls12	0.27	1.18	0.63	0.68	0.0	3.20	3.30	1.54
Ls13	0.13	0.97	0.50	0.45	0.0	1.26	1.32	0.77
Ls14	0.27	1.17	0.60	0.67	0.0	2.59	2.47	1.29
Ls15	0.17	0.93	0.63	0.38	0.0	2.21	1.72	1.01
Ls16	0.20	1.15	0.53	0.55	0.0	1.65	1.49	0.93
Ls17	0.40	1.22	0.50	0.74	0.0	3.28	2.47	1.43
Ls18	0.03	0.28	0.57	0.46	0.0	0.85	0.70	0.48
Ls19	0.50	0.98	0.83	1.40	0.65	3.09	3.00	1.63
Mean	0.19	0.99	0.55	0.59	0.04	1.91	1.62	0.98
SE	0.03	0.08	0.02	0.03	0.03	0.14	0.14	0.05

The combined analysis of variance for grain yield and number of broomrapes per plant revealed that all main effects (environments (E), genotypes (G) and G\*E interaction) were statistically significant (Tables 4 and 5). Environment was the most important factor for both traits (Tables 4 and 5), retaining more than a half of the variance contribution while, G and G\*E showed similar contribution. Environment explained for 76% of the total variation (G + E + G\*E sum of squares (SS)) for yield, whereas G and G\*E accounted for 9% and 15%, respectively (Table 4). For number of broomrapes per plant, E, G and G\*E accounted for 60%, 17% and 23%, respectively (Table 5).

**Table 4.** Analysis of variance for yield (kg ha<sup>-1</sup>) of 19 accessions of grass pea in 7 environments (DF: degrees of freedom; SS: sum of squares; MS: mean square; G\*E: term of the genotype-by-environment interaction).

Source	DF	SS	MS	% Variation
Environment (E)	6	594,938,551	99,156,425 ***	76 <sup>A</sup>
Replication/E	14	10,326,066	737,576	
Genotype (G)	18	68,746,582	3,819,254 ***	9 <sup>A</sup>
G*E	108	113,679,415	1,052,587 ***	15 <sup>A</sup>
IACP1	23	82,737,353	3,597,276 ***	73 <sup>B</sup>
IACP2	21	14,543,665	692,555	13 <sup>B</sup>
Residual	64	16,398,397	256,224	
Error	252	120,081,873	476,515	
Total	398	907,772,488		

\*\*\* Significant at  $p < 0.0001$  level of probability, IPCA: principal component of interaction. <sup>A</sup> % variation respect (E + G + G\*E) SS. <sup>B</sup> % variation respect (G\*E) SS.

**Table 5.** Analysis of variance for number of broomrape per plant of 19 genotypes of grass pea in 6 environments (DF: degrees of freedom; SS: sum of squares; MS: mean square; G\*E: term of the genotype \* environment interaction).

Source	DF	SS	MS	% Variation
Environment (E)	6	171	28.5 ***	60 <sup>A</sup>
Replication/E	14	3	0.19	
Genotype (G)	18	48	2.64 ***	17 <sup>A</sup>
G*E	108	64	0.58 ***	23 <sup>A</sup>
IACP1	23	54	2.32 ***	84 <sup>B</sup>
IACP2	21	5	0.23	8 <sup>B</sup>
Residual	64	5	0.08	
Error	252	38	0.15	
<b>Total</b>	<b>398</b>	<b>322</b>		

