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Some studies on the Selection of Forage pea (*Pisum sativum* L.) to increase the Symbiotic nitrogen fixing Potential

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Abstract

The trial was carried out during the period of 2011-2013 on the experimental field of the Institute of Forage Crops, Pleven, Bulgaria. Populations of P_1, P_2, F_2 and F_1 of the crosses Shtambovyi x Pleven 10 and Rosacrono x Pleven 4 were investigated. It was found the performance of heterosis effect in regard to characteristics studied in F1 hybrids. Shtambovyi x Pleven 10 showed the highest positive true heterosis in regard to fresh weight of aboveground mass, nodule number and root length. Rosacrono x Pleven 4 shoved the highest positive true heterosis in regard to fresh weight of aboveground mass, seeds weight and fresh root weight. Plants from the two hybrids were the most depressed in F2 in regard to fresh weight of root and aboveground mass. The traits root length, fresh weight of root mass and seeds weight per plant in both crosses inherited positively over dominantly. There were dominated genes determining longer root system and higher dry weight, as well as higher seeds weight. As a result of prevailing negative epistatic interactions to reduce the degree of phenotypic expression of these characteristics in comparison to their full additive inheritance can be expected. Positively correlation interactions between specific nodulating ability and nodule weight per plant (r=0.957), and root length and fresh root weight (0.858) were found. Fresh root weight and root length had a maximal direct effect on fresh mass and grain productivity. The mass selection for seed weight, nodule weight, aboveground fresh weight and nodule number could start in F_2-F_3 for Shtambovyi x Pleven 10. For Rosacrono x Pleven 4 the mass selection for phenotype of the characteristics studied will be more effective if started as early as in F_6-F_7 or by multiple individual selection applying.

Key-Words: *Pisum sativum* L., Genetic control, Nitrogen fixation

Introduction

Legume-rhizobium symbiosis is a major source of nitrogen and has important ecological and agronomic importance in the sustainable agriculture [1, 2]. Nitrogen from biological nitrogen fixation is a major source of nitrogen in the soil which is used directly by the plants and is less susceptible to denitrification and leaching [3, 4]. Most of legumes through biological nitrogen fixation can meet between 50 and 80% of the nitrogen requirements [5].

To increase biological nitrogen fixation and plant productivity should be working on the selection of high productive genotypes with increased nitrogen fixing potential. Associated with the rise of genetic analysis, genetic of symbiotic systems are increasingly attracting the attention of researchers [6, 7, 8].

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The appropriateness of the selection directed to increasing of symbiotic potential and selection of promising genotypes for including in breeding process has been demonstrated in studies of many authors [9, 10, 11].

In study of pea genotypes was found that dual inoculation, fungal arbuscular micorrhiza and bacterial symbiosis, increased seed productivity and dry mass of plants (chosen as key parameters to assess the symbiosis), and exceeded the effect from mineral fertilization in majority of genotypes [9]. The possibility to conduct simultaneous selection for increasing both, nodulating ability and productivity in pea was demonstrated in studies of Sidorova et al. (2012) [12].

Pea is one of the main food legume crops (green mass and grain). It is preferred in the crop rotations and has a positive effect on the next crops [13, 14, 15, 16]. It can fixed to 150 kg N/ha and under favourable conditions to accumulate in the soil 45-70 kg N/ha [17, 18]. It was found winter forms formed 1.22 times more nodules as

compared to spring ones [19].

Pea is one of the best models to study the plant symbiosis. Most intensive research was carried out in regard to the genetic control of the process of interaction with nitrogen fixing bacteria [20]. More than 40 symbiotic genes controlling nodulation were identified in *Pisum sativum* L. [21]. There were described genes which both, decreased or increased symbiotic ability [8, 22]

The aim of this study was genetic assessment of plant material from pea (*Pisum sativum* L.) for the selection of high productive genotypes with increased symbiotical potential.

