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Analysis Based on Morphological, Protein and SRAP Markers in Pea (*Pisum sativum* L).

María Andrea Espósito[1*] • Leonardo Andrés Milanesi[1] • Eugenia Alejandra Martin[2] • Vanina Pamela Cravero[2] • Fernando Sebastian López Anido[1] • Enrique Luis Cointry[1]

[1] Genetic and Plant Breeding Department, Rosario National University (UNR), CC 14, Zavalla S2125ZAA, Argentina
[2] CONICET – Av. Rivadavia 1917. C1033AAJ. Buenos Aires, Argentina

Corresponding author: * **mesposi@unr.edu.ar**

ABSTRACT

Morphological, protein and SRAP markers among fourteen pea varieties (*Pisum sativum* L) were studied. Data on 15 morphological traits were collected and analyzed. A total of 32 protein bands and 162 polymorphic SRAP fragments were scored. A comparison between morphological and molecular data and morphological and protein data was carried out through a Procrustes Generalized Method. Previously we carried out a Principal Component Analysis (PCA) for morphological data and Principal Coordinates Analysis (PCoA) for the protein and SRAP data. The correlation between SRAP and morphological data was R=0.56 for 2005 and R=0.80 for 2006 showing the highest correlation between both data sets for the second year what presented unfavourable environmental conditions. Meanwhile, the correlation between protein and morphological data was R=0.56 for 2005 and R=0.63 for 2006. These results suggest that exposure to a combination of environmental stresses may increase the expression of genetic variability for productive traits. Genetic diversity is the basis for successful crop improvement and can be estimated by different methods such as protein or molecular markers but DNA markers provide an opportunity to characterize genotypes more precisely than proteins. Molecular markers were significantly correlated with markers based on agronomic traits, suggesting that the two systems give similar estimates of genetic relations among the varieties.

Keywords: breeding; consensus analysis; legumes; genetic diversity

INTRODUCTION

Since the beginning of agriculture, a considerable amount of biodiversity has built up in crop production. With the application of scientific methods to plant breeding, the world's agricultural output has increased immensely. The application of tools such as plant breeding, however, has led to the substitution of traditional local varieties by widespread genetically homogeneous varieties, and a loss of non-sustainable diversity.

The importance of hybridization in crop improvement varies greatly from one crop to another and this is particularly true when dealing with autogamous species as is the case for most grain legumes. However, the relatively narrow gene pool (Heath and Hebblethwaite 1985) and the heavy use of a small number of parents by competing breeding programs have led to low genetic diversity among pea varieties (Gantotti and Kartha 1986; Simioniuc *et al*. 2002; Baranger *et al*. 2004). The latter occurred particularly after the introduction of the semileafless trait about 25 years ago (Heath and Hebblethwaite 1985). Extensive use of closely related cultivars by producers could result in vulnerability to pests and diseases (Duvick 1984; Cox *et al*. 1986). The lack of genetic diversity also led to a plateau in the genetic improvement of yield in pea (McClean *et al*. 1993; Tar'an *et al*. 2005). There are situations where lack of useful genetic diversity is considered the potential rate-limiting factor for genetic progress (Shands and Weisner 1991, 1992; Simmonds 1993; Kannerber and Falk 1995). Genetic diversity is the basis for successful crop improvement and can be estimated by different methods such as morphological traits, end-use quality traits, and molecular markers (Fufa *et al*. 2005).

The current pea variety identification system is based mainly on morphological and phenological characters. Although these descriptors are useful, they are limited in number and may be affected by environmental factors. Seed storage protein profiles could be useful markers in cultivar identification, registration of new varieties, pedigree analysis, and in the studies of genetic diversity and classification of adapted cultivars, thereby improving the efficiency of pea breeding programs in cultivar development.

DNA markers are useful complements to morphological and phenological characters because they are plentiful, independent of tissue or environmental effects, and allow cultivar identification in the early stages of development. The use of DNA markers for diversity analysis can also serve as a tool to discriminate between closely related individuals from different breeding sources. Sequence-related amplified polymorphism (SRAP) technology has been recognized as a new and useful molecular marker system for mapping and gene tagging in *Brassica oleracea* L. (Li and Quiros 2001), *Curcubita moschata* (Duchesne ex Lam.) Duchesne ex Poir (Ferriol *et al*. 2004), *Cynara cardunculus* complex (Cravero *et al*. 2007) and *Pisum sativum* L. (Espósito *et al*. 2007).

The objective of this work was to characterize different accessions of pea through protein reserve profiles and DNA markers and their association with productive traits comparing the efficiency of both methods.

