

GENOTYPE x ENVIRONMENT INTERACTION IN GRASS PEA (*Lathyrus sativus* L.) LINES

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INTRODUCTION

Lathyrus sativus L. (grass pea, in Italian “cicerchia”) has been a traditional crop for both animal consumption as forage and grain, and as a human food grain. The main qualities of this grain legume consist of its rusticity, drought tolerance and adaptability to a wide range of soil types, including the marginal ones. Also, high protein content makes their species interesting as a forage crop (Polignano et al., 2003; Crinò et al., 2004; Polignano et al., 2005a; Polignano, 2007). Although rich in protein the utilization of grass pea grain is limited by the presence of a water soluble, non protein amino acid β -N-oxalyldiaminopropionic acid (β -ODAP) which acts as a neurotoxin crippling the lower limbs when consumed in large amounts causing the disease lathyrism (Sharma et al., 2000). This has led to the crop being excluded from agricultural improvement efforts. In fact, growing of *Lathyrus* has been officially banned in some countries (Riley, 1996). In Italy, since the early ‘70s crop has decreased alarmingly and has almost disappeared. More recently, a renewed interest in grass pea cultivation is justified by the need to recover marginal lands and to provide an efficient alternative to wheat in the areas overexploited by cereal cultivation. Also, research on the use of grass pea for animal feeding will be of great importance in order to stimulate the expansion of grass pea cultivation in sustainable and low-input agricultural systems (Crinò et al., 2004). Breeding programmes evolving genotypes combining high yield with high protein content and low or no neuro-toxin (β -ODAP) are in progress all over the world (Dorrestein et al., 1998; Addis and Narayan, 2000; Hambury et al., 2000; Robertson and Abd El Moneim, 1996; Mehta and Santha, 1996; Crinò et al., 2004; Poma et al. 2007). For these reasons several research programs aimed at the collection, characterization and evaluation of grass pea germplasm have been conducted. Considerable genetic diversity, as revealed by phenological, morphological, agronomical, biochemical, molecular and quality polymorphism, exists in grass pea throughout the world (Alfaro et al.,

1997; Chowdhury and Slinkard, 2000; Chtourou-Ghorbel et al., 2001; Przybylska et al., 2000; Alba et al., 2001; Siddique et al., 1996; Bisignano et al., 2002; Polignano et al., 2003; Granati et al., 2001, 2003; De La Rosa and Varela, 1998; Costa et al., 2007; Sardinha et al., 2007; Pankiewicz., 2007; Polignano et al., 2005b; Polignano, 2007). In our previous work we have developed a core collection to include accessions relevant for genetic studies and breeding (Polignano et al., 2004). In addition we have evaluated and identified a set of elite grass pea lines useful for breeders and farmers (Polignano et al., 2005b). Genotype x environment interaction is one of the most important steps in order to encourage the utilization of the most stable genotypes from users. Numerical classificatory or pattern analysis methods have been applied more widely in comparing the responses of cultivars and/or breeding lines across environments (Mungomery et al., 1974; Shorter et al., 1977; Polignano et al., 1989; Polignano and Alba, 1995; Alba et al., 1996; Hambury et al., 1999). The present research provided additional information concerning the behaviour of a set of selected grass pea lines over different years.

MATERIALS AND METHODS

Eight elite lines extracted from the Bari grass pea core collection were compared in a replicated randomized complete block design in Matera’s Experimental Field at the “Chiancalata” Farm of the Basilicata Region in South Italy during three growing seasons: 2003-2006 (Table 1).

The lines were grown in six-row plots, each 30 m². Distance between the rows was 1.50 m with a plot density of 360, 300 and 210 g respectively for small, medium and large seed size. The soil is a clay-loam of generally medium nutrient status, so pre-sowing adequate fertilizer applications were made. A brief summary of both effective rainfall and mean monthly temperatures for each season is given in Fig. 1. Rainfall and temperature patterns for three growing seasons, as usually in the Mediterranean countries, have showed rainy autumns and declining temperatures with a final drought period and increasing temperatures in late spring. Growing season rainfall (November to June) was greater in 2005-06 relative to

the other two seasons 2004-05 and 2005-06, being 725 mm, 321 mm and 447 mm respectively.

