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Prediction of heterotic crosses for yield in *Pisum sativum* L.

María Andrea Espósito[a,b], Carolina Bermejo[b], Ileana Gatti[a,*], María Fernanda Guindón[a,b], Vanina Cravero[a,b], Enrique Luis Cointry[a]

[a] Department of Plant Breeding, Rosario National University (UNR), CC 14, Zavalla S2125ZAA, Argentina
[b] CONICET, Zavalla, Argentina

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ABSTRACT

Efficient parent's selection and heterosis prediction in pea have been of great interest for breeders in order to determine with anticipation, those crosses capable of producing a high frequency of transgresive recombinant lines. The combining ability is the most frequently used criteria to select parents, though its estimation is costly and time-consuming. In this study we examine heterotic crosses for yield determined by morphological and molecular genetic distances between parents, and compare these with the heterotic crosses established by specific combing ability in a line × tester design. To select parental lines that would generate heterotic hybrids, different criteria were applied: genetic distances between lines obtained through morphological traits and molecular markers and cluster analysis. Inspite genetic distances were more efficient than cluster analysis, the correlations values between them and heterosis were weak, and so, we propose to change the way to measure the importance of the genetic distances using the percentage of heterotic crosses that can be successfully predicted as parameter to evaluate. The percentage of heterotic crosses comparing best parent heterosis was higher than the one obtained comparing midparent heterosis. SRAP methodology based on Dice distance between the parental lines and the Euclidean distances based in morphological data collection are the best alternative to SCA grouping association, since 69% and 65% of heterotic crosses can be predicted.

1. Introduction

The pea (*Pisum sativum*) is the second most important legume crop worldwide after common bean (*Phaseolus vulgaris* L.) (Gowhar et al., 2010) and has been considered lately as an interesting protein source (Santalla et al., 2001) for animal and human nutrition.

Breeding efforts are mainly concentrated in the selection of superior individuals derived from the hybridization of parents showing high *per se* performance, in order to establish pure lines with good characteristics. For this reason, efficient parent selection and prediction of heterosis is of major interest for breeders.

Parent selection may be approached in two ways: *a priori* and *a posteriori* (Dos Santos Dias et al., 2004). The first approach consists in the selection of parents based on *per se* performance, such as mean values or divergence between them. Nevertheless, parent selection based on phenotypic performance is not a suitable procedure since superior lines may produce low yield progenies and no better lines can be obtained in further generations. In the *a posteriori* parent selection approach, the parents are evaluated on basis of the performance of the F_1 and advanced generations in order to identify highly heterotic combinations when the objective is to obtain lines with transgresive characteristics.

Line × tester analysis (Kempthrone, 1957) provides information about the effects of general combining ability (GCA) and specific combining ability (SCA) of parents, and is also helpful in estimating various types of gene actions (Falconer and Mackay, 1996; Sharma et al., 2007; Ceyhan et al., 2008; Basal et al., 2011 and Espósito et al., 2013). Commonly, lines that show high general combining ability produce high performance hybrids (Castañón-Najera et al., 2011) when crossed with lines that show large genetic distances between them, and homozygous lines equal to or better than the F 1 have been reported to have been developed from highly heterotic crosses in self-pollinated crops as stated by Singh (1980) in mungbean (*Vigna radiata*). Singh (1974) also suggested the possibility of deriving pure lines performing better than or as well as F 1 hybrids in chickpea.

But combining ability estimations based on morphological characters is costly and time-consuming; moreover, these traits are largely influenced by environment factors making the estimation more imprecise. The efficiency of breeding programs could be

* Corresponding author. Tel.: +54 341 49700801254.
E-mail address: ileana1111@gmail.com (I. Gatti).

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Table 1
Studied pea accessions.

Cultivars	Origen
Females	
CAN A	Canada
KEOMA (KEO)	Canada
EXLORER (EXPL)	Canada
EI	Canada
DDR11	India
DMR7	India
APA	Local breeding program
C2001	Local breeding program
ZAV10	Syria
ZAV26	Syria
ZAV5	Syria
ZAV20	Syria
ZAV17	Syria
ZAV25	Syria
ZAV12	Syria
ZAV15	Syria
MARINA (MAR)	Romania
VIPER (VIP)	Holland
TURF	Russia
Testers	
ZAV23	Syria
AMA	Local breeding program
COME	France
DDR14	India

increased if the performance of the lines *per se* and the superior crosses could be predicted before field evaluation (Melchinger et al., 1990).

Heterosis is the result of genetic complementation between divergent parents. In pea, different authors have analyzed the existence of heterosis for various agronomic traits (Sarawat et al., 1994a,b; Ceyhan et al., 2008; Karnwal and Kushwaha, 2010). The quantitative genetics' explanation for this phenomenon relies directly on the effect of dominance at different loci in the hybrids. If hybrid vigor results from the accumulation of dominant alleles, then heterosis could be accumulated *via* selection in homozygous genotipes (Sarawat et al., 1994a,b). If heterosis is a function of heterozygosity, it can be considered also as a function of parental diversity. Therefore, parental genotypes morphologically and/or molecularly divergent may originate crosses with high heterosis. Several works emphasized the importance of genetic divergence for desirable parent selection in many crops (Cai and Lan, 2005; Zhao et al., 2009).

In this study, heterotic crosses for yield predicted by genetic distance between parents using morphological and molecular data where compared with heterotic crosses predicted by Specific Combing Ability with the aim of identifying if *a priori* strategies are as useful as *a posteriori* ones for parental selection.

2. Material and methods

2.1. Vegetal material and field experiments

An assessment was made of the pea collection owned by Rosario University during two seasons (2008 and 2009), where morphological traits were analyzed and a cluster analysis was performed (dendrogram shown in Fig. 1) (Espósito et al., 2007) in order to select divergent parents. As a result, 4 pea accessions of different genetic stocks were selected to be used as male parents (AMA, DDR14, ZAV23 and COME) and 19 ones as females, originating 76 hybrids (Table 1). The experiment was designed according to the line × tester method (Kempthrone, 1957).

Morphological markers (color of cotyledon and flower, leafless and normal folioles) or molecular markers (SSR) where used to ensure the hybrid nature of the progeny. All hybrids and their parents were evaluated during 2010 and 2011 in the Experimental Field of the Faculty of Agronomy of Rosario University (33°1′S and 60°53′W). The plots of parents and F_1's consisted of three rows of 1 m long, with 50 cm between rows and 10 cm between plants (30 plants per plot) arranged in a complete randomized design with two replications. Conventional sowing was made on 6/25/2010 and 6/30/2011, 5 cm deep, and irrigation was used until flowering and pod setting stages.

Table 2a
Sequences of primers for SRAP markers.