MATERIALS AND METHODS

Fourteen accessions of pea from North and South America, Europe, Australia, India and local breeding programs (**Table 1**) were sown in plots of twenty plants per accession in a completely randomized design with two replications in the Experimental Field of Rosario University (33º 1′S and 60º 53′W) during the 2005 and 2006 cropping seasons. Plots were arranged in ten rows 2 m long with an inter- and intra row spacing of 70 and 10 cm, respectively.

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Table 1 Origin of the entire collection.

Cultivars	Origin
Sring Pea	Australia
Canadá A	Canada
Erik 1	Canada
Viper	France
DDR11	India
C2001	Local breeding program
B2001	Local breeding program
Zav15	Local breeding program
Amarilla	Local breeding program
Aparecida	Local breeding program
Marina	Romania
Sprut	Russia
Turf	Russia
Inca	South America

Morphological traits

In order to characterize the diversity in plant morphology, several traits were analyzed. Data were collected for fifteen traits. Length (LS) and width (WS) of stipule, leaflets (LL, WL), length of the internodes (LI), plant height (PH), number of nodes at the first flower (NF) and at the first pod (NV), and numbers of days to flowering (DF) were measured in period the flowering while that length and width of pod (LP, WP), numbers of pods (NP) and seeds per plot (NS), yield (Y) and grain diameter (GD) were measured at the dry seed stage.

Four traits (plant height, size of pods and stipule, and diameter of grain), were recorded in cm, while the nodes at the first flower and at the first pod were counted with the average of three plants randomly selected in the centre of rows. The yield was estimated in g per plot at harvest. Seeds and pod per plot were counted. The days to flowering were estimated as days from sowing time to the day on which at least 50% of the plants in the plot had started to flower.

The 2005 cropping season represented an agronomically well-managed environment with irrigation and well-drained soils. Urea at the rate of 150 kg/ha and super phosphate at the rate 150 kg/ha were applied prior to sowing. The temperatures were appropriate during flowering stage (13-18°C). Low-input and abiotic stress caused by rainfed conditions, by using fields that were depleted of mineral nitrogen and with high average temperatures (25°C) constituted the 2006 environment conditions. This total situation represents a stressful environment.

Principal components analysis (PCA) was used for reducing set of correlated variables to a simpler set of orthogonal components. PCA is the well-known method, described in many statistical text books, by Jolliffe (1986) and was carried out in both seasons. This method leads to synthetic variables which are a combination of the original ones.

Protein extract and SDS-PAGE procedure

Total pea protein extract was prepared by stirring 100 mg of ground pea seed in 1.5 mL of 0.125 M Tris HCl and SDS-Mg in 1% buffer for 10 min at 14000 rpm (or 12700 × *g*) at room temperature to precipitate insoluble material. The protein composition of the supernatant, referred to as total pea protein extract (TPPE) was determined by SDS-PAGE (Laemmli 1970) on a Mini-Protein II electrophoresis system (Bio-Rad, Hercules, USA). Five hundred µL of TPPE was diluted 1+1 (v/v) in sample buffer, consisting of 0.21 M Tris-HCl buffer (pH 8), 13.33 ml glycerol, 6.6% SDS, 8.4 ml Tris 1 M (pH 6.8) and 20 mg bromophenol blue. The samples, sealed in 1.5 mL tubes, were heated at 95ºC in boiling water for 3 min and proteins were separated using 12% Tris-HCl polyacrylamide. The gels were stained in a solution of 0.1% Coomassie Brilliant Blue R250, 40% methanol, and 10% acetic acid. In order to check reproducibility of the method three separate gels were run under similar electrophoretic conditions. Electrophoretograms for each variety were scored and the presence (1) or absence (0) of each band noted. Presence and absence of bands were entered in a binary data matrix for calculate the distances Dice's and these matrix of genetic distances was subjected to Principal Coordinate Analysis (PcoA) (Gower 1966).

DNA extraction and SRAP procedure

For DNA extraction and SRAP procedure, about 100 mg of fresh leaf was ground in liquid nitrogen and the total genomic DNA was extracted using the commercial kit PureLink™ (Invitrogen, California, USA). The amplifications were carried out in a thermocycler MyCycler™ (Bio-Rad). At the beginning of the PCR reaction, the annealing temperature was set at 35°C and run for 5 cycles. Then the annealing temperature was raised to 50°C for another 35 cycles. Denaturing was done at 94°C for 1 min, while extension was carried out at 72°C for 1 min in all cycles. A total of 15 primer combinations between 5 primers forward and 5 primers reverse (**Table 2**) were assayed on all accessions. Primer banding patterns that were difficult to score and those that failed to amplify consistently in all genotypes was excluded. Consequently, only seven combinations were selected. The amplified fragments separated by denaturing acrylamide sequencing gels (6% w/v) and revealed with silver. SRAP fragments were scored for presence (1) or absence (0) in each sample. The procedure was done times for each combinations. Finally the distances Dice's were calculated and these matrices of genetic distances were subjected to Principal Coordinate Analysis (PcoA) (Gower 1966).