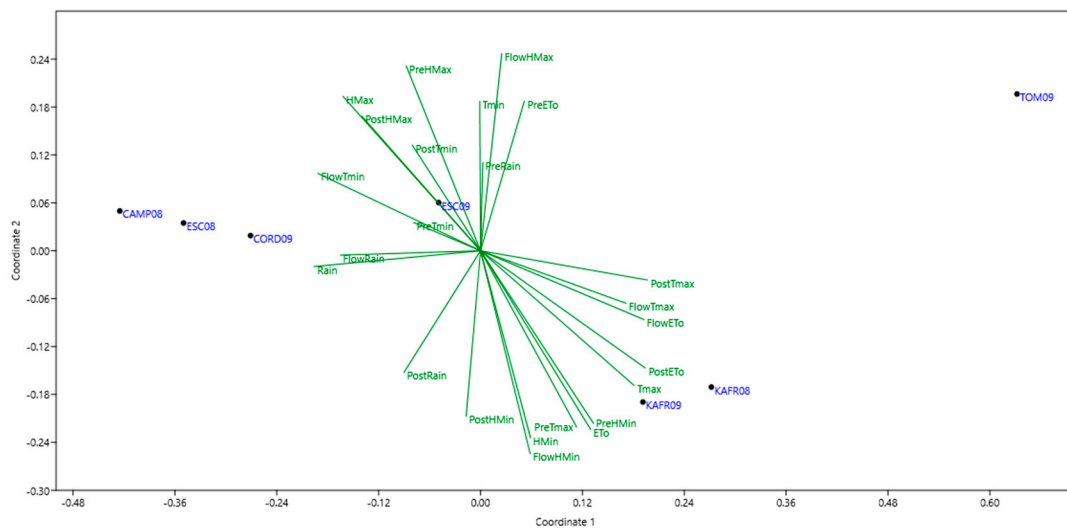
\*\*\* Significant at  $p < 0.0001$  level of probability, IPCA: principal component of interaction. <sup>A</sup> % variation respect (E + G + G\*E) SS. <sup>B</sup> % variation respect (G\*E) SS.

Tables 4 and 5 also show the degrees of freedom (DF) for the two first principal components of interaction (IPCAs) assigned according to the method of Gollob [27]. In Table 4, the SS for G is 68,746,582, for  $GE_N = 108 \times 476,515 = 51,463,620$ , and for  $GE_S = 113,679,415 - 51,463,620 = 62,215,795$ . In Table 5, the SS for G is 48, for  $GE_N = 108 \times 0.15 = 16.2$ , and for  $GE_S = 64 - 16.2 = 47.8$ . In both cases, the  $GE_S$  was as large as the SS of G. Therefore, AMMI analysis was quite likely to be used worthwhile [13].