Primer forward		Primer reverse	
Name	Sequence	Name	Sequence
me1	5′-tgagtccaaaccggata-3′	em1	5′-gactgcgtacgaattaat-3′
me2	5′-tgagtccaaaccggagc-3′	em2	5′-gactgcgtacgaatttgc-3′
me3	5′-tgagtccaaaccggaat-3′	em3	5′-gactgcgtacgaattgac-3′
me4	5′-tgagtccaaaccggacc-3′	em4	5′-gactgcgtacgaatttga-3′
me5	5′-tgagtccaaaccggaag-3′	em5	5′-gactgcgtacgaattaac-3′

The climate of the region is typically Mediterranean, with 950 mm of mean precipitation per year and average temperature of 13.1 °C, with an average minimum of 3.0 °C in July and an average maximum of 28.6 °C in January. Soil reaction (pH) is neutral (6.6–7.3) and is representative of the major production area of pea in Argentina. Due to the typical loamy soil of the Argentinean pampas, which is well provided in phosphorus and potassium, there was no need of macro-element supply. Herbicides linuron at a 600 g ai ha^{-1} dose (ai = active ingredient), applied in pre-emergence and bentazon at a 20 g ai ha^{-1} dose applied in post-emergence were used to control common weeds.

Phenotypic data for the fourteen following traits: length (SL) and width (SW) of stipule, leaflets (LL, LW) and pod (PL, PW) (cm); length of the internodes (LI) (cm); plant height (PH) (m); number of nodes at the first pod (NFP); numbers of days from sowing to 50% of plot flowering (DF); numbers of pods (NP) and seeds per plot (NS); yield (g per plot) (Y) and weight of 100 seeds (WS) measured at the dry seed stage was measured in 20 plants located in the middle of the plot. Mean values over replications were calculated for each trait and used in data analysis.

2.2. Sequence-related amplified polymorphism (SRAP) and microsatellite assays

To perform the DNA extraction, about 100 mg of fresh leaf tissue taken from young plants of 23 parental lines were grounded in liquid nitrogen and the total genomic DNA was extracted using the CTAB method (Smykal et al., 2008). A total of 25 SRAP primer combinations and 14 SSR primer combinations were assayed on all accessions (Tables 2a and 2b).

The amplifications were carried out in a thermo-cycler MyCyclerTM (BIO-RAD). At the beginning of the PCR reaction, the annealing temperature was set at 35 °C and run for five 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. The amplified fragments were separated by denaturing acrylamide sequencing gels and reveled with silver (Li and Quiros, 2001).

SRAP and SSR fragments were scored for presence or absence in each sample.

2.3. Statistical methods

General (GCA) and Specific combining ability (SCA) effects were estimated for yield according to Hallauer and Miranda (1988).

Heterosis for yield was calculated by the Kempthorne method (1957) using the software "Genes" (Cruz, 2006). Mid-parent

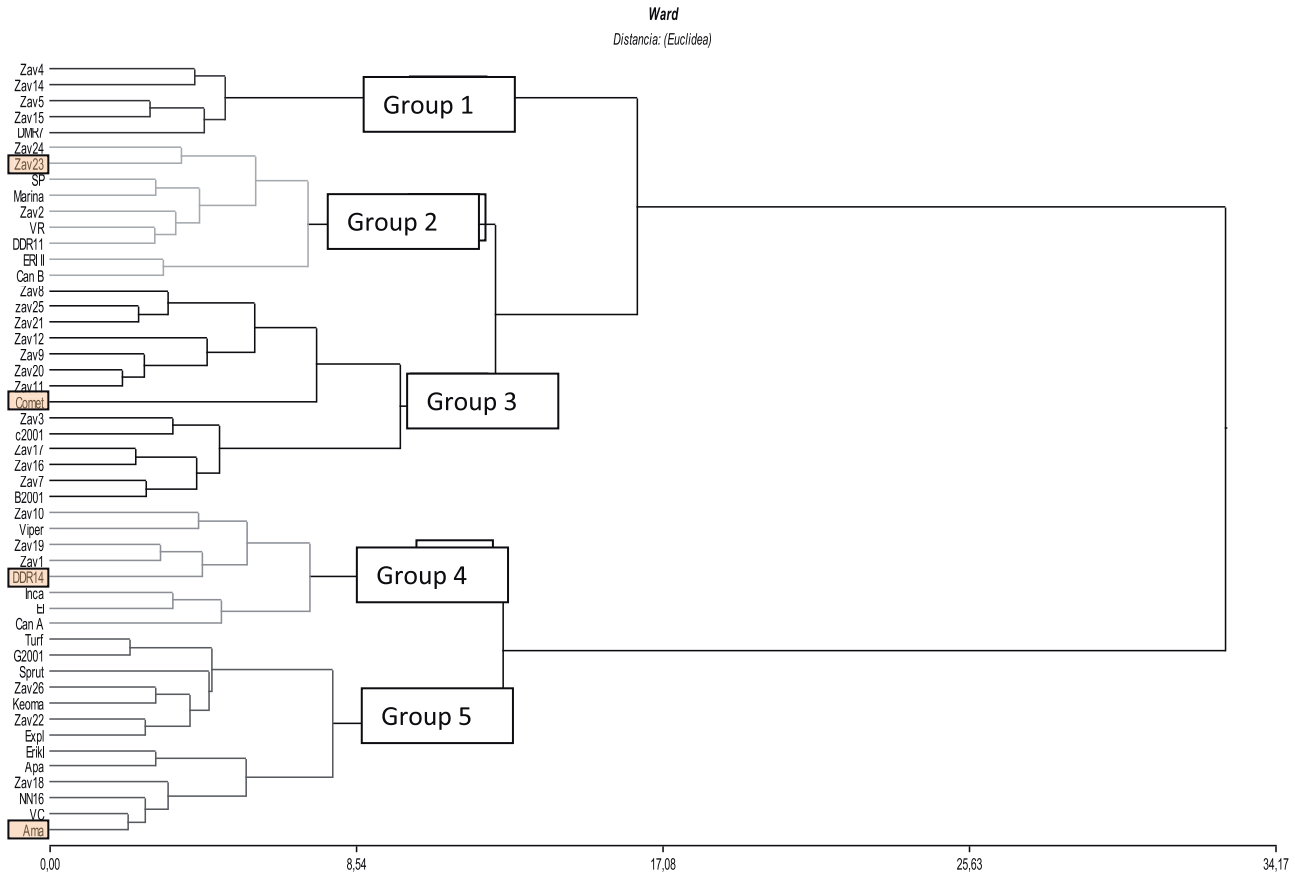

Fig. 1. Dendrogram of 49 accessions based on morphological data using Euclidean distances. Values followed by same letter are not significantly different at $p \leq 0.05$, Duncan's multiple range test.

Table 2b
Sequences of primers for 10 microsatellite (SSR) loci, position on chromosomes, number of detected alleles and Polymorphic information content (PIC) values.