Table 2 Primer sequences used to generate molecular data.

Name	Sequence
Primers F	
me1	5′-TGAGTCCAAACCGGATA-3′
me2	5′-TGAGTCCAAACCGGAGC-3′
me3	5′-TGAGTCCAAACCGGAAT-3′
me4	5′-TGAGTCCAAACCGGACC-3′
me5	5′-TGAGTCCAAACCGGAAG-3′
Primers R	
em1	5′-GACTGCGTACGAATTAAT-3′
em2	5′-GACTGCGTACGAATTTGC-3′
em3	5′-GACTGCGTACGAATTGAC-3′
em4	5′-GACTGCGTACGAATTTGA-3′
em5	5′-GACTGCGTACGAATTAAC-3′

Comparisons between morphological traits, protein and SRAPs

A comparison between morphological and protein and SRAP data was carried out using Procrustes Generalized Analysis (PGA) obtaining previously Principal Component Analysis (PCA) for morphological data and Principal Coordinates Analysis (PCoA) for protein and SRAP data. These analyses were carried out with InfoGen software (Balzarini and di Renzo 2003). The PGA proposed by Gower (1975) harmonizes the individual configurations, or geometrical representations in a plane, through iterative algebraic steps that transform each individual configuration. These steps include translation, rotation, reflection, and scaling of their point's coordinates under two premises: to maintain the relative distance among elements of the individual configurations and to minimize the sums of squares between analogous points, i.e., points that correspond to the same element under different configurations. The consensus configuration is obtained as the average of all these transformed individual configurations. The Gower's general similarity coefficient (R) estimates the correlation between the sets data.

RESULTS

Morphological traits

Morphological characters, especially quantitative traits, are subject to environmental influences. The mean values of productive traits were lower in the second season than in 2005 (**Table 3**).

Associations among the 14 accessions were examined by means of PCA. In this analysis, four PCs explained

Table 3 Means and standard errors for each morphological trait in the entire pea collection.

