

## Morpho-agronomic diversity in grasspea (*Lathyrus sativus* L.)

G. B. Polignano<sup>1\*</sup>, P. Uggenti<sup>1</sup>, V. Alba<sup>1</sup>, V. Bisignano<sup>1</sup> and C. Della Gatta<sup>2</sup>

<sup>1</sup>Istituto di Genetica Vegetale, C.N.R., Via Amendola 165/A, 70126 Bari, Italy and

<sup>2</sup>Dipartimento di Biologia e Chimica Agro-Forestale ed Ambientale, Sez. di Genetica e Miglioramento Genetico, University of Bari, Bari, Italy

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### Abstract

In order to describe the phenotypic diversity in a core collection of grasspea, 47 progenies of single plants (pure lines) selected from 25 accessions were evaluated. Sixteen morpho-agronomic characters including seed 3-( $\beta$ -*N*-oxaly)-L-2,3 diaminopropionic acid ( $\beta$ -ODAP) content were studied. Univariate and multivariate analyses (principal component analysis and cluster analysis) were performed to estimate differences between progenies. Some high-yielding lines could be used directly for cultivation as new varieties and/or as parental lines in crossing programmes. Seed  $\beta$ -ODAP concentration varied from 0.24% to 0.64%. However, a medium to high seed  $\beta$ -ODAP level was recorded for most of the lines, and no significant correlation was observed between this and other morphological traits.

**Keywords:** grasspea; multivariate analysis;  $\beta$ -ODAP; variation

### Introduction

Grasspea (*Lathyrus sativus* L.) is a self-pollinated, annual herbaceous legume, belonging to the tribe Viciae. Thanks to its stress tolerance, adaptability to different soils and adverse climatic conditions, it is an important pulse crop across Asia and Africa (Campbell *et al.*, 1994; Yadav and Mehta, 1995; Hanbury *et al.*, 1999; Sharma *et al.*, 2000; Gongke *et al.*, 2001), and in marginal lands of the Mediterranean basin (Granati *et al.*, 2003). It is grown either for stockfeed or for human consumption. Grasspea was once widely grown in central and southern regions of Italy, but soon after the Second World War its production decreased, as evident from official production statistics since 1971, which show that it occupied only 1800 ha with an average yield of 639 kg/ha (Infantino *et al.*, 1994). Today, its survival relies on a small

number of farmers in very limited areas of central-south Italy (Alba *et al.*, 2001). Although rich in seed proteins (up to 35%; Williams *et al.*, 1994), the crop is of little interest to farmers as a result of low productivity, difficulties in mechanization and the presence of the compound 3-( $\beta$ -*N*-oxaly)-L-2,3 diaminopropionic acid ( $\beta$ -ODAP), a non-protein free amino acid, which is known to cause neurolathyrism, an irreversible paralysis of the lower limbs which can occur both in animals and humans (Rao *et al.*, 1964; Hanbury *et al.*, 2000).

The need for sustainable and environmentally sound agricultural systems has stimulated interest in the role of grain legumes and of this species in particular (Campbell *et al.*, 1994; Bozzini, 1997; Polignano and Uggenti, 1996; Tavoletti *et al.*, 1998). Its importance in developing countries has focused on the possibility of reducing seed  $\beta$ -ODAP levels. As for other neglected grain legumes, the lack of good varieties has meant that breeding programmes are forced to resort to local landraces. On the research side, various programmes have been initiated to safeguard, col-

\* Corresponding author. E-mail: giambattista.polignano@igv.cnr.it

lect and establish collections, complemented by diversity evaluations (Sarker *et al.*, 2000).

Field studies to evaluate the Bari collection of grasspea were initiated in 1999 on 332 entries (Polignano *et al.*, 2001). Additional details of this study can be found elsewhere (Bisignano *et al.*, 2002; Granati *et al.*, 2003; Polignano *et al.*, 2003). Recently we have developed a core collection (representing 12% of the total collection) to include accessions relevant for genetic studies and breeding (Polignano *et al.*, 2003). The prime objective of the present study was to identify a set of improved grasspea lines with adequate diversity for use by breeders and farmers.