Name	Sequence primer forward	Sequence primer reverse	Linkage group	Number of alleles	PIC
AA205	tacgcaatcatagagtttggaa	aatcaagtcaatgaaacaagca	II	8	0.84
AB23	tcagcctttatcctccgaacta	gaacccttgtgcagaagcatta	V	7	0.81
AC58	tccgcaatttggtaacactg	cgtccatttcttttatgctgag	V	6	0.77
AD56	gaaacattggttgaagagcgag	gttgtcgcgtgaacacaagtaa	VII	10	0.83
AD61	ctcattcaatgatgataatccta	atgaggtacttgtgtgagataaa	IV	8	0.8
AD146	tgctcaagtcaatatatgaaga	caagcaaatagttgttttgtta	VII	9	0.82
AD147	agcccaagtttcttctgaatcc	aaattcgcagagcgtttgttac	I	10	0.87
AD148	gaaacatcattgtgtcttcttg	ttccatcacttgattgataaac	II	10	0.84
D21	tattctcctccaaaatttcctt	gtcaaaattagccaaattcctc	I	11	0.83
AD59	ttggagaatgtcttctctttag	gtatattttcactcagaggcac	VI	8	0.79

heterosis (MPH) and Better Parent Heterosis (BPH) were calculated as follows:

$$\mathrm{MPH} = \left(\frac{(F_1 - \mathrm{MP})}{\mathrm{MP}}\right) \times 100,$$

$$\mathrm{BPH} = \frac{(F_1 - \mathrm{BP})}{\mathrm{BP}} \times 100$$

where F_1 were the means for F_1 hybrids; $\mathrm{MP} = (P_1 + P_2)/2$, where P_1 and P_2 were the means for the parental lines; and BP the best parent performance.

Based on specific combining ability (SCA) for yield, the varieties were classified in different heterotic groups. The consistence of the proposed heterotic groups was confirmed comparing intra- and inter-group mid-parent heterosis.

In order to select parental lines to generate heterotic hybrids, different criteria were applied:

(1) Genetics distance (GD) between lines obtained through:

- morphological traits (Euclidean distance)
- molecular markers (Dice distance).
- morphological and molecular data together (Gower distance).

Euclidean or straight-line measure of distance is the most commonly used statistic for estimating genetic distance (GD) between individuals (genotypes or populations) by morphological data. Euclidean distance between two individuals i and j, having observations on morphological characters (p) denoted by $x_1, x_2, \ldots, x_p$ and $y_1, y_2, \ldots, y_p$ for i and j, respectively, can be calculated by the following formula:

$$d(i,j) = \left[(x_1 - y_1)^2 + (x_2 - y_2)^2 + \cdots \left(x_p - y_p\right)^2\right]^{1/2}$$

Table 3
Pooled analysis of variance for yield in pea.

F.V.	Df	MS	F
Years	1	10,679,765.24	62.35***
Crosses	75	705,885.30	4.12***
Lines (GCA)	18	460,754.75	2.69**
Testers (GCA)	3	397,379.55	2.32*
Cross (SCA)	54	647,454.62	3.78***
Cross* year	75	409,368.45	1.39
Pooled error	196	171,284.29	

*** Significant at $p \leq 0.001$.
** significant at $p \leq 0.001$.
* Significant at $p \leq 0.05$.

Genetic distances (GD) for each kind of molecular marker independently and for both markers together were calculated using the Dice similarity index (DSI) (Dice, 1945):

$$\mathrm{DSI} = \frac{2a}{(2a + b + c)}$$

Being (a) the number of total matches between lines and (b and c) single matches.

Gower (1971) described a general coefficient for measuring genetic distance between individuals on the basis of various types of characters, such as dichotomous, qualitative, and quantitative.

Gower's measure of distance between individuals (i and j) is defined as $\mathrm{DG}_{ij} = 1/p \sum \mathrm{w}_k\, d_{ijk}$ where p is number of characters, d_{ijk} is the contribution of the kth character to the total distance between two individuals; $d_{ijk} = |x_{ik} - x_{jk}|$, where x_{ik}, x_{jk} are the values of the kth character on the individuals i and j, respectively, and $w_k = 1/R_k$, where R_k is the range of the kth character in the sample (Franco et al., 1997).

All distances were calculated using InfoGen software (Balzarini and Di Renzo, 2003)

(2) *Cluster analysis:* Cluster analysis was performed based on UPGMA using the software XLSTAT (Addinsoft, 2012) and dendrograms were constructed on the basis of genetics distances (Euclidean, Dice and Gower).

Lines that showed the highest GD values and lines from clusters with the greatest distance between centroids were selected and crossed to produce potentially heterotic hybrids. The percentage of crosses that showed MPH greater than 50% and BPH heterosis greater than 20% was calculated. A Mantel test using XLSTAT software was performed in order to study the correlation between distances matrices.

The Pearson correlation coefficient between heterosis and parental lines' genetic distances was calculated using Genes software (Cruz, 2006).

3. Results and discussion

3.1. Hybrid performance and heterosis for yield

Yield is the most important character in all crops, thus heterosis was measured only for this trait and the rest of the variables analyzed were used for distances calculation between parental lines. The line × tester analysis can be used to estimate general and specific combining abilities in both self and cross-pollinated plants (Kempthrone, 1957). It provides useful information for the identification of appropriate parents and superior crosses; therefore, this analysis has been widely used by plant breeders as selection criteria in early generations. Pooled analysis of variance over environments for yield (Table 3) indicates significant differences among lines and testers ($F = 2.7$; $p < 0.01$; $F = 2.3$; $p < 0.05$, respectively) revealing genetic diversity between parental lines. Significant differences were observed also between crosses ($F = 3.8$; $p < 0.01$) and no interactions between materials and year was found ($F = 1.4$ ns).

Table 4a
General combining ability for lines and testers for yield in pea.

Materials	GCA
Parental lines	
VIPER	−46.046
CANA	185.227
ZAV12	205.411
C2001	141.356
ZAV20	366.032
EI	29.845
APA	−63.151
EXPLORER	287.745
KEOMA	18.472
ZAV10	−370.031
DMR7	56.332
ZAV25	13.495
ZAV17	−243.696
ZAV15	−286.018
TURF	−391.258
ZAV5	86.141
MARINA	246.332
DDR11	−304.614
ZAV26	68.424
Tester	
AMA	−50.94
DDR14	−12.189
ZAV23	79.085
COME	−15.956

The estimates of general combining ability effects (Table 4a) of the parental lines revealed that Can A, ZAV 12, ZAV 20, Explorer, Marina and ZAV23 exhibited desirable (high) *gca* effects for yield, suggesting that these lines have a higher frequency of favorable alleles for this trait and might produce the best hybrid combinations. The specific combining ability effects (Table 4b) showed a wide range of variation, from −938.5 (DMR 7 × ZAV 23) to 633.4 (ZAV 5 × ZAV 23). MPH varied from −51.4% (ZAV 10 × DDR 14) to 303.8% (ZAV 20 × ZAV 23) and BPH from −61% (ZAV7 × DDR14) to 274.3% (DMR7 × COME). Espósito et al. (2013) evaluated GCA and SCA for the rest of the variables using the same material in order to conform heterotic groups, identify appropriate parents in crosses for these traits and assess their potential use in pea breeding programs.

If an inbred line express negative SCA effects when it is crossed to a tester is considerer that they belong to the same heterotic group and the inverse is also true (Vasal et al., 1992). According to the SCA values for crosses between lines and testers, four heterotic groups were made (Table 5). Inter-group hybrids showed greater heterosis values than intra-groups ones.

Combining ability values are determinant for plant breeders in order to select parents that produce heterotic hybrids. However, parental genetic distance is equally important, as pointed by Zhang et al. (2010) for yield in rice.