Cultivar	Year	PH (m)	LP (cm)	WP (cm)	Y (g/plot)	NP	NS	NF	LI (cm)	WS (cm)	LS (cm)	NV	WL (cm)	LL (cm)	GD (cm)	DF
Amarilla	2005	0.8±0.1	6.7±0.2	1.2±0.1	457.8±2.2	608.9±58.8	2797.8±395.5	11.7±0.1	3.7±0.4	3.9±0.3	6.9±0.4	12.9±1.0	0	0	0.6±0.0	87.0±0.6
Amarilla	2006	0.6±0.1	6.0±0.1	1.2±00	313.3±52.0	640.0±70.2	2643.3±525.6	13.4±0.3	3.8±0.7	2.7±0.2	4.5±0.4	12.8±0.6	0	0	0.6±0.0	97.0±2.3
Aparecida	2005	0.8±0.1	7.9±0.1	1.1±0.1	513.3±34.8	767.8±63.9	2716.7±164.6	13.3±1.4	3.1±0.4	4.4±0.5	6.8±0.8	16.0±0.6	0	0	0.6±0.0	98.7±0.3
Aparecida	2006	0.6±0.1	7.6±0.1	1.2±0.1	154.4±8.7	355.6±8.0	1231.1±41.5	8.3±0.3	2.3±0.4	2.1±0.2	3.8±0.3	15.6±0.3	0	0	0.6±0.1	107.0±0.6
B2001	2005	1.2±0.1	6.3±0.5	1.2±0.1	534.5±47.8	707.8±41.3	2663.3±271.3	10.0±2.7	7.7±0.9	5.0±0.3	7.3±1.0	13.9±0.9	3.1±0.1	5.1±0.2	0.7±0.1	77.3±1.2
B2001	2006	0.8±0.1	5.4±0.1	1.2±00	370.0±10.0	557.8±26.2	2293.3±476.8	11.7±0.5	4.1±0.9	2.8±0.3	5.2±0.5	10.6±1.4	2.2±0.1	3.5±0.4	0.7±0.1	88.0±0.6
C2001	2005	1.5±0.1	5.8±0.2	1.2±0.1	563.0±69.1	617.8±87.8	2506.7±590.7	12.7±2.4	7.1±0.9	4.8±0.5	7.6±0.5	14.1±0.8	3.0±0.14	5.2±0.2	0.8±0.1	84.7±1.7
C2001	2006	0.6±0.1	6.0±0.1	1.2±0.1	283.3±28.4	722.2±101.1	2520.0±225.2	13.8±2.5	5.7±0.4	2.8±0.4	5.3±0.3	12.2±1.7	1.8±0.0	3.2±0.2	0.6±0.1	88.0±0.7
Canada A	2005	0.9±0.1	6.9±0.1	1.2±0.1	676.7±58.8	546.7±12.0	1980.0±150.4	13.2±1.2	4.1±0.6	4.4±0.2	7.7±0.6	12.3±1.2	0	0	0.7±0.1	91.0±2.5
CanadaA	2006	0.6±0.1	6.5±0.1	1.1±0.1	223.3±44.1	368.9±11.1	1228.9±106.6	14.0±0.7	2.7±0.3	3.6±0.8	6.1±1.2	15.7±1.4	0	0	0.7±0.0	91.0±1.3
DDR11	2005	0.7±0.1	6.2±0.1	1.1±0.1	370.0±15.3	586.7±49.1	1976.7±149.5	10.6±1.6	3.3±0.4	4.7±0.3	7.4±0.3	12.6±0.9	3.2±0.1	5.0±0.2	0.7±0.1	87.7±0.9
DDR11	2006	0.4±0.1	6.1±0.1	1.3±00	330.0±45.8	413.3±26.9	1588.9±192.2	12.2±0.2	3.0±0.1	3.5±0.3	5.6±0.2	13.9±1.1	2.2±0.2	3.4±0.3	0.7±0.1	92.0±1.5
EI	2005	0.9±0.1	7.8±0.2	1.2±0.1	803.3±72.2	786.7±12.0	5046.7±421.5	11.2±1.3	3.5±0.1	4.4±0.2	7.7±0.3	12.9±1.6	0	0	0.7±0.0	94.3±0.9
EI	2006	0.7±0.1	7.3±0.1	1.2±0.1	313.3±17.6	571.1±40.4	2353.3±144.4	8.2±0.1	2.4±0.3	3.6±0.1	5.8±0.2	16.9±2.8	0	0	0.6±0.1	102.0±3.5
Inca	2005	0.8±0.1	7.4±0.1	1.1±0.1	533.3±98.4	713.3±58.9	4251.1±381.2	13.2±1.2	3.1±0.2	5.0±0.1	8.0±0.3	16.2±1.0	0	0	0.7±0.1	96.3±0.3
Inca	2006	0.7±0.1	6.6±0.1	1.2±0.1	353.3±37.1	458.9±39.7	2840.0±270.1	11.4±1.1	2.8±0.2	3.4±0.1	6.4±0.1	14.9±0.1	0	0	0.6±0.1	79.0±0.9
Marina	2005	0.9±0.1	7.6±0.5	1.3±0.1	499.3±93.7	594.4±37.2	2800.0±440.2	8.9±0.4	3.8±0.4	4.8±0.1	7.6±0.2	11.4±0.7	3.4±0.3	5.5±0.4	0.7±0.0	87.0±1.2
Marina	2006	0.8±0.1	5.9±0.3	1.1±0.1	381.1±39.7	695.6±27.8	2762.2±531.2	14.3±1.0	2.8±0.3	3.3±0.3	5.4±0.4	9.6±0.2	1.9±0.3	3.2±0.4	0.7±0.1	86.0±0.6
Sring Pea	2005	0.8±0.1	6.9±0.1	1.2±0.1	460.0±11.6	522.2±36.6	1984.4±38.6	7.58±0.5	4.2±0.1	4.1±0.1	6.7±0.1	12.0±1.0	2.4±0.1	5.1±0.3	0.7±0.0	83.0±0.1
SringPea	2006	0.8±0.1	6.3±0.1	1.2±0.1	636.7±31.8	1151±93.1	3373.3±235.0	11.6±0.3	4.3±0.6	3.1±0.2	5.9±0.2	12.3±1.50	2.0±0.3	3.3±0.2	0.7±0.1	70.0±3.8
Sprut	2005	0.8±0.1	7.4±0.3	1.4±0.1	486.7±63.6	780.0±23.1	3126.7±148.1	12.3±2.5	4.7±0.8	5.6±0.2	8.6±0.4	14.0±1.2	0	0	0.8±0.1	90.7±2.3
Sprut	2006	0.4±0.1	6.0±0.2	1.4±0.1	190.0±37.9	329.0±103.7	684.4±270.3	14.4±0.2	2.2±0.1	3.4±0.2	6.0±0.3	15.8±0.5	0	0	0.7±0.1	92.0±2.1
Turf	2005	0.8±0.1	6.9±0.2	1.2±0.1	465.6±84.3	497.8±86.2	2557.8±450.1	11.4±1.6	3.4±0.3	3.5±0.1	6.6±0.1	16.2±0.6	0	0	0.7±0.0	86.0±1.5
Turf	2006	0.5±0.1	6.1±0.1	1.1±0.1	173.3±10.2	388.9±8.0	826.7±24.0	13.4±0.8	3.7±0.9	2.8±0.2	5.4±0.3	18.0±0.5	0	0	0.6±0.1	92.0±1.8
Viper	2005	0.7±0.2	6.1±0.1	1.1±0.1	684.4±61.6	1113.3±92.5	5404.4±531.7	13.7±0.8	3.1±0.4	4.0±0.1	7.0±0.4	16.9±0.9	0	0	0.7±0.0	93.3±0.9
Viper	2006	0.6±0.0	5.9±0.1	1.1±0.1	191.1±4.4	424.4±37.0	1795.6±85.4	18.3±0.7	2.7±0.2	2.2±0.1	4.0±0.1	20.7±0.3	0	0	0.6±0.0	100.0±1.3
Zav15	2005	1.4±0.1	6.6±0.2	1.2±0.1	995.6±54.4	1180.0±75.1	4226.7±394.8	12.0±1.7	7.3±0.3	5.1±0.2	7.7±0.5	12.9±0.6	3.6±0.1	5.4±0.2	0.7±0.0	96.3±1.3
Zav15	2006	0.9±0.1	6.3±0.2	1.4±0.1	608.9±18.6	895.6±178.1	2795.6±454.3	11.6±0.1	4.9±0.6	3.3±0.1	5.2±0.1	13.9±0.4	2.1±0.2	3.3±0.4	0.7±0.0	73.0±0.9
Mean	2005	0.93	6.9	1.2	574.5	716	3145.6	11.6	4.5	4.5	7.4	13.9	1.3	2.2	0.7	89.5
Mean	2006	0.65	6.2	1.2	323.0	569	2061	12.6	3.4	3	5.3	14.5	0.9	1.4	0.65	89.7