## Materials and methods

Forty-seven single plant progenies characterized by large white seeds, high yield and high biomass were grown during 2001/2 in the Matera's Experimental Field at the 'Chiancalata' Farm of the Basilicata region of south Italy. The progenies were generated from 25 accessions in the Core Collection. The materials were planted in a randomized complete-block design with two replicates. Seeds of each progeny were sown in a single row plot with an inter-plant distance of 30 cm and an inter-row distance of 1.50 m. Five plants were chosen randomly from each line and used to measure the following 16 morpho-agronomic and seed quality traits according to the IPGRI descriptor list (2000):

- pod length, average of five dry pods per plant (cm);
- pod width, average of five dry pods per plant (cm);
- pod curvature (1 = straight, 2 = slightly curved, 3 = curved);
- pod shape (1 = oblong-elliptical, 2 = medium oblong-elliptical, 3 = curved, 4 = beaded, 5 = broad linear, 6 = broad elliptical, 99 = others);
- pods per plant (number);
- seeds per pod, average of five pods per plant (number);
- seed shape (1 = oblong, 2 = triangular, 3 = rhomboid, 4 = square, 5 = oblong-triangular, 6 = spherical, 99 = other);
- seed coat colour (1 = greyed-white, 2 = yellow-white, 3 = grey, 4 = brown, 5 = yellow green, 6 = pink, 7 = red-purple, 8 = black, 9 = grey-mottled, 10 = green mottled, 99 = other);
- yield per plant (g);
- 100-seed weight (g);
- seed coat pattern (0 = absent, 1 = marbled, 2 = dotted, 3 = streaked, 4 = mixture);
- seed length, average of five seeds randomly collected from five pods (cm);
- seed width, average of five seeds randomly collected from five pods (cm);
- seed thickness, average of five seeds randomly collected from five pods (cm);
- seed size (1 = small, 2 = intermediate, 3 = large);
- $\beta$ -ODAP content (% on dry matter basis).

Seed  $\beta$ -ODAP content was analysed following a modified Rao's procedure using 200 mg of finely milled flour obtained from 15 seeds (Rao, 1978; Granati *et al.*, 2003).

Both quantitative and qualitative data were subjected to univariate and multivariate analyses as described elsewhere (Polignano *et al.*, 1993; Granati *et al.*, 2003). A hierarchical clustering procedure using Ward's minimum variance method was carried out to define groups and to establish similarity and dissimilarity among progenies. The results of clustering were combined with those of the principal component analysis as a visual aid for discerning clusters in subsequent graphical presentations. All computations were performed using SAS (SAS Institute, 1987) and STATISTICA for Windows (StatSoft, 1995).

## Results

The univariate analysis revealed significant variation between lines for all the quantitative descriptors. Means, coefficient of variation, minimum and maximum values are presented in Table 1. Yield per plant and pod number per plant showed the highest coefficient of variation, while pod width showed the lowest coefficient of variation. Seed  $\beta$ -ODAP content was medium to high, varying from 0.24 to 0.60%, with a mean of 0.39% and a coefficient of variation of 20%.

The frequency distribution of six qualitative pod and seed characters are given in Table 2. The majority of progenies produced straight pods. The oblong-elliptical pod shape dominated in all progenies. Rhomboid seed was recorded with highest frequency. The most frequent cotyledon–testa colour combinations were yellow–

**Table 1.** Mean, minimum and maximum values, and coefficients of variation, observed in 47 grasspea progenies

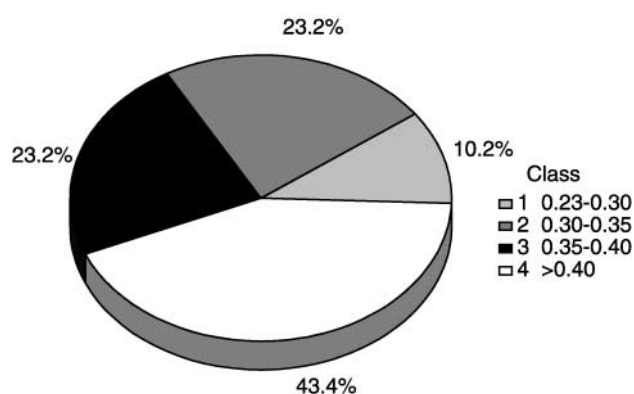
Descriptor	Mean	Min.	Max.	CV (%)
Pod length (cm)	3.9	1.3	5.3	8.8
Pod width (cm)	1.4	0.7	1.9	8.3
Pods per plant	103.3	6.0	303.0	41.7
Seeds per pod	2.4	1.0	5.0	21.5
Yield per plant (g)	60.7	3.0	191.0	43.3
100-seed weight (g)	27.1	10.0	57.7	16.1
Seed length (cm)	0.9	0.1	1.4	11.1
Seed width (cm)	0.8	0.5	1.3	10.3
Seed thickness (cm)	0.5	0.3	0.7	10.1
$\beta$ -ODAP (%)	0.39	0.24	0.60	20.5