Mean monthly temperatures at the end of each season were similar in the three seasons: 11.5 °C in 2004-05, 11.4 °C in 2005-06 and 11.3 °C in 2003-04. Five traits selected for their agronomic interest were recorded as average of five plants randomly chosen in each plot: plant height (cm from ground level to plant tip when plants were fully mature), flowering time (as days from January 1st to 50% plants with flowers), 100-seeds weight, seed yield and biomass. A univariate analysis of variance was performed with the years assumed as random, and the lines as the fixed effect. Thereafter the plot means were processed using an analysis which combined multivariate methods (Mungomery et al., 1974; Polignano et al., 1989; Alba et al., 1996) A pattern analysis approach, based on ordination and classification, is presented to identify differences among lines in mean performances and response across seasons. In particular, data analysis followed two steps: 1) – an analysis of the main components in order to summarise the information contained in the original traits in a smaller and unrelated number of variables to be represented on a smaller number of orthogonal axes; 2) – a cluster analysis utilising the first three principal components in order to differentiate the behaviour of the lines during the growing seasons. With this approach a stable line could show similar behaviour in different years. In other words, each line is represented by three vectors whose elements correspond to the behaviour of each line in each year. The analysis starts with a cluster containing the two most similar behaviour and continues for the remaining ones until it reaches a single cluster. For statistical analysis, ANOVA, PRIN COMP and PROC CLUSTER procedures from the SAS (1989) statistical software package were performed. In addition, the Stat Graph procedure from the STATISTICA for Windows software was performed to get a 3D graphical presentation.

RESULTS AND DISCUSSION

Mean values and least minimum differences (LSD) of five traits for height grass pea lines estimated in each year of cultivation are presented in table 1. Lines did show differences among growing seasons. Growing conditions were fairly typical in 2003-04 and 2005-06 with slightly warmer than normal temperatures in 2004-05. There was sufficient moisture for productive plant growth in 2003-04 and 2005-06, but not in 2004-05, when a severe drought occurred and temperatures were above normal. So, the growing season 2005-06 gave higher mean values for all traits. On the contrary, the growing season 2004-05 gave lower mean values. Intermediate mean values were

showed for the year 2003-04. Variance, mean square and significance for lines and their interaction with years are given in Table 2.

Table 1 Means and LSD for five characters observed in 8 grass pea (*Lathyrus sativus* L.) lines over three consecutive years (2003-2006)

Lines	Code	Years			Mean	LSD
		2003/ 2004	2004/ 2005	2005/ 2006		
		Flowering time (days)				
1. MG 110437-4	A	119	117	123	119	1.26
2. MG 112251-3	B	119	118	122	119	0.99
3. MG 110435-3	C	120	119	123	120	1.24
4. MG 113873-1	D	120	118	122	120	1.37
5. MG 113089-5	E	120	118	124	120	1.12
6. MG 110957-4	F	121	121	124	122	0.99
7. MG 103203-1	G	119	118	120	119	0.84
8. MG 110492-4	H	117	117	118	117	0.76
Mean		119	118	122		
LSD		1.02	1.26	0.98		
		Plant heigh (cm)				
1. MG 110437-4	A	55.9	54.9	60.3	57.0	2.60
2. MG 112251-3	B	58.1	53.3	60.2	57.2	2.30
3. MG 110435-3	C	53.3	47.3	56.6	52.0	1.83
4. MG 113873-1	D	56.0	46.7	57.9	53.5	3.48
5. MG 113089-5	E	58.1	56.7	60.0	58.2	3.27
6. MG 110957-4	F	59.6	49.9	62.4	57.3	3.52
7. MG 103203-1	G	45.2	42.4	46.0	44.5	1.20
8. MG 110492-4	H	41.8	41.2	45.7	42.9	1.16
Mean		53.1	49.5	56.1		
LSD		3.01	1.36	2.52		
		Seed Yield (kg/plot)				
1. MG 110437-4	A	3.3	4.8	5.4	4.5	0.90
2. MG 112251-3	B	3.6	3.0	3.9	3.6	0.60
3. MG 110435-3	C	3.3	3.0	4.2	3.6	0.42
4. MG 113873-1	D	3.6	3.3	4.2	3.6	0.33
5. MG 113089-5	E	3.6	3.3	3.9	3.6	0.57
6. MG 110957-4	F	3.0	2.4	3.6	3.0	0.39
7. MG 103203-1	G	4.2	4.2	4.5	4.2	0.42
8. MG 110492-4	H	4.5	3.6	4.8	4.2	0.30
Mean		3.6	3.3	1.4		
LSD		0.39	0.39	0.23		
		Biomass (kg/plot)				
1. MG 110437-4	A	9.6	9.0	11.7	10.2	0.42
2. MG 112251-3	B	8.1	6.3	9.0	7.8	0.60
3. MG 110435-3	C	9.3	7.8	10.8	9.3	0.48
4. MG 113873-1	D	9.9	10.5	11.1	10.2	0.93
5. MG 113089-5	E	8.4	7.8	9.9	8.7	0.60
6. MG 110957-4	F	7.8	6.9	9.0	7.8	0.63
7. MG 103203-1	G	9.9	9.3	11.1	10.2	0.51
8. MG 110492-4	H	9.9	9.3	10.5	9.9	0.54
Mean		9.0	8.4	10.5		
LSD		0.48	0.66	0.66		
		100-Seeds weight (g)				
1. MG 110437-4	A	23.8	23.3	23.9	23.7	4.06
2. MG 112251-3	B	30.4	30.1	31.0	30.5	7.01
3. MG 110435-3	C	38.5	38.3	39.3	38.7	6.20
4. MG 113873-1	D	40.7	39.9	41.0	40.5	5.49
5. MG 113089-5	E	26.1	26.9	26.7	26.6	7.57
6. MG 110957-4	F	27.9	27.0	27.6	27.5	0.96
7. MG 103203-1	G	34.2	34.1	34.2	34.2	1.32
8. MG 110492-4	H	32.6	32.5	32.6	32.6	0.86
Mean		31.8	31.5	32.0		
LSD		3.30	3.46	3.31		