81.0% of the total variation observed among the varieties for both seasons.

The first component (PC_1) for 2005 accounted for 34.0% of the variation and was characterized by length of the internodes, length and width of leaflets and days at flowering. The second component (PC_2) accounted for 24.0% of the variation and was characterized by plant height and the yield and its components. The third component (PC_3) represented for 12.0% of the variation and was characterized by length and width of stipules and length of pod. The fourth component (PC_4) accounted for 11.0% of the variation and was characterized by number of nodes at the first flower and at the first pod, width of pod and grain diameter.

For 2006, the first component (PC_1), accounted for 44.0% of the variation and was characterized by the yield and its components and length and width of leaflets. The second component (PC_2) accounted for 16.0% of the variation and was characterized by length of the internodes, length and width of stipules and grain diameter. The third component (PC_3) represented for 14.0% of the variation and was characterized by plant height, length and width of pod and number of nodes at the first flower. Finally the fourth component (PC_4) accounted for 0.070% of the variation and was characterized by number of nodes at the first pod and days at flowering.

Protein extract and SDS-PAGE procedure

In this study SDS-PAGE of grain storage proteins was performed in order to analyze molecular weight subunits and investigate genetic diversity among different pea varieties. The electrophoretogram showing proteins banding pattern of different pea varieties are given in **Fig. 1**. The banding pattern drawn from the photograph of gel is quite evident of variation among genotypes. A total of 31 bands were obtained among which 20 bands were common in all varieties but the others 11 bands show variation. The analysis of protein profiles showed a polymorphism level of 33%.

The four main eigenvalues of the PcoA on the simple

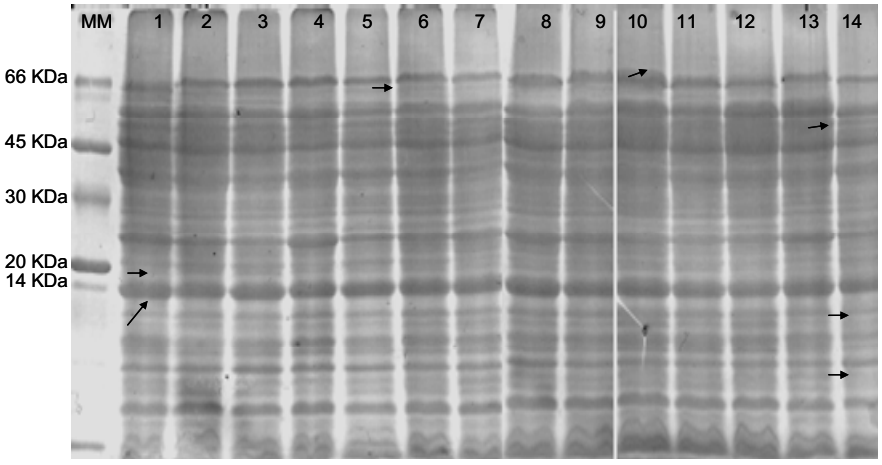

Fig. 1 Seed protein profile in pea. Arrows indicate some polymorphic bands. MM) Molecular weight marker. 1) B2001, 2) Sprut, 3) Amarilla, 4) DDR11, 5) C2001, 6) Zav 15, 7) Canadá A, 8) Marina, 9) Aparecida, 10) Erik 1, 11) Spring Pea, 12) Inca, 13) Turf, 14) Viper.