**Table 2.** Frequency distribution of discrete descriptors in 47 grasspea progenies

Descriptor	Score code	Descriptor state	Frequency (%)
Pod curvature	1	Straight	90.9
	2	Slightly curved	8.9
	3	Curved	0.2
Pod shape	1	Oblong-elliptical	60.0
	2	Medium oblong-elliptical	33.4
	3	Curved	0.9
	4	Beaded	3.2
	5	Broad-linear	1.9
	6	Broad-elliptical	0.6
Seed shape	1	Oblate or flattened	0.4
	2	Triangular	2.8
	3	Rhomboid	84.3
	4	Square	3.2
	5	Oblong-triangular	9.4
Seed coat colour	1	Greyed-white	23.2
	2	Yellow-white	34.7
	3	Grey	2.8
	4	Brown	4.5
	5	Yellow-brown	31.7
	6	Pink	0.9
	7	Red-purple	1.1
	8	Black	0.2
	9	Grey mottled	1.1
Seed pattern	0	Absent	87.0
	1	Marbled	1.7
	2	Dotted	7.7
	3	Streaked	3.2
Seed size	4	Mixture	0.4
	3	Small	19.6
	5	Medium	64.3
	7	Large	16.2

white, yellow–brown and green–white. The majority of progenies had no seed coat pattern. Medium-sized seed appeared with the highest frequency. The seed  $\beta$ -ODAP content of most of the lines was high ( $>0.40\%$ ; Fig. 1). Only few lines had a low seed  $\beta$ -ODAP content.

Using principal component analysis, we calculated eigenvalues, percentage of variation and load coefficients



**Fig. 1.** Classes of ODAP percentage in 47 elite grasspea lines (% on dry matter basis).

of the first three components for 10 pod and seed characters, which accounted for 77% of the total diversity (Table 3). Based on correlations between the principal components and traits, seed length, seed width, 100-seed weight, pod width and length were the primary determinants of the first principal component, while yield per plant, pod number per plant and seed number per pod dominated the second component. The third principal component involved seed thickness and seed  $\beta$ -ODAP content.

Ward's clustering procedure allowed the identification of three groups which account for 37% of the original phenotypic variation. Groups I (25 lines) and II (18 lines) are the most similar to one another, while the smallest group III (four lines) is distinct. Groups, progeny numbers and origins are reported in Table 4, while a detailed description of each progeny can be found on the website <http://www.igv.cnr.it/bis/bancadati.html>.

The principal component analysis was combined with the cluster analysis in a three-dimensional matrix of the first three components (Fig. 2). As explained by Granati *et al.* (2003), three-dimensional distances between lines reflect a summation of the differences, based on the 10

**Table 3.** Principal component analysis of characters associated with 47 grasspea progenies showing eigenvalues and proportion of variation associated with the first three principal component axes and eigenvectors of characters

Descriptor	Principal component		
	PRIN 1	PRIN 2	PRIN 3
	Eigenvectors		
Pod length	0.37	0.11	-0.03
Pod width	0.39	-0.02	0.22
Pods per plant	-0.13	0.55	0.27
Seeds per pod	-0.19	0.40	0.04
Yield per plant	0.07	0.59	0.31
100-seed weight	0.44	0.04	0.03
Seed length	0.44	-0.03	0.16
Seed width	0.45	0.03	0.04
Seed thickness	0.24	0.18	-0.61
$\beta$ -ODAP	-0.01	-0.37	0.62
Eigenvalue	4.53	2.17	1.04
Variation (%)	0.45	0.22	0.10
Cumulative variation (%)		0.67	0.77

descriptors, measured to the extent that the first three PCs are effective in capturing the combined variance. Cluster assignments were added to a three-dimensional matrix to clearly define the groups. The four group III lines are

characterized by individuals with high scores for pod and seed characters, but a low number of pods per plant and seeds per pod, and a low  $\beta$ -ODAP content. In contrast, group I lines have low pod length, pod width, yield per plant, 100-seed weight, seed length, seed width and seed thickness, but high  $\beta$ -ODAP content. Group II lines have intermediate mean values for all characters, except for pod number per plant, seed number per pod and yield per plant.