Material and Methods

The study was conducted during the 2011-2013 period in the experimental field of the Institute of Forage Crops, Pleven, Bulgaria. Parental forms used for crossing were from our collection: spring forms (*Pisum sativum ssp. sativum*) – Shtambovyi and Pleven 4, winter types (Pisum sativum ssp. arvense) – Rosacrono and Pleven 10. Characteristics of the varieties are shown in Table 1. These forms were crossed by hand in 2011. The parental forms (P1 and P2) and first and second generation (F1 and F2) were sown at scheme P1, P2, F2, F1 on a row spacing 70 cm and distance in a row 5 cm. Sowing by hand was applied with depth of 5 cm. The forage pea was grown according to the technology approved in the Institute of Forage Crops, Pleven. The samples were taken by taking the soil monoliths [19] at three phonological stages of plant development – budding, beginning of the flowering and maturity. There where 15 plants from each hybrid or parent fell into the monolith. Biometrical assessment of the parental forms (P1 and P2) and crosses of first and second hybrid generation (F1 and F2) was done by the following characteristics: specific nodulating ability (g nodules/g roots), nodule number, nodule weight per plant (g), root length (cm), fresh root mass (g), fresh aboveground mass (g), seeds weight from plant (g). For all the characteristics studied an average arithmetical (x); heterosis effect in F1 - (hypothetic and true), inbred depression by Omarov (1975) [23]; degree of dominance in F1 (hp1) and in F2 (hp2) by Romero and Frey (1973) [24]; coefficient of inheritance in narrow (H2) and wide sense (h2) by Konstantinov et al. (1979) [25] in F2 were calculated. Using method of Sobolev (1976) [26] the characteristic of transgression (Tn); number of genes, in which the parents differ (N); dominance (D); epistasis (E) and coefficient of effectiveness of the mass of genotypes by phenotypical performance of the trait (Pp) were found. The next statistical methods were used to process the

experimental data: factor analysis by the method of principal components Vandev, 2003) [27]; hierarchical cluster analysis by the method of Ward (1963) [28] – for the grouping of genotypes by similarity as a measure for the difference (the genetic distance), the Euclidean distance between them was used, having previously standardization the data carried out; correlation and path coefficient analysis according to the procedure given by Dewey and Lu (1959) [29] were calculated. All experimental data were processed statistically using the computer software GENES 2009.7.0 and Excel for Windows XP.

Results and Discussion

Biometric data for specific nodulating ability (Table 2) showed that all hybrids exhibited negative hypothetical and true heterosis highly expressed in Shtambovyi x Pleven 10. This cross was also characterized by strong negative depression (-92.77 %) in the plants from the second generation. Epistatic gene effects were exhibited for inheritance of the trial in Shtambovyi x Pleven 10 ($h_{p2}>h_{p1}$). In Rosacrono x Pleven 4 the dominance ($h_{p1}>h_{p2}$), i.e. dominated genes determining stronger nodulating ability played more importance. Analogical results were received for the nodule weight per plant heterosis for both hybrids being negative. The same was for the depression in plants from Shtambovyi x Pleven 10 (-100 %). Similar inheritance was found for nodule number per plant. Shtambovyi x Pleven 10 showed higher positive heterosis and lower depression (-30.07%) as compared to Rosacrono x Pleven 4.

There was relatively high positive hypothetical heterosis for root length in all hybrids, strongly expressed in Shtambovyi x Pleven 10, where the true heterosis was positive, too but the depression was positive (16.24 %).

The values for degree of dominance for fresh weight of root mass showed that the inheritance was epistatic for two hybrids. Hybrids showed positive heterosis and relatively high depression, more pronounced at the crossing Rosacrono x Pleven 4.

Positive dominance in inheritance of number of nodules, size of nodules and root mass weight were reported after study on mutant forms pea and received using the recurrent selection [20].

Positive over dominance ($h_{p1}>h_{p2}$) was the inheritance of fresh weight of aboveground mass for the two hybrids where the true heterosis was high. Statistically significant value of the trial in first generation of Rosacrono x Pleven 4 (40.89) and the level of h_{p1} (15.62) suggest that the genes of Rosacrono cultivar dominated.The trait seeds from one plant inherited under the influence of epistatic gene actions.

Shtambovyi x Pleven 10 showed negative and Rosacrono x Pleven 4 positive heterosis with very close values between hypothetical and true one, both crosses showed negative depression.