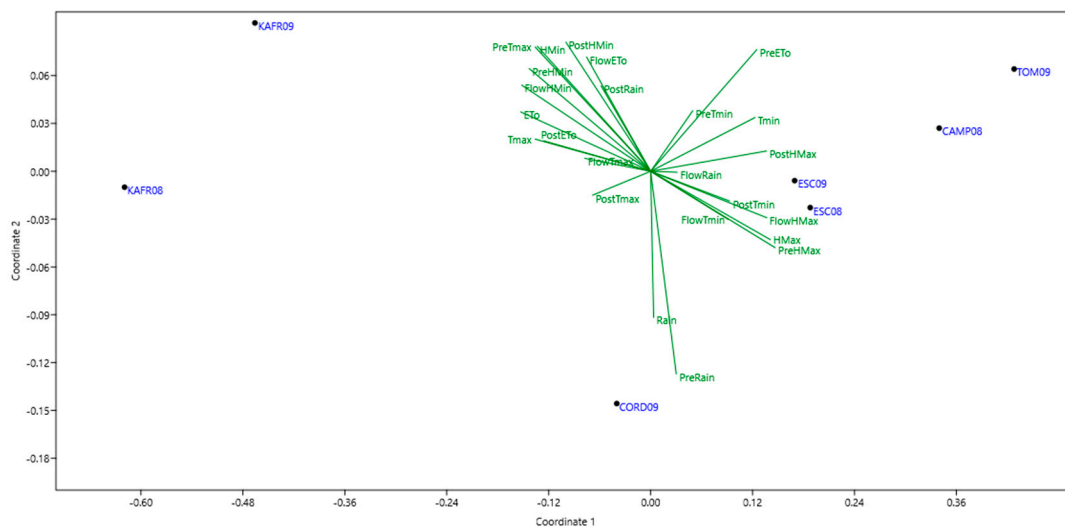
### 3.1. Model Diagnosis

According to Malik et al. [25] it is crucial to decide how many significant multiplicative interaction terms to retain. They propose resampling-based methods for multi-environment trial data with replicates, which are free from the distributional assumptions. The  $p$  values of the resampling-based tests were derived using  $B = 5000$  bootstrap samples. For yield and  $K + 1 = 1$  the test statistic was of 0.2296693 and a  $p$ -value of 0.0000 whereas, for  $k + 1 = 2$  the test statistic was of 0.04037152 and a  $p$ -value of 0.5420. For the number of broomrapes per plant and  $K + 1 = 1$  the test statistic was of 0.4515335 and a  $p$ -value of 0.0000 whereas, for  $k + 1 = 2$  the test statistic was of 0.04538442 and a  $p$ -value of 0.490. For both traits, the resampling-based methods resulted in the same number of significant multiplicative terms. This test suggested that an AMMI model with only one multiplicative term was appropriate for the grass pea dataset. Therefore, the most precise model was the AMMI1.

Correlation coefficients can be calculated between IPCA scores and various genetic or environmental measurements to discern causal factors. For grain yield environmental IPCA1 was positive significantly correlated with evapotranspiration and minimum humidity ( $r^2 = 0.87$ ,  $p < 0.01$  and  $r^2 = 0.93$ ,  $p < 0.01$ , respectively), and maximum temperature in pre-flowering ( $r^2 = 0.90$ ,  $p < 0.01$ ). It was also negative significantly correlated to the maximum humidity in growing season ( $r^2 = -0.83$ ,  $p < 0.01$ ). For the number of broomrapes per plant environmental IPCA1 was positive significantly correlated to the growing season evapotranspiration and minimum humidity ( $r^2 = 0.97$ ,  $p < 0.01$  and  $r^2 = 0.88$ ,  $p < 0.01$ , respectively), and maximum temperature in pre-flowering ( $r^2 = 0.89$ ,  $p < 0.01$ ) and it also was negative significantly correlated to the maximum humidity in growing season ( $r^2 = -0.88$ ,  $p < 0.01$ ) and minimum temperature in flowering ( $r^2 = -0.82$ ,  $p < 0.01$ ). These results are in agreement with NMDS (Figures 1 and 2) and with the Principal Components analysis with climatic parameters (not shown).



**Figure 1.** Non metric multidimensional scaling (NMDS) analysis of climate variables including: maximum temperature (Tmax), minimum temperature (Tmin), maximum humidity (Hmax), minimum humidity (Hmin), Evapotranspiration (ETo) and rain during different growing stages [pre-flowering (Pre), flowering (Flow), post-flowering (Post) and complete growing season] characterizing the seven environments, used for phenotyping grain yield.



**Figure 2.** Non metric multidimensional scaling (NMDS) analysis of climate variables including: maximum temperature (Tmax), minimum temperature (Tmin), maximum humidity (Hmax), minimum humidity (Hmin), Evapotranspiration (ETo) and rain during different growing stages [pre-flowering (Pre), flowering (Flow), post-flowering (Post) and complete growing season] characterizing the seven environments, used for phenotyping the number of broomrapes per plant.