Table 2 Analysis of variance for five characters observed in 8 grass pea lines over three consecutive years (2003-2006).

Source	Year (Y)	Error a	Line (L)	L x Y	Error b	Total
d. of f.	2	6	7	14	331	359
Character						
Flowering time (days)	489.91 ***	11.29	85.16 ***	11.51 ***	2.46	
Plant height (cm)	1538.82 ***	118.50	1635.31 ***	67.42 ***	11.84	
100-Seeds weight (g)	7.50 n.s.	96.4	1567.09 ***	1.61 n.s.	24.96	
Biomass (t ha ⁻¹)	1356.22***	34.20	480.53 ***	50.71 ***	8.99	
Seed Yield (t ha ⁻¹)	317.59 ***	10.12	134.04 ***	31.92 ***	5.95	

*** $P \leq 0.001$; n. s. = not significant

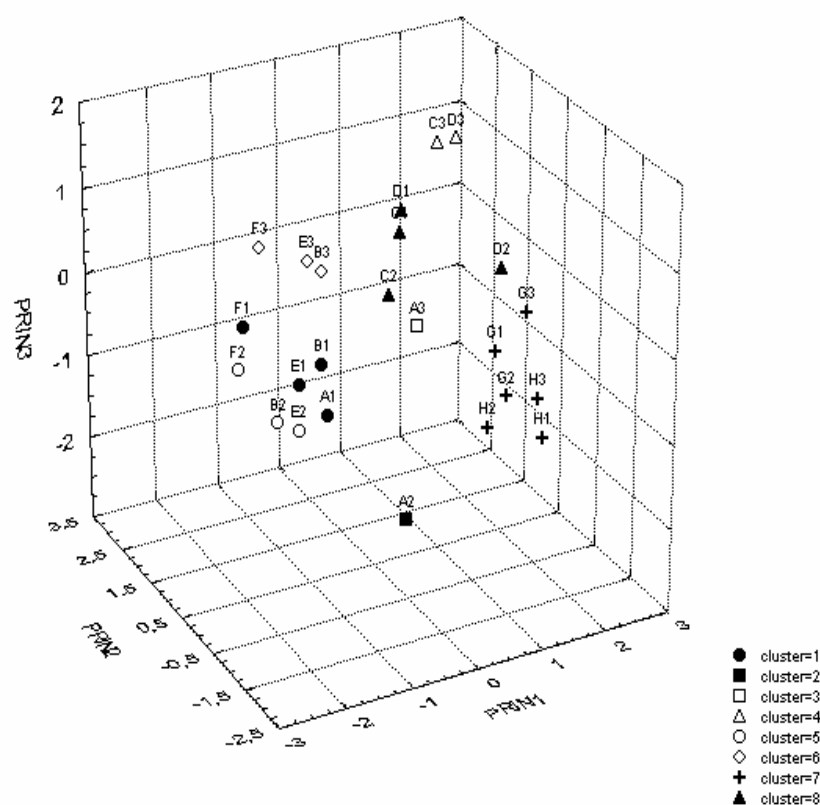


Fig. 1 Rainfall and monthly temperatures during the growing seasons.