3.2. Genetics distances

Euclidean distances between lines were calculated from morphological data (Table 4b). Seventeen combinations had parents with Euclidean distances greater than 6.0. Among these, 11 hybrids (65%) showed high values for mid-parent heterosis (MPH) and best parent heterosis (BPH) (Table 6a).

For the calculation of Dice distances, fourteen microsatellite markers were selected because of their high polymorphism (Loridon et al., 2005), but only ten of them revealed successful amplifications of expected allele sizes. Therefore, genetic diversity between the 23 genotypes was assessed by ten SSR markers and 87 alleles were detected. The alleles per locus ranged from 6 (AC58) to 11 (D21) with an average of 8.7 alleles. PIC ranged from 0.77 (AC58) to 0.87 (AD147) with an average of 0.82. A high level of genetic

Table 4b
Specific combining ability effects, Euclidean distances, Dice distances for SSR, SRAP and SRAP + SSR markers, Gower distances, mid-parent Heterosis (MPH), and best parent heterosis (BPH) for all hybrid combinations.

		Distances						
HYBRIDS	SCA	Euclidean	Dice SSR	Dice SRAP	Dice SRAP SSR	Gower	MPH	BPH
APAXAMA	−83.433	3.32	0.95	0.67	0.70	0.55	29.06	27.7
APAXCOME	42.568	6.33	1	0.76	0.78	0.62	86.47	49.1
APAXDDR14	−288.5	5.17	0.71	0.62	0.62	0.48	5.04	−0.5
APAXZAV23	−434.87	4.98	1	0.72	0.74	0.57	21.54	4.8
C2001XAMA	−95.18	5.77	0.95	0.77	0.78	0.62	60.98	49.5
C2001XCOME	−380.21	6.83	1	0.73	0.75	0.61	67.88	41.3
C2001XDDR14	179.878	4.96	0.89	0.77	0.78	0.60	89.65	69.2
C2001XZAV23	−468.73	4.81	0.95	0.72	0.74	0.58	43.16	42.2
CAN AXAMA	−308.33	4.64	1.00	0.67	0.69	0.58	31.84	20.2
CAN AXCOME	190.504	6.51	0.89	0.64	0.65	0.55	136.84	75.9
CAN AXDDR14	−526.51	4.1	0.95	0.74	0.76	0.60	7.51	0.6
CAN AXZAV23	−119.9	4.95	1.00	0.67	0.69	0.57	88.98	53.4
DDR11XAMA	−144.47	4.98	1.00	0.68	0.70	0.59	16.91	−6.6
DDR11XCOME	−369.99	4.88	0.95	0.66	0.69	0.55	21.31	20.1
DDR11XDDR14	−337.42	4.42	0.89	0.70	0.71	0.56	−10.22	−30.5
DDR11XZAV23	87.64	2.72	0.89	0.73	0.73	0.58	98.67	62.7
DMR7XAMA	−550.68	7.26	0.90	0.64	0.66	0.58	34.40	−11.7
DMR7XCOME	572.084	6.83	0.80	0.59	0.60	0.53	237.85	274.3
DMR7XDDR14	152.817	7.52	0.95	0.69	0.71	0.59	144.85	57.6
DMR7XZAV23	−938.46	4.51	0.95	0.68	0.70	0.60	16.10	−28.4
EIXAMA	−576.59	5.48	0.82	0.63	0.64	0.58	4.29	−17.6
EIXCOME	203.971	7.02	1.00	0.67	0.69	0.63	199.74	198.1
EIXDDR14	227.355	7.24	0.87	0.64	0.66	0.57	112.49	62.6
EIXZAV23	−618.97	5.19	0.92	0.64	0.65	0.59	81.65	11.7
EXPLXAMA	115.656	2.94	0.95	0.66	0.69	0.54	132.65	90.0
EXPLXCOME	−508.33	4.18	1.00	0.69	0.71	0.58	102.19	94.7
EXPLXDDR14	−549.7	5.88	1.00	0.74	0.76	0.60	37.82	8.8
EXPLXZAV23	178.131	4.89	1.00	0.68	0.71	0.56	199.92	156.3
KEOXAMA	−597.82	3.3	0.89	0.67	0.70	0.55	−4.77	−21.3
KEOXCOME	65.929	4.91	0.95	0.68	0.68	0.56	154.79	141.8
KEOXDDR14	208.752	6.59	0.95	0.73	0.75	0.59	99.74	59.5
KEOXZAV23	−441.1	4.38	1.00	0.73	0.75	0.59	59.91	35.4
MARXAMA	510.334	3.63	0.95	0.64	0.66	0.55	148.09	130.0
MARXCOME	−456.93	4.84	0.95	0.64	0.65	0.55	72.75	45.7
MARXDDR14	−859.26	3.86	0.95	0.67	0.69	0.56	−18.61	−30.6
MARXZAV23	41.618	3.22	0.89	0.68	0.69	0.56	136.52	124.4
TURFXAMA	89.009	5.12	0.89	0.68	0.69	0.57	75.12	10.1
TURFXCOME	−486.95	4.36	1.00	0.67	0.69	0.58	15.74	−16.7
TURFXDDR14	−237.84	7.25	0.84	0.69	0.70	0.57	14.64	−22.6
TURFXZAV23	−128.45	5.32	1.00	0.69	0.71	0.58	98	21.2
VIPXAMA	−172.4	3.47	0.76	0.65	0.65	0.56	36	19.6
VIPXCOME	−348.04	5.35	1.00	0.66	0.68	0.60	20.46	36.6
VIPXDDR14	239.946	5.74	0.95	0.67	0.69	0.57	84.26	56.0
VIPXZAV23	−483.74	5.39	1.00	0.68	0.70	0.60	33.92	19.9
ZAV10XAMA	−168.48	2.84	0.95	0.66	0.68	0.60	−13.29	−16.7
ZAV10XCOME	−488.47	5.69	0.96	0.61	0.64	0.57	−32.31	−44.6
ZAV10XDDR14	−510.32	6.07	1.00	0.71	0.73	0.62	−51.39	−55.2
ZAV10XZAV23	403.032	5.58	0.86	0.66	0.67	0.57	94.35	76.6
ZAV12XAMA	63.74	3.98	1.00	0.66	0.68	0.56	97.7	74.8
ZAV12XCOME	−789.18	4.51	0.85	0.62	0.64	0.53	21.31	6.9
ZAV12XDDR14	207.988	4.26	0.90	0.71	0.73	0.57	109.99	78.3
ZAV12XZAV23	−246.79	3.72	0.90	0.62	0.64	0.51	102.48	86.8
ZAV15XAMA	402.419	6.82	0.90	0.64	0.66	0.59	152.34	57.5
ZAV15XCOME	−358.67	7.41	0.91	0.58	0.60	0.56	80.36	28.4
ZAV15XDDR14	−256.38	6.74	0.96	0.73	0.75	0.65	30.04	−20.2
ZAV15XZAV23	−551.61	5.79	0.75	0.62	0.64	0.55	32.61	−22.5
ZAV17XAMA	−8.253	5.89	0.95	0.70	0.72	0.60	54.86	15.8
ZAV17XCOME	14.363	4.62	0.95	0.68	0.71	0.57	126.19	108.6
ZAV17XDDR14	−692.9	5.33	0.95	0.71	0.74	0.57	24.91	−61.0
ZAV17XZAV23	−77.443	3.2	0.90	0.65	0.67	0.52	96.37	48.5
ZAV20XAMA	−842.93	5.31	0.84	0.56	0.57	0.49	20.9	−9.7
ZAV20XCOME	226.184	5.11	0.95	0.64	0.66	0.57	262.07	267.3
ZAV20XDDR14	−579.83	5.19	0.95	0.72	0.73	0.60	58.48	13.8
ZAV20XZAV23	432.343	4.17	0.90	0.67	0.69	0.57	303.77	202.0
ZAV25XAMA	−302.79	5.88	1.00	0.69	0.71	0.59	7.68	4.1
ZAV25XCOME	−419.18	7.29	0.95	0.69	0.76	0.60	6.35	−4.5
ZAV25XDDR14	133.905	5.33	0.89	0.74	0.74	0.59	52.74	51.2
ZAV25XZAV23	−176.17	5.11	1.00	0.73	0.74	0.60	55.63	31.2
ZAV26XAMA	−333.07	3.32	1.00	0.64	0.66	0.58	22.05	14.3
ZAV26XCOME	236.593	4.33	0.84	0.60	0.61	0.54	150.48	109.3
ZAV26XDDR14	52.291	5.75	1.00	0.71	0.73	0.62	47.78	48.4
ZAV26XZAV23	−720.05	5.46	0.77	0.67	0.67	0.57	8.61	−2.4
ZAV5XAMA	−626.84	6.04	0.95	0.7	0.71	0.61	5.97	−16.9
ZAV5XCOME	−576.39	6.21	0.89	0.66	0.69	0.59	60.74	58.2
ZAV5XDDR14	−194.39	5.27	0.89	0.74	0.75	0.62	64.22	24.7
ZAV5XZAV23	633.385	3.87	0.84	0.67	0.66	0.55	255.25	191.1