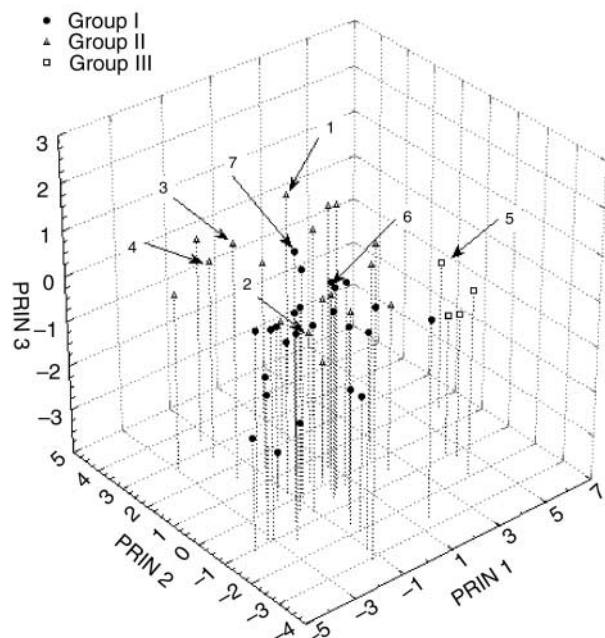
### Discussion

The cluster analysis reported here differentiates between lines on the basis of their similarity, thus providing a hierarchical classification. We have used this to identify two main groupings with small differences among the three groups. No significant differences were observed with respect to geographic origin of the material, most of which was derived from Italy. Group III was distinct, while groups I and II were similar to one another. The grouping pattern indicates a narrow genetic base. Nevertheless, the analysis indicates that genetic improvement through crossing and selection should be possible. A few lines with useful traits such as large and light-coloured

**Table 4.** Cluster memberships of 47 grasspea progenies

Group I (25 lines)		Group II (18 lines)		Group III (4 lines)	
Progeny	Origin	Progeny	Origin	Progeny	Origin
MG 110492-1 <sup>a</sup>	Italy	MG 110437-4	Italy	MG 106385-3	Algeria
MG 110957-4	Italy	MG 110492-4	Italy	MG 113873-1	Italy
MG 109680-3	Spain	MG 113089-1	Italy	MG 115242-1	Italy
MG 113089-5	Italy	MG 112252-5	Italy	MG 113874-2	Italy
MG 109680-1	Spain	MG 106434-1	Italy		
MG 103641-5	Italy	MG 110437-1	Italy		
MG 113873-2	Italy	MG 113090-2	Italy		
MG 115833-2	Unknown	MG 113090-4	Italy		
MG 115094-2	Italy	MG 113089-3	Italy		
MG 106434-4	Italy	MG 113090-1	Italy		
MG 115833-5	Unknown	MG 103212-5	Italy		
MG 112251-3	Italy	MG 110437-5	Italy		
MG 110435-1	Italy	MG 100293-3	UK		
MG 113873-5	Italy	MG 103237-4	Italy		
MG 115834-5	Unknown	MG 103212-4	Italy		
MG 103376-3	Italy	MG 110435-3	Italy		
MG 115834-2	Unknown	MG 112252-4	Italy		
MG 110437-3	Italy	MG 110434-1	Italy		
MG 113089-2	Italy				
MG 115833-1	Unknown				
MG 103579-1	Ethiopia				
MG 115094-3	Italy				
MG 100288-3	Spain				
MG 100293-2	UK				
MG 103212-1	Italy				

<sup>a</sup>Mediterranean germplasm number.



**Fig. 2.** Three-dimensional plot of the first three principal components (PRIN 1, PRIN 2 and PRIN 3). Arrows indicate the entries: 1, MG 110435-3; 2, MG 110437-1; 3, MG 113090-4; 4, MG 113090-1; 5, MG 115242-1; 6, MG 115834-2; 7, MG 110437-3.

seeds, low  $\beta$ -ODAP content and/or good yield potential were identified, specifically MG 110435-3, MG 110437-1, MG 113090-4 and MG 113090-1 (group II); MG 115242-1 (group III); and MG 115834-2 and MG 110437-3 (group D). Germplasm with large white seeds and high yield are of particular interest, since these characters are attractive to both consumer and producer. The future of grasspea as a protein-rich food and feed in Italian agriculture depends on the development of high-yielding varieties with low seed  $\beta$ -ODAP. However, as reported elsewhere (Capitani *et al.*, 2001; Granati *et al.*, 2003),  $\beta$ -ODAP levels in Italian germplasm are medium to high (0.24–0.57%), so that its reduction remains a high priority in the selection of materials suitable for animal and human consumption (Hanbury *et al.*, 1995; Robertson *et al.*, 1996).

The present study suggests that more basic information regarding both the genetic diversity and the genetic control of  $\beta$ -ODAP content will be required to properly evaluate grasspea germplasm. As demonstrated in earlier studies (Campbell and Briggs, 1987; Hanbury *et al.*, 1995; Zhou and Arora, 1996; Polignano *et al.*, 2003) primary, secondary and tertiary gene pools may play a major role in this process. With this in mind, a modest hybridization programme, including lines MG 110437 and MG 110435, has been initiated to attempt to combine good yield with low  $\beta$ -ODAP seed content. More field studies are required to evaluate these lines for agronomic traits and seed quality characters in a range of environments.

Future investigations using new germplasm will focus on the development of molecular markers to allow a genetic analysis of the control of seed  $\beta$ -ODAP. Provided that this character is relatively simply inherited, a marker-assisted selection strategy can then be undertaken to breed for low  $\beta$ -ODAP materials.

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