### 3.2. Mega-Environments (MEs) Delineation and Description

In the next step of our analysis, we employed the most precise AMMI model to determine the existence of mega-environments [22]. Table 6 is a ranking table listing the top 6 accessions in each of the 7 environments according to AMMI1. This model is of particular interest because is suitable for mega-environment delineation [22]. In our case, AMMI1 is also the model which optimizes predictive accuracy according to the resampling-based method [25]. According to AMMI1 analysis, accessions Ls10 and Ls11 were ranked winner for grain yield in most locations (Group 1, sites suffering

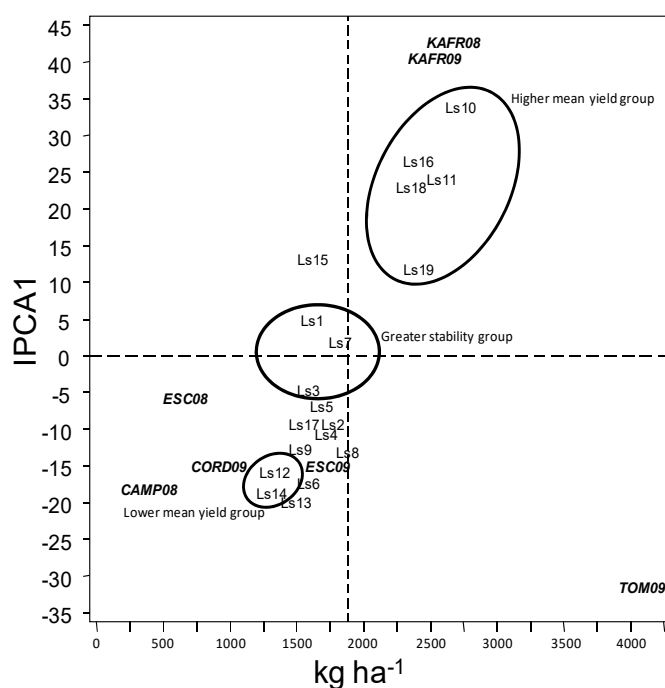
from broomrape infestation), whereas accessions Ls8 and Ls13 were the winners for this trait in TOM09 (Group 2, the only site with little broomrape). Different cultivars were winners in the case of the number of broomrapes per plant, being Ls3 and Ls1 in the first rank positions (lowest infection) for most environments, with no groups detected.

**Table 6.** Ranking tables showing the top 6 accessions with highest grain yield and lowest broomrape infection in each environment, according to AMMI1.

Environment	AMMI1 Rank for Grain Yield						AMMI1 Rank for Number Broomrapes Per Plant					
	1	2	3	4	5	6	1	2	3	4	5	6
CAMP08	Ls11	Ls19	Ls10	Ls8	Ls16	Ls18	Ls3	Ls1	Ls18	Ls11	Ls8	Ls5
CORD09	Ls11	Ls10	Ls19	Ls8	Ls16	Ls18	Ls3	Ls1	Ls18	Ls8	Ls7	Ls11
ESC08	Ls10	Ls11	Ls19	Ls8	Ls16	Ls18	Ls18	Ls3	Ls11	Ls1	Ls8	Ls15
ESC09	Ls10	Ls11	Ls19	Ls16	Ls8	Ls18	Ls3	Ls1	Ls18	Ls11	Ls8	Ls13
KAFR08	Ls10	Ls11	Ls16	Ls18	Ls19	Ls15	Ls3	Ls1	Ls18	Ls7	Ls10	Ls8
KAFR09	Ls10	Ls11	Ls16	Ls18	Ls19	Ls15	Ls3	Ls1	Ls18	Ls7	Ls10	Ls8
TOM09	Ls8	Ls13	Ls6	Ls2	Ls4	Ls9						

### 3.3. Selection or Recommendation of the Best Accessions

Accessions falling almost on a horizontal line in the AMMI1 biplot (Figure 3) have similar interaction pattern lines or environments. For instance, accessions Ls8 and Ls9 were clearly observed from the biplot for similar interaction patterns. Similarly, environments KAFR08 and KFR09 showed similar interaction patterns. On the other hand, accession or environments appearing almost on the vertical line have similar means. For instance, accessions Ls19, Ls18 and Ls16 are shown by the biplot having similar means above general mean of the trial. Also, accession or environment on the right side of the midpoint on the vertical lines have higher mean yield than those on the left side. As a result, it is quite evident from the biplot (Figure 3) that accessions with highest yield were Ls10 and Ls11, followed close by Ls16, Ls18 and Ls19. In contrast, Ls12 and Ls14 were those with lowest yield.