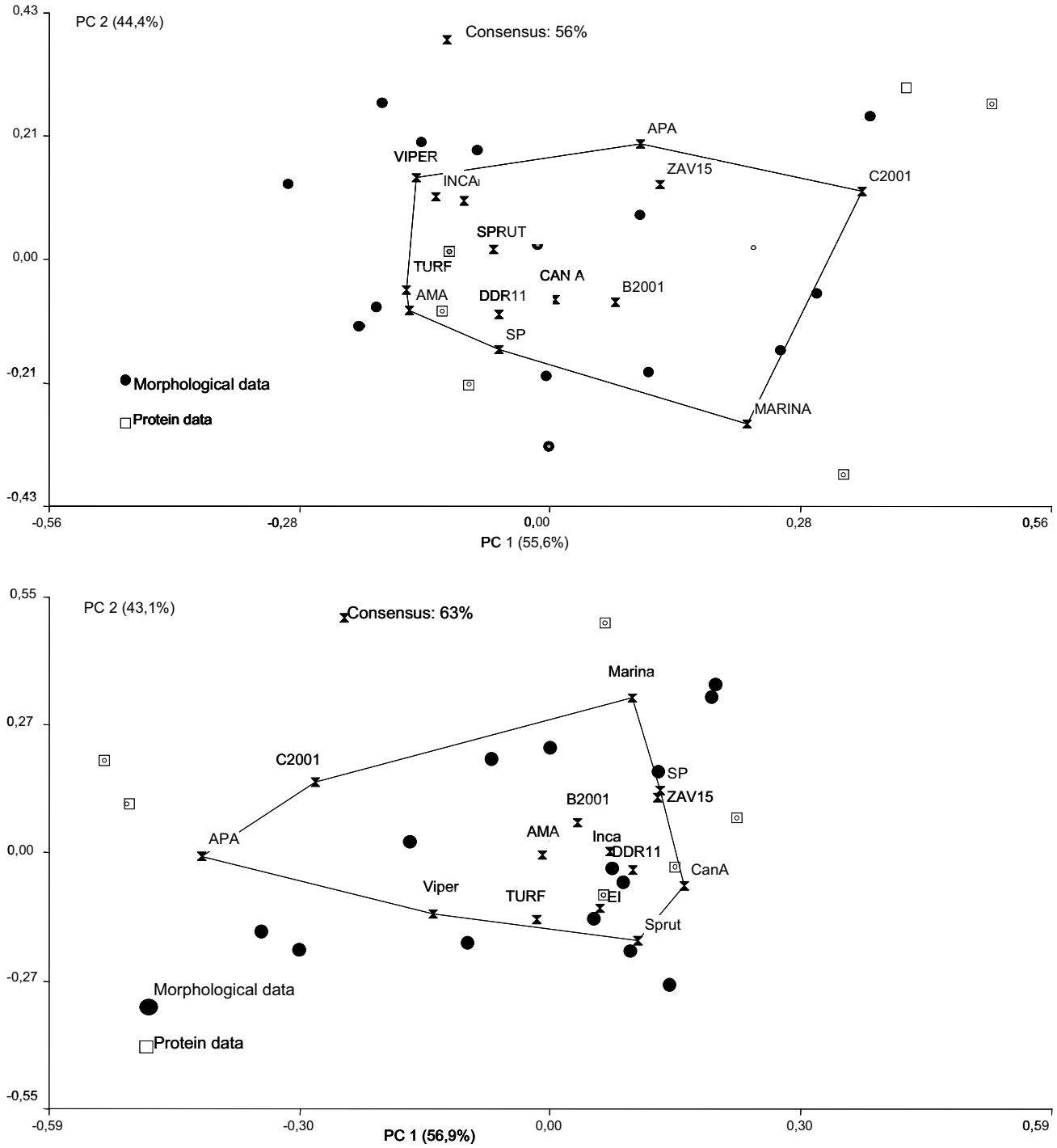

Fig. 2 Procrustes Generalized analysis between protein and morphological data in 2005 (above) and 2006 (below).

matching similarity matrix explained 38%, 60%, 75% and 87% of the total variation, respectively.