From the negative values of transgression (Table 3) for specific nodulating ability (Shtambovyi x Pleven 10), nodule weight per plant and fresh weight of aboveground mass (Rosacrono x Pleven 4), it can be assumed that in the generations from the homozygous genotypes, a large percentage will be with lower characteristics than the initial parental forms. According to number of nodules per plant, seed weight per plant and root length for both hybrids, homozygous genotypes with higher values of these characteristics can be expected. This is probably due to the different hereditary basis of the initial parental forms. In regard to the fresh weight of aboveground mass Shtambovyi x Pleven 10 only showed positive degree of transgression and in the next generations plants with bigger weight of aboveground mass can be received.

The part of overall volatility determined from the genetic differences was found by coefficient of inheritance in wide (H^2) and in narrow sense (h^2). Inheritance is a characteristic of the relative part of genetic differences and those resulting from the action of external environment of the phenotypic variability. When changing the genotype or the environment, the assessment of inheritance should change. For all traits studied (Table 3) low value of coefficient of inheritance was found, and the coefficient in narrow sense was less than coefficient in wide sense.

For Rosacrono x Pleven 4 there were statistically significant and relatively higher values in regard to seeds weight per plant, specific nodulating ability and nodule weight per plant, and for Shtambovyi x Pleven 10 for the fresh weight of aboveground mass.

Parental components participating in the crosses differ in number of genes for the most of characteristics studied, probably due to the hereditary traits of the initial cultivars involved in the selection of parents.

Significant differences in regard to this characteristic was found between cultivars Rosacrono and Pleven 4 for specific nodulating ability, nodule number per plant, nodule weight per plant, root length, fresh weight of aboveground mass and seeds weight per plant, and for the crossing between Shtambovyi and Pleven 10, for the fresh weight of aboveground mass.

From the values of characteristic for allelic interactions (N) positive over dominance of the dominated alleles, determining the expression of root length and fresh weight of root mass was found. For the specific nodualating ability, nodule weight and fresh weight of aboveground mass, dominated alleles, determining weaker nodulating ability and lower nodule and aboveground mass weight.

From the analysis of the between alleles interactions (E) (Table 3) it can be seen that in the part of the characteristics studied, the epistasis was negative and can be assumed that this will reduce the extend of their phenotypic expression in comparison to the full additive inheritance. There are positive epistatic interactions in Shtambovyi x Pleven 10 for fresh weight of aboveground mass (43.42) and root length (0.37); in Rosacrono x Pleven 4 for fresh weight of aboveground mass (60.84), nodule weight per plant (9.30), specific nodulating ability (6.36) and nodule number per plant (3.96).

The variation of studied quantitive traits due to the fact that parental varieties used in the hybridization schemes contained alleles of different genes from the polygenic set. Hybrids will combine more different alleles of the genes of the respective sets as the initial forms are more contrasted on its phenotype. There were positive values of the coefficients for effectiveness of the mass found in Shtambovyi x Pleven 10 for seeds weight per plant (0.63), nodule weight (0.61), fresh weight of aboveground mass (0.53) and nodule number per plant (0.33). Coefficients for effectiveness of the mass were low and negative for Rosacrono x Pleven 4.

The high coefficients of effectiveness of the mass for these characteristics give us the reason to suggest that there is a real probability for a selection of homozygote genotypes in their phenotypic expression. Mass selection in these hybrids can start even in F_2-F_3. Having in a mind the values of gene parameters for other traits as well as the coefficients of inheritance and effectiveness of the mass found, better results can be expected if desirable genotypes are selected after mass selection in the late generations (F_6-F_7) or if multiply individual selection was applied.

The analysis showed (Table 4) strong positive correlation between specific nodulating ability and nodule weight per plant (r=0.957); root length and root weight (0.858). Path analysis for the productivity of fresh mass (Table 5), fresh weight of root mass and root length showed positive direct effect (4.20, 2.50) by the indirect influence of specific nodulating ability (22.11). Path coefficient analysis for grain productivity (Table 6) showed that fresh root weight (4.04), root length (3.31) and specific nodulating ability (2.63) are the components having maximal positive direct effect on seed productivity. They can be important breeding criteria in the genetic improvement of forage pea. The

strongest indirect effect had nodule weight (9.67) and nodule number per plant (0.19).