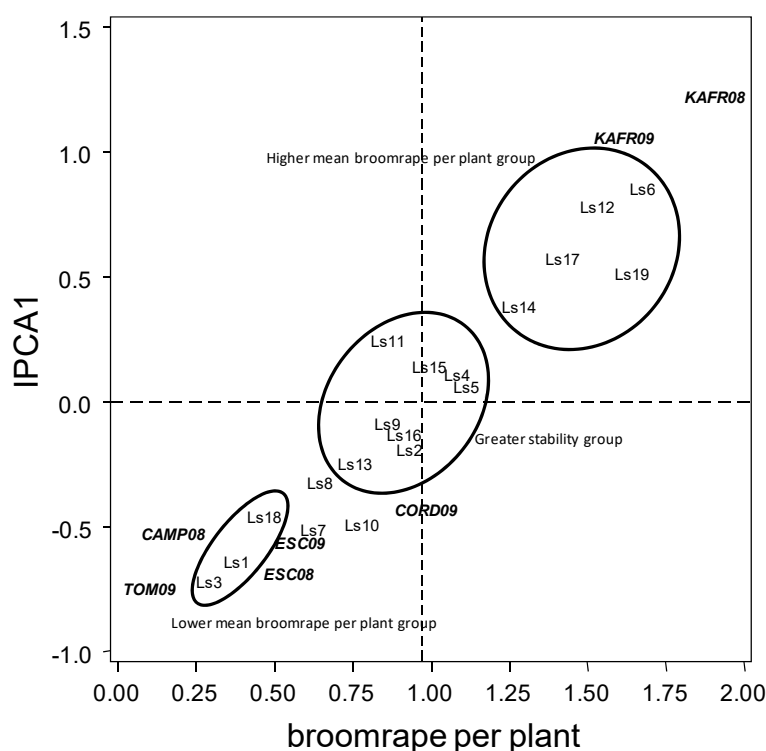
**Figure 3.** AMMI1 Biplot display for mean yield and IPCA 1 scores of the 19 grass pea lines tested across seven environments. Vertical line at the centre of biplot is the general grand mean.



Environments on the right hand side of the midpoint of the main effect axis suggested that these environments were more favorable for yield (TOM09, KAFR08 and KAFR09), whereas those to the left (CAMP08, ESC08 and CORD09) were less favorable. Likewise, lines or environments with IPCA1 scores of zero or near zero (either positive or negative) have small interactions and show greater stability and adaptation over the test environment. On the contrary, a large IPCA1 scores indicate high interaction effect and reflect adaptation to specific environments. In this present case, ESC08 was the environment with lowest IPCA1 scores whereas KAFR08, KAFR09 and TOM09 were those with highest scores, therefore there were accessions specifically adapted to these environments.

Accessions Ls7, Ls1 and Ls3 recorded the lowest IPCA1 scores and showed small interactions (close to vertical axis). On the contrary, accessions Ls18, Ls16, Ls11 and Ls10 recorded the highest IPCA1 score. This implied that high interaction effect of lines had specific stability and adaptations to particular environments.

The stability of responses to broomrape can be seen in the AMMI1 biplot (Figure 4). Accessions Ls6, Ls19, Ls12, Ls17 and Ls14 interacted positively with KAFR08 and KAFR09 (all in the right upper corner of the biplot), indicating that they are best suited for these environments, but negatively with the remaining environments. Similarly, accessions Ls3, Ls1, Ls18, Ls7 and Ls10 interacted positively with CAMP08, ESC08, ESC09, and CORD09 (all in the left down corner), but negatively with KAFR08 and KAFR09. The group of lines Ls11, Ls15, Ls4 and Ls5 and the group of lines Ls9, Ls16, Ls2 and Ls13 have low interaction and hence stable over environments.