The PGA between morphological data, evaluated in both seasons, and the protein data showed a consensus value of R=56% for 2005 and R= 63% for 2006 (**Fig. 2A, 2B**).

DNA extraction and SRAP procedure

Fifteen primer combinations were initially tested for amplification of pea genomic DNA. Eight of them showed inconsistent amplification or low polymorphism and were discarded. Hence, the analysis of the 14 pea accessions was performed with seven primer combinations. We found a total of 266 fragments which 162 showed variation (an average of 23 polymorphic bands per primer combination) ranging in size from 150 bp to 800 bp. The analysis of the SRAP revealed a polymorphism of the 60% (**Fig. 4**).

The four main eigenvalues of the PcoA on the simple matching similarity matrix explained 54%, 60%, 65% and 70% of the total variation, respectively.

Comparisons between morphological traits and SRAPs

The PGA analysis between morphological and molecular data showed a consensus of R=56% in the first season (2005) and R=80% in the second, i.e. 2006 (**Fig. 3A, 3B**, respectively).

DISCUSSION

According to the results of the SDS-PAGE, the overall pattern of seed storage-proteins shows low degree of hetero-

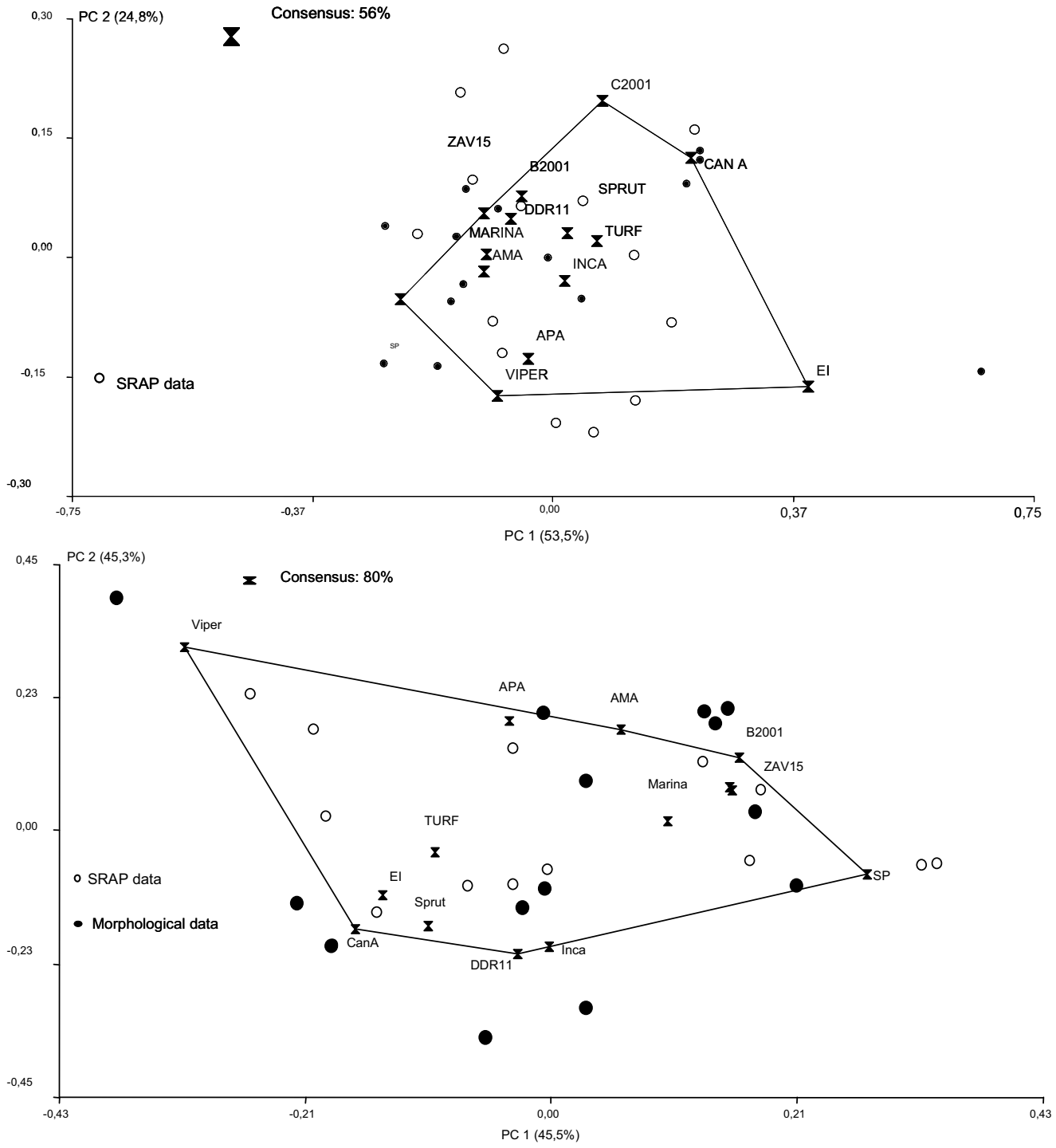

Fig. 3 Procrustes Generalized analysis between SRAP and morphological data in 2005 (**A**) and 2006 (**B**).