Table 5
Heterotic groups according to SCA for MPH and BPH.

	MPH		BPH	
Group	Intra	Inter	Intra	Inter
AMA	6.60	59.90	−16.38	31.91
DDR14	−0.71	75.74	−24.05	39.88
ZAV23	26.88	120.93	1.76	79.00
COME	14.47	109.24	−4.77	94.05

Table 6a
Percentage of crosses successfully predicted for mid-parent heterosis (MPH) and best parent heterosis (BPH).

DISTANCES	MPH	BPH
SRAP	60	68.8
SSR	49	65.2
SRAP + SSR	57	59.1
Euclidean	65	65.0
Gower	45	66.7

diversity at the ten SSR loci was observed. The degree of polymorphism observed across the 23 lines was similar to that found by Smykal et al. (2008) who analyzed 21 commercial varieties of field pea (*P. sativum* subsp. *sativum*) and 4 varieties of fodder pea (*P. sativum* subsp. *arvense*). Based in Dice distances (greater than 0.95), 45 combinations can be predicted to be heterotic. From these, 49% showed high MPH values and 65.2% high BPH ones (Table 6a).

Using SRAPs, from a total of 25 primer combinations, 18 were selected according to their easy scoring banding patterns and capacity to amplify consistently in all genotypes. We found a total of 213 polymorphic fragments ranging in size from 300 to 900 bp with an average of 12 bands per combination. Dice distances between lines were obtained and twenty combinations had distances higher than 0.7. The percentage of hybrids with high MPH and BPH were 60% and 68.8%, respectively (Table 6a).

The SSR and SRAP genetic distance matrices were correlated ($r = 0.28$; $p < 0.01$) with each other. Using the combination of both molecular markers, Dice distances were obtained. Thirty combinations between lines with distance values higher than 0.71 were predicted. The percentage of combinations that really showed high MPH was 57% and for BPH, 59.1% (Table 6a).

Gower distances were obtained using morphological and molecular data. Twenty combinations between lines and testers with distance values higher than 0.6 were planned. The percentages for MPH and BPH were 45% and 66.7%, respectively (Table 6a).

The correlation coefficient between GD and MPH using Euclidean distances was moderate ($r = 0.26$, $p < 0.01$) whereas between GD and BPH was $r = 0.11$; $p < 0.01$. No correlations were found between Dice distance and mid-parent heterosis (MPH) using microsatellites, but we found moderate correlation with best parent heterosis ($r = 0.36$, $p < 0.01$). On the other hand, the correlation coefficient between Dice distance and heterosis using SRAPs was $r = 0.33$; $p < 0.01$ for MPH and $r = 0.41$; $p < 0.01$ for BPH. Thus, SRAP markers could be more advantageous over SSR marker due to occasional loss of amplification sites of SSR primers as found by Amar (2012) in a comparative analysis of SSR and SRAP in citrus.

Using SSR and SRAPs in combination, negative correlation was found for MPH ($r = -0.17$; $p < 0.05$) and positive for BPH ($r = 0.18$; $p < 0.01$). The correlation coefficient for Gower distances was $r = 0.27$; $p < 0.05$ for MPH and non-significant correlations was found for BPH.

Heterosis depends on genetic differences between parents and traits' complementarities. In general, when the genetic difference between parents is high, the heterosis observed in the progeny is also high (Falconer and Mackay, 1996). The procedure most frequently used has been the selection of parental using DNA-marker-based genetic distance between inbred lines, and the estimation of its relationship with heterosis (Lee et al., 2007) due to its simple application (Dreisigacker et al., 2005; Shanthi et al., 2006; Biswas et al., 2008; Gvozdenović et al., 2009; Selvaraj et al., 2010). However, the researchers have different views on molecular marker based prediction. Studies about the relationship between genetic diversity and hybrid performance were developed in several crops. Researches made in corn, *Zea mays* L., showed that the genetic diversity between parents was significantly correlated with hybrid performance and that yield heterosis could be predicted using molecular markers (Betrán et al., 2003; Reif et al., 2003; Schrag et al., 2006). Conversely, low correlations between genetic distance and hybrid performance and heterosis were reported in oilseed rape, *Brassica napus* L. (Diers et al., 1996), pepper, *Capsicum annuum* L. (Geleta et al., 2004), faba bean, *Vicia faba* L. (Zeid et al., 2004) alfalfa, *Medicago sativa* L. (Riday et al., 2003), and aromatic rice, *Oriza sativa* (Zhang et al., 2006). Until now, no studies were developed in pea.

3.3. Cluster analysis

Cluster analysis was performed based on different genetic distances and heterosis intra group and inter group for MPH and BPH are shown in Table 6b.

Cluster analysis based on Euclidean distances (Fig. 2) reveal three principal clusters. Group 3 contained seven lines related with the tester ZAV 23, Group 2 consisted of five lines associated with DDR 14 and Group 3 with seven lines related to AMA and COME. Hybrids from crosses between genotypes from Group 2 with lines of groups 1 and/or 3are supposed to have a superior performance

For Dice distances by Microsatellites, clustering was performed using the Dice similarity index values throughout UPGMA and a dendrogram was constructed (Fig. 3). The 23 parental lines were grouped in three clusters. The first cluster consisted of nine genotypes including two testers (AMA and COME). The second cluster included nine genotypes with ZAV23 and the remaining lines were grouped in cluster 3 which included also DDR14. Based in the greatest distances between clusters' centroids, the crosses between genotypes of cluster 2 with genotypes of cluster 1 and 3 are proposed to be heterotic.