All main effects and interaction were highly ($P \leq 0.001$) significant. For all traits, except for 100-seeds weight, the effects associated with years were most important in determining differential line responses. In other words, the year component of variance was larger than the line component for days to flowering, biomass and seed yield; similar results for seed yield are reported by Hanbury et al. (1999) and Tadesse (2003). On the contrary, the line effect was larger for plant height and 100-seeds weight. For all traits the interaction line x year was lower than the main effects. These interactions indicate that from a statistical view, the relative performance among lines was not the same from

one year to the next, which is not surprising considering the climatic differences among growing seasons. This indicates that genetic variation for flowering time, plant height, seed yield and biomass existed among the lines and that selection should be effective for these traits in future work improvement. For 100 seeds weight there was no interaction between years and lines indicating that the lines behaved similarly in all years. The mean values of each line in the three growing seasons were used in the subsequent pattern analysis based on ordination and classification procedures. The principal component analysis was done to reduce efficiently the information on response across the three growing seasons to a smaller number

of dimensions. In other words, the ordination procedure allowed the relative proximity of line performances to be visualized in a spatial model of reduced dimensions, and also indicate directions of major variation. The first three vectors obtained by the ordination procedure for all traits accounted for 93% of total variation (Table 3).

Table 3 Principal component analysis: eigenvalues, eigenvectors and percent of variation accounted for the first three principal components (PCs).

	PC1	PC2	PC3
<i>Eigenvalue</i>	1.85	1.81	0.98
Variance (%)	37	36	20
Cumul. (%)		73	93
Character		<i>Eigenvector</i>	
Seed yield	0.62	0.18	-.41
Flowering time	-.00	0.65	0.39
Plant height	-.26	0.64	0.08
Biomass	0.68	0.20	0.04
100-Seeds weight	0.29	-.28	0.82

In particular, if we consider the association coefficients between the original and transformed variables ("eigenvectors"), the first component (37%) displayed differences in the behaviour of the lines for the following traits: seed yield (.62) and biomass (.68); the second component (36%) showed different behaviour for the following traits: plant height (.64) and flowering time (.65); while, 100-seeds weight (.83) showed high loadings in the third component. A cluster analysis arranged the line performances into groups that were differentiable in terms of means and stability. In the classification of the lines, the hierarchy was truncated at 8-groups level according the number of lines tested. All lines responses in each cluster were closely related. The results of clustering were combined with those of the principal component analysis as a visual aid for discerning clusters in subsequent graphical presentation (Fig. 2).

MG 103203-1 and MG 110492-4 grass pea lines showing similar behaviour in three different years, turned out to be the one characterized by a great stability than the other lines. Lower uniformity of behaviour was displayed by MG 110437-4, MG 112251-3, MG 113089-5 and MG 110957-4 lines. The remaining lines showed less similar behaviour and therefore are present in all clusters. Our results confirmed that the grouping and ordination procedures were effective in delimiting groups of lines which differed in their environmental responses and within which the individual lines had a relatively homogeneous response.