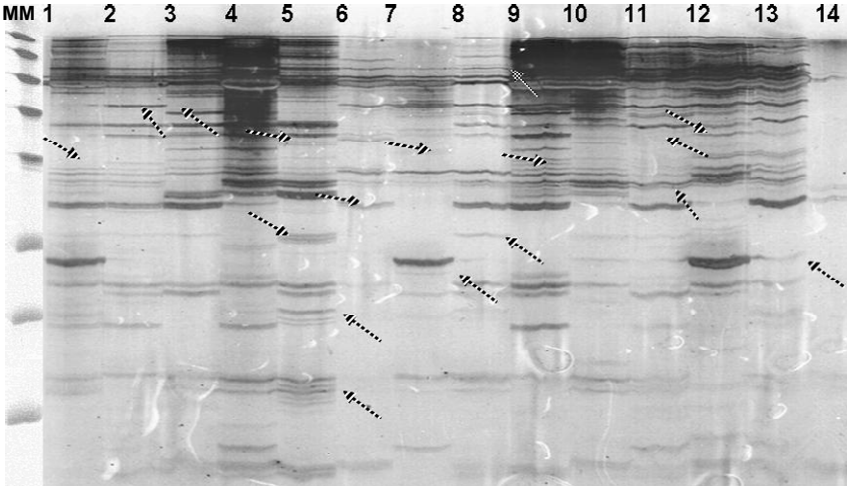

Fig. 4 Srap profiles for 14 accessions of pea. Arrows indicate some polymorphic fragments. MM) Molecular weight Marker, 1) B2001, 2) Sprut, 3) Amarilla, 4) DDR11, 5) C2001, 6) Zav 15, 7) Canadá A, 8) Marina, 9) Aparecida, 10) Erik 1, 11) Spring Pea, 12) Inca, 13) Turf, 14) Viper.

geneity between the different accessions of analyzed pea.

The PGA between morphological data, evaluated in both seasons, and the protein data showed a consensus value of 56% for 2005 and of 63% for 2006. These facts demonstrate that as biochemical marker storage protein is not very influenced by the environment. Gepts (1989) and Murphy *et al*. (1990) showed that the SDS-PAGE is a particularly reliable method because storage proteins are largely independent of environmental fluctuations. Nevertheless, according to several authors (Casey *et al*. 1982; Schroeder 1982; Cousin 1983; Guéguen and Barbot 1988; Turner *et al*. 1990) the variability in pea seed composition results from both genotypic and phenotypic factors. Ours results suggest that environmental conditions did have a slight effect on the synthesis of storage proteins. This may be mainly due to the synthesis of storage proteins in pea cultivars, mostly genetic in nature (Casey *et al*. 1986; Gepts 1990; Hany *et al*. 2000).

Several molecular approaches have been employed to assess genetic diversity and taxonomic relationships. SRAP combines simplicity, repeatability and easy sequencing of select fragments in the characterization of pea germplasm (Espósito *et al*. 2007). SRAP analysis also revealed genetic variability between accessions but the level of polymorphism found was 60%, clearly superior to that shown for protein analysis.

The relationship between protein and DNA markers showed a superior level of association when the environment conditions are stressful. The exposure to conditions of environmental stresses may increase the expression of genetic variability for productive traits. There is also evidence that heritable variation in quantitative traits can be increased by stressful conditions (Parsons 1987; Hoffmann and Parsons 1991).

The initial intention of this study was to identify protein markers that could be used to separate accessions of pea but SDS-PAGE was not a sufficiently powerful technique to distinguish a specific cultivar but DNA markers provide an opportunity to characterize genotypes and to measure genetic relationships more precisely than biochemical markers. Such information could be useful to determine optimal breeding strategies to allow continued progress in pea breeding.

CONCLUSION

Genetic diversity is the basis for successful crop improvement and can be estimated by different methods such as protein or molecular markers but DNA markers provide an opportunity to characterize genotypes more precisely than proteins.

Protein and SRAP markers showed a superior level of association with morphological traits when the environment conditions are stressful, suggesting that exposure to combinations of environmental stresses may increase the expression of genetic variability for productive traits.

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