The relationships between the 23 accessions revealed by cluster analysis based on Dice distance by SRAPs are showed in Fig. 4. Four main clusters can be observed. In the first group we found seven lines associated with ZAV 23; three lines related to DDR14 were included in the second cluster and five accessions associated with AMA and COME were included in the third cluster. The others four lines formed another group without tester association. Fifteen crosses between genotypes of cluster 2 with genotypes of cluster 3 and 4 were proposed based in the greatest distances between the centroid of each cluster.

Three groups were formed by cluster methodology using Dice distances by SSR's and SRAPs together (Fig. 5). In the first group we found 11 lines related to COME and ZAV23, in the second group only two lines associated with DDR 14 were included. All the other lines were grouped with AMA in cluster 3.

Finally, three groups were constructed using Gower Distances in cluster analysis (Fig. 6). The first group included 6 lines related to AMA, the second group; two genotypes associated DDR14 and the third group, 11 lines and two testers (COME and ZAV 23). Crosses between genotypes of cluster 2 with genotypes of cluster 3 were supposed to be heterotic.

Across all methods of cluster analysis, heterosis intra-group was higher than intra-group heterosis determined by SCA, demonstrating the presence of a higher percentage of heterotic crosses than those detected by SCA. The percentage of heterotic crossed predicted varied from 40% for cluster analysis using Dice distances by

Table 6b
Intra and intergroup heterosis and percentage of crosses predicted by cluster analysis.

	METHODS	Intragroup heterosis	Intergroup heterosis	% Crosses predicted
Mid-parent heterosis	SCA	11.81	91.45	100
	Euclidean cluster	58.13	54.83	50
	SRAP cluster	80.00	48.87	40
	SSR cluster	80.39	55.33	52
	SRAP + SSR cluster	62.35	52.37	48
	GOWER cluster	69.22	58.22	45
Best parent heterosis	SCA	−10.86	61.21	100
	Euclidean cluster	31.67	15.73	50
	SRAP cluster	35.4	22.69	60
	SSR cluster	50.97	22.36	48
	SRAP + SSR cluster	35.4	22.69	48
	Gower cluster	35.4	26.37	53

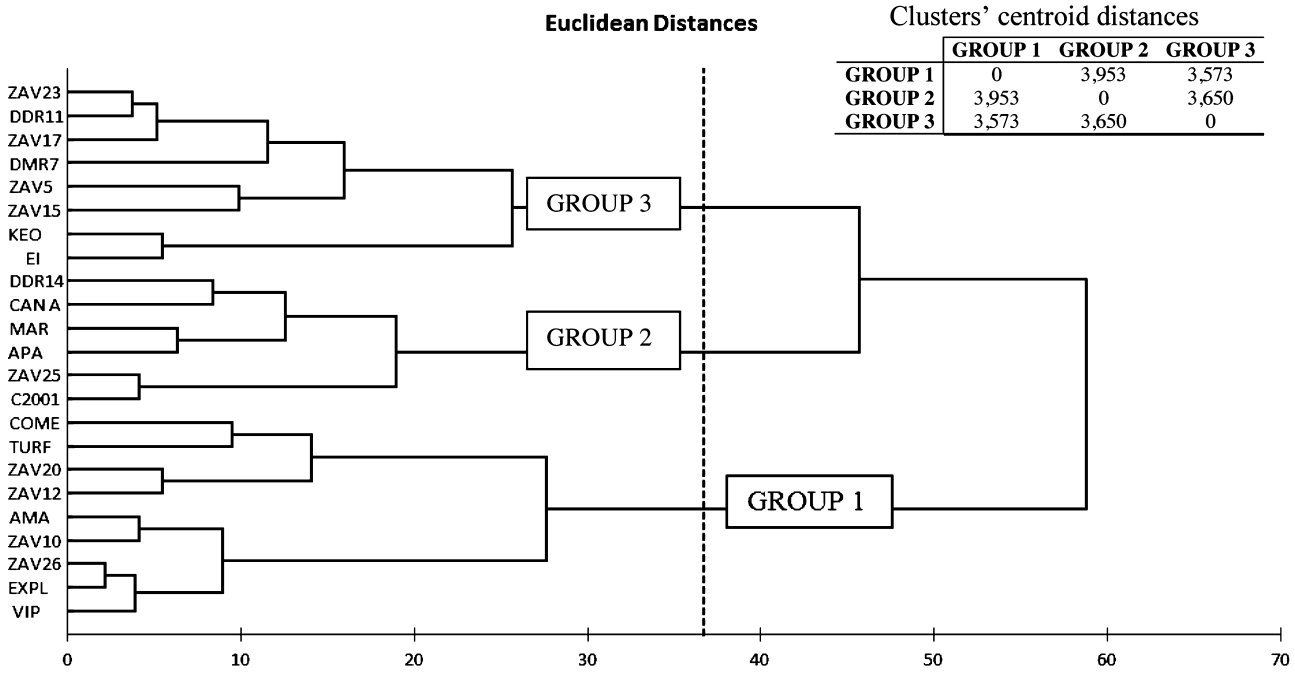

Fig. 2. Dendrogram of Euclidean distances for morphological characters. Clusters' centroid distances

SRAPs for MPH to a maximum of 60% using the same method for BPH.

The concept of heterotic groups was widely used in maize breeding (Tracy and Chandler, 2006). Generally, inbred lines are divided into different heterotic groups and new inbred lines are derived by crosses within lines in the same heterotic group. Then, the new lines are crossed to testers from opposite heterotic groups to evaluate their performance. There was evidence (Melchinger, 1999; Tracy and Chandler, 2006) that crosses within an heterotic group tended to exhibit lower heterosis than crosses between heterotic groups. Early research suggested that low genetic diversity will result in low yield heterosis (Moll et al., 1965) leading some researchers to think that genetic distance may be a good predictor of heterosis. While some data support a positive relationship between genetic diversity and heterosis, this seems to hold only for closely related inbred lines.

Molecular markers present information on relict diversity, most probably not related to fitness traits, and also of limited value for predicting performance (Dreisigacker et al., 2005). Melchinger (1999) pointed out that a correlation between MPH and molecular distance was more likely to be found in intra-group crosses than in inter-group crosses. Our data support this last statement—the correlation between the two parents' genetic distance and the hybrid heterosis for yield was no statistically significant.

However, more than the correlations values between genetics distance and heterosis, it is interesting the proportion of heterotic crosses that can be predicted. We propose to change the way to measure the importance of the genetics distances using the percentage of heterotic crosses as parameter to evaluate.

Using this parameter, the values obtained for different methodologies are shown in Tables 6a and 6b).

The percentage of heterotic crosses predicted for BPH was superior to the one for MPH. Mid parent heterosis concept is rather generic, as stated by Lamkey and Edwards (1998), because it does not refer to the genetic architecture of the parents crossed to produce the hybrids, for this reason is more suitable to use BPH in the calculation of the percentage of heterotic crosses.