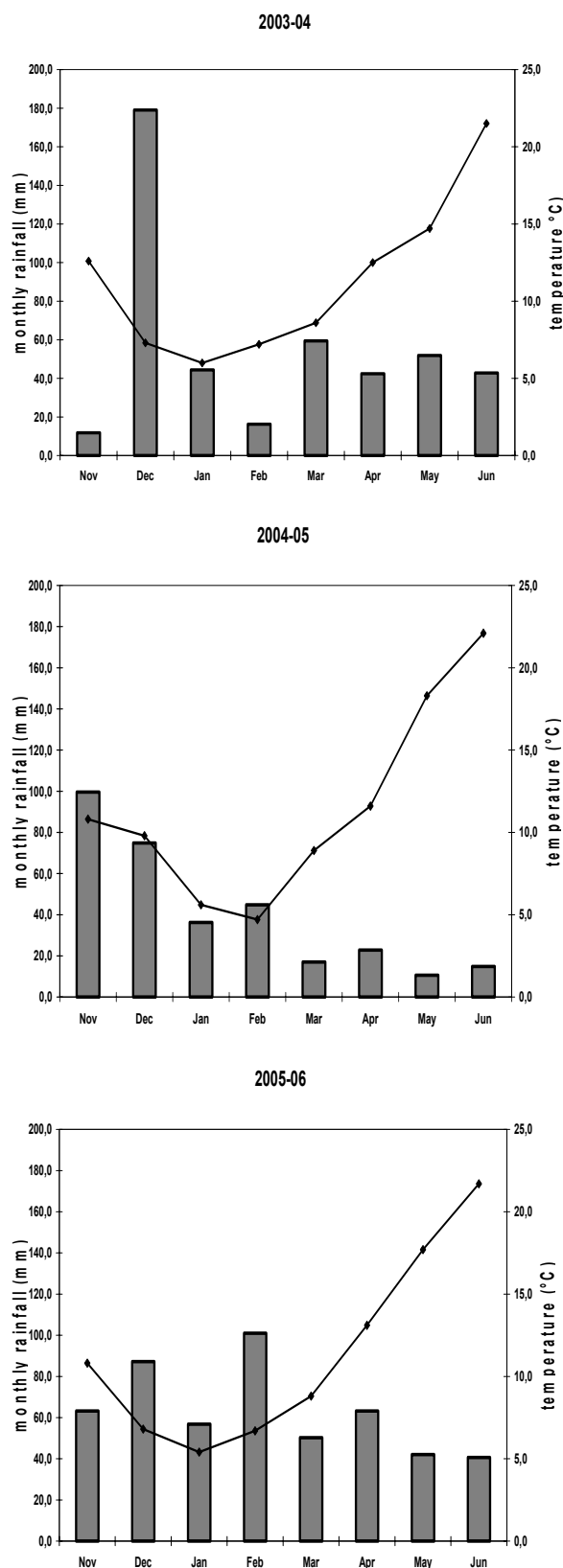


Fig. 2 A graphic representation of the behaviour of the lines according the first three principal components and identification of clusters. The behaviour in each year is represented by a letter (line code) and a number (1=2004; 2=2005; 3=2006).

ABSTRACT

Eight grass pea lines grown in three different seasons were evaluated for the stability of seed yield, 100-seeds weight, flowering time, plant height and biomass. Significant differences existed among years, lines and lines x years interaction for all traits except for 100-seeds weight. Two methods of multivariate analysis cluster and principal components were utilized to determine: firstly, whether a pattern existed among lines in their response across years and secondly to examine the relationships among them. In both analysis each line was presented as a vector whose elements were given by the performance of lines in each year. The analyses used arranged the lines into groups that were differentiable in terms of performances and stability.

CONCLUSIONS

The results presented in this paper have given an idea of the relative stability of selected grass pea lines in three different growing seasons.

The range of climatic conditions was sufficiently broad to provide for a substantial test of the lines. In fact, the effect of year was much more important than the other effects at least for flowering time, seed yield and biomass. Clear differences for these traits between the grass pea lines across growing seasons were evident.

On the contrary, the effect of line on 100-seed weight is clearly most important, while the year and year x line interaction effects were of little importance. This suggests that the seed size is a stable trait in the tested grass pea lines which have showed a good potential to respond better in most favourable growing conditions. Utilising a combined principal components and cluster analyses to examine genotype performance, the advanced grass pea lines that had a significant stability over different years were identified (MG 103203-1; MG 110492-4).

These lines were relatively indifferent to environmental variation and always had good performance.

The analysis also identified lines with average sensitivity (MG 110435-3; MG 113873-1) and lines with extreme or undesirable sensitivity (MG 110437-4; MG 112251-3; MG 113089-5; MG 110957-4). Consequently, the stable grass pea lines could be considered suitable for a broad "general" adaptability; while, development of specific grass pea lines for specific regions of production would utilize to advantage lines with narrow "specific" adaptability.

The pattern analyses used provided effective method to systematically investigate the response patterns of a set of genotypes.

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