**Figure 4.** AMMI1 Biplot display for broomrape per plant and IPCA 1 scores of the 19 grass pea lines tested across seven environments. Vertical line at the centre of biplot is the general grand mean.

With the help of the rankings shown in Table 6 we can recommend for broomrape infection Ls3, Ls1 and Ls18 in spite of their low value in Figure 4, showing that they are better adapted to Egyptian than to Spanish locations. The most interesting accessions would be those with high yield and low broomrape infection, particularly Ls10, Ls11 and Ls18.

#### 4. Discussion

Common experience in Mediterranean countries is a severe decrease in grass pea cultivation during the last 50 years, with little or negligible trade. However, farmers share an appreciation on derived food products and show interest in the crop, provided that better adapted cultivars are made available. As a highly nutritive food/feed crop and its high drought tolerance with minimal input requirements for its cultivation, grass pea provides food and nutrition security to many low-income communities.

Great morphological variation is reported in grass pea, showing a clear grouping in two major types, one covering blue flower and colored seeds, and other white flowers and seeds [28]. The first group is typical of Indian subcontinent whereas the second has more western distribution. Our Ls accessions correspond to the first group, whereas Ls19 has larger and whiter seeds, the type preferred by Mediterranean consumers. Mediterranean accessions are regarded also to have lower content of  $\beta$ -ODAP [13], although we did not assess this trait. The  $\beta$ -ODAP content is highly influenced by climatic and edaphic conditions and has a strong G\*E effect [3,14,15]. This, together with the current understanding that the disease is caused only when there is overconsumption of grass pea in a non-balanced diet, made breeders reconsider yield stability as a higher concern. We therefore focused in this study on adaptation of current breeding lines to different farming systems in the Mediterranean Basin, identifying the more productive for each of the studies locations and the more stable ones. AMMI1 biplot (Figure 3) allowed identification of the accessions better adapted to Delta Nile conditions (Ls10, Ls11, Ls16, Ls18 and Ls19) and to rain fed Spanish conditions (Ls12 and Ls14) as well as those most stable over environments (Ls7, Ls1 and Ls3).

We also paid attention to other factors potentially affecting yield, such as pests and diseases. Grass pea has been reported to be affected by a number of pests and diseases, including aphid, weevil, rust, powdery mildew or fusarium wilt [6,29]. However, in Mediterranean regions, the weedy root parasite crenate broomrape is regarded as a major constraint for grass pea cultivation [30]. Our current results confirm this, as we did not identify any other pest or disease at worrying levels in any of the locations. Grass pea appears as very sensitive crop to broomrape infection, suffering higher yield penalty than pea or faba bean [31], with 100% reduction at severities higher than four parasites per plant. In addition to the host, infection severity of broomrape strongly depends on parasitic seedbank density and on environmental factors such as temperature and rain. Level of broomrape infection was favored by moderate temperatures and high humidity at crop flowering (Figure 2), what coincides with broomrape seed germination and establishment, and by higher pluviometry after crop flowering, what coincides with broomrape development and emergence. Temperature effects have already been reported, with cool winters acknowledged to reduce infection [32–34]. Water availability is indeed acknowledged as an important factor for broomrape development [32,33,35]. Small levels of resistance have been reported in grass pea [20] which would be the result of a combination of different escape and resistance mechanisms, acting each one at different phases of the infection process as is the case in most crops [36]. AMMI1 biplot (Figure 4) allowed the identification of accessions performing better in Egypt (Ls6, Ls12, Ls14, Ls17 and Ls19), those performing better in Spain (Ls1, Ls3, Ls7, Ls10 and Ls18) and those stable over environments ((Ls2, Ls4, Ls5, Ls9, Ls11, Ls13, Ls15 and Ls16). The most interesting accessions would be those with high yield and low broomrape infection (Table 6), particularly Ls10, Ls11 and Ls18. Availability of accessions with high and stable yield offers hope for the reintroduction of the crop in Mediterranean conditions.

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