The a priori strategies have been shown to be efficient to predict a high percentage of heterotic crosses determined by SCA. Among these a priori strategies, SRAP methodology based on Dice distance between the parental lines and the Euclidean distances based in morphological data collection, are the best alternative to SCA grouping association, since 69% and 65% of heterotic crosses could be predicted owing to its simplicity in use as Zhang et al. (2010) stated.

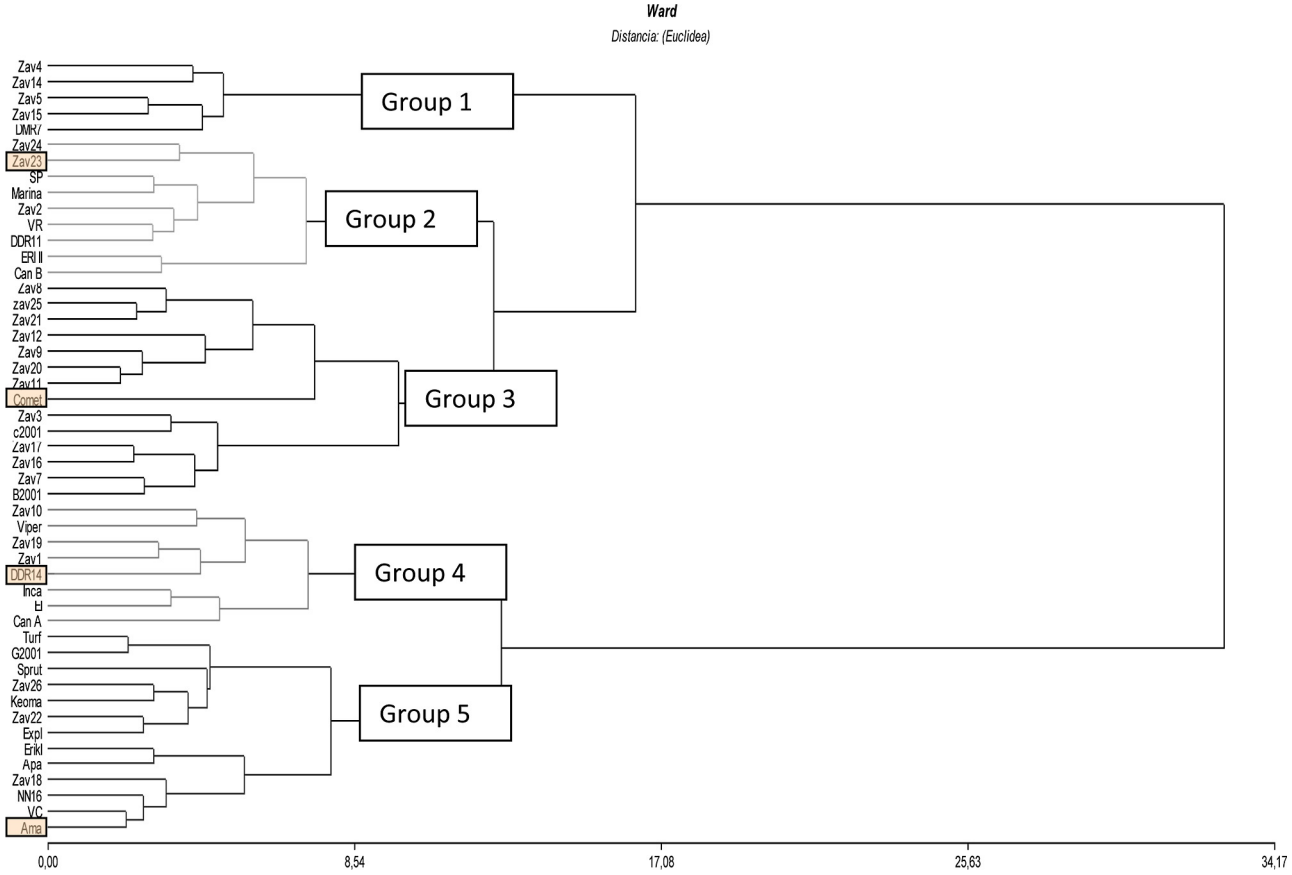

Fig. 3. Dendrogram of Dice distances for microsatellite (SSR) markers. Clusters' centroid distances

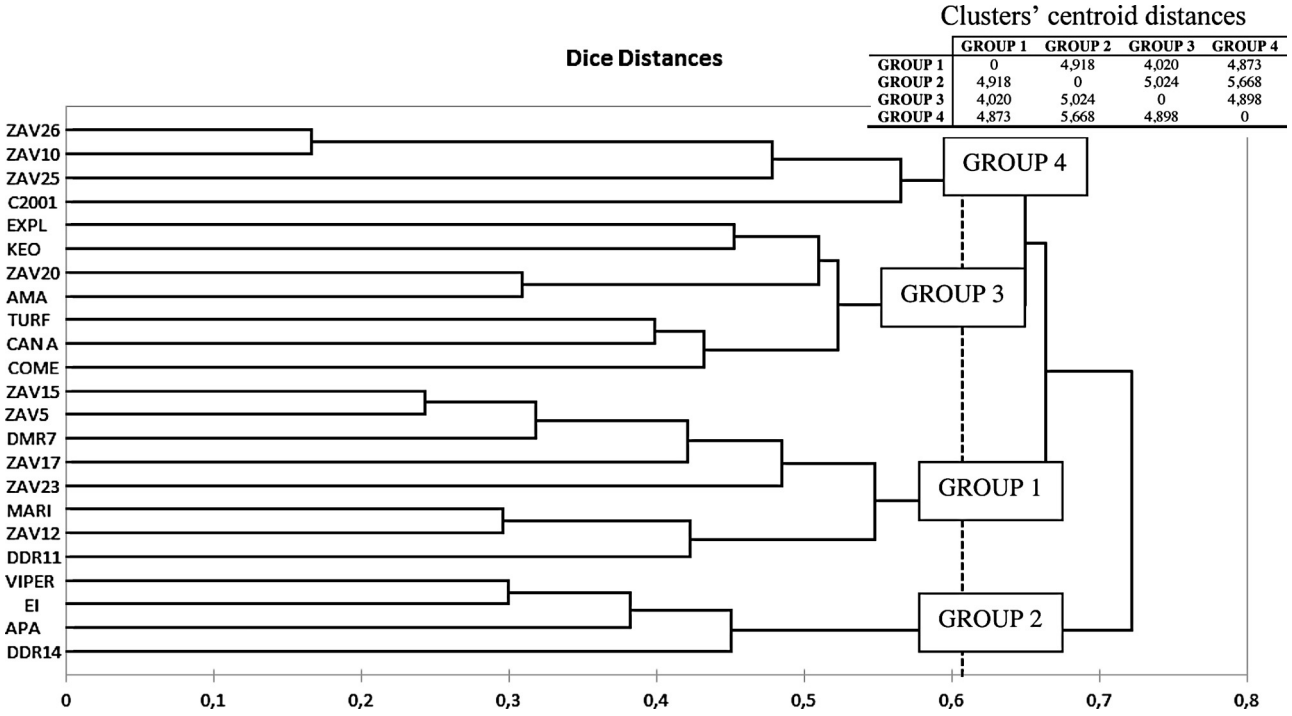

Fig. 4. Demdrogram of Dice distances for SRAP markers. Clusters' centroid distances

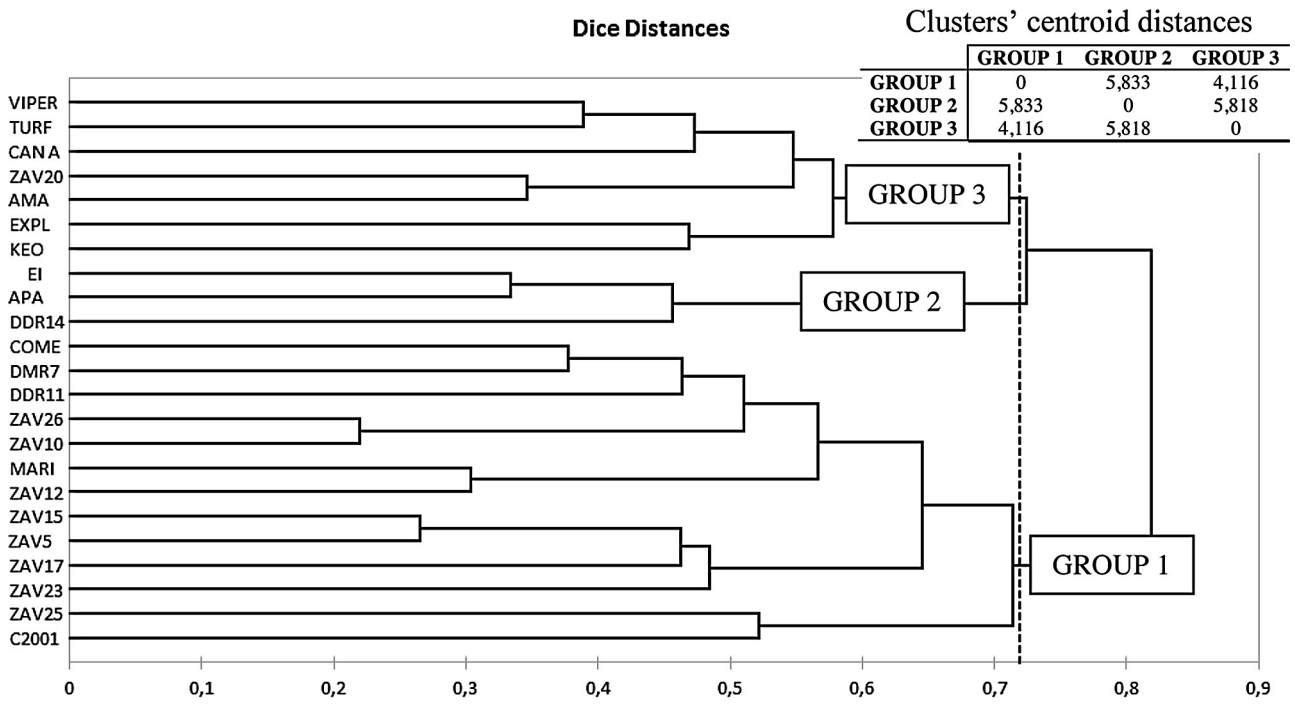

Fig. 5. Dendrogram of Dice distances for both SRAP and SSR markers. Clusters' centroid distances

In summary these results indicate that genetic distances based on SRAP's markers may be useful for predicting heterotic combinations in the pea genotypes selected, and are supportive of the idea that the level of correlation between hybrid performance and genetic divergence is dependent of the germplasm employed. In pea breeding, as in all self-pollinated species, the prediction of heterotic crosses makes more efficient the obtaining of superior lines that are transgressive segregants that can be identified in advanced generations by concentrating the efforts on the most promising cross-combinations. When a large quantity of possible parents is available and the posteriori method is used, a high number of manual crosses must be done and a large number of progenies must be evaluated, making this phase the most costly and time consuming. Any procedure capable to make more efficient this phase is of great interest to pea breeders, so the results in this study are very promising for breeders.

Anyway, it is necessary to include a greater number of combinations of SRAP to determine if a greater number of heterotic crosses can be predicted.

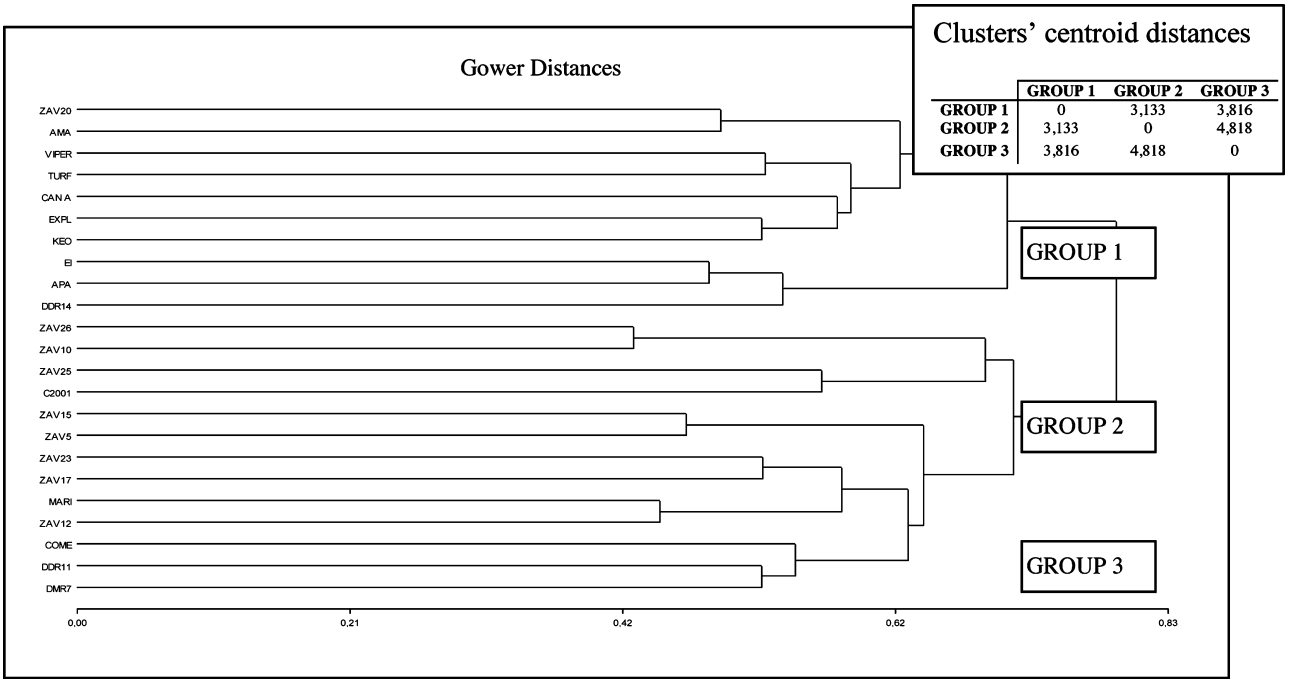

Fig. 6. Dendrogram of Gower distances for molecular and morphological data. Clusters' centroid distances

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