Hierarchical cluster analysis of the varieties and their hybrids was done on the basis of the values of the traits studied. The Euclidean distance was used for as a measure for genetic distance.

Results shown as a dendrogram (Figure 1) indicated the different grouping of the varieties by similarity and difference. Samples were clustered into two main groups (A and B). Data showed that there was observed significant genetic distance between Rosacrono x Pleven 4 (F_1) and Shtambovyi x Pleven 10 (F_1), and all other genotypes. The plants from hybrids were characterized by high values of the traits fresh weight of the aboveground mass and nodule number per plant and are separated into group "A". In the subgroup of "B1" based on the main group "B" fall varieties Pleven 4 and Rosacrono, which are genetically closed to Rosacrono x Pleven 4 (F_2) and Shtambovyi x Pleven 10 (F_2), forming the second and third branch in the same subgroup. In the subgroup "B2" fall Pleven 10 and Shtambovyi cultivars which are genetically closed by root length and fresh weight of root mass.

To obtain more pronounced transgressive forms in hybrid combinations should be genotypes from different groups included to expect better combination of the favourable genes in one genotype. The hierarchical cluster analysis can be used in the selection to plan the initial parent combinations [30].

Genetic diversity between hybrids and initial parental forms was shown by factorial analysis applying using method of the main components (PC analysis) based on seven traits studied. There are three eigenvalues greater than 1 which determined the choice of the three main components. The first component explained 46.46 %, the second - 28.26 % and the third - 16.13 % of the total variance. The main component (F1 and F2) determined 74.72 % of the total variation of genotype × trait. The first component was mainly related to the characteristics E, D and B. In the formation of the second component characteristics A and C have participated. Characteristic having importance for the third component formation was G. Varieties were phenotypically very different between them (Figure 2). Rosacrono characterized by positive values of the both components and Shtambovyi, Shtambovyi x Pleven 10 (F_2) and Pleven 4 which were arranged in the first quadrant only of component F2. Pleven 10 and Shtambovyi x Pleven 10 (F_1) are situated in fourth quadrant showing negative values of both components. In the third quadrant hybrids from first and second

generation were arranged and they were derived from the crossing between Rosacrono and Pleven 4. Apart position of the hybrids in the first, third and fourth quadrant showed that together they are phenotypically similar in a small number of characteristics. From the location of the varieties and hybrids in bipolar plane it was found that from the characteristics studied A, C and B was defining. The vectors with the greatest length and determining the level of diversity were E, D and A.

Espinosa and Ligarreto (2005) [31] reported for the occurrence of negative heterosis for 1000 seeds weight and different (negative and positive) for seed weight per plant.

According to some authors [32, 33] even small difference between the coefficients of heritability in a wide and narrow sense suggested the genetically variability was highly heritable and the mass by phenotype will be effective, as well as in the next generations the consolidated forms with desirable parameters of the trials can be separated.

Conclusion
The performance of heterosis effect in regard to characteristics studied in F1 hybrids was found. Shtambovyi x Pleven 10 showed the highest positive true heterosis in regard to fresh weight of aboveground mass, nodule number and root length. Rosacrono x Pleven 4 shoved the highest positive true heterosis in regard to fresh weight of aboveground mass, seeds weight and fresh root weight. Plants from the two hybrids were the most depressed in F2 in regard to fresh weight of root and aboveground mass. The traits root length, fresh weight of root mass and seeds weight per plant in both crosses inherited positively over dominantly. There were dominated genes determining longer root system and higher dry weight, as well as higher seeds weight. As a result of prevailing negative epistatic interactions to reduce the degree of phenotypic expression of these characteristics in comparison to their full additive inheritance can be expected. Positively correlation interactions between specific nodulating ability and nodule weight per plant (r=0.957), and root length and fresh root weight (0.858) were found. Fresh root weight and root length had a maximal direct effect on fresh mass and grain productivity. The mass selection for seed weight, nodule weight, aboveground fresh weight and nodule number could start in F_2-F_3 for Shtambovyi x Pleven 10. For Rosacrono x Pleven 4 the mass selection for phenotype of the characteristics studied will be more effective if started as early as in F_6-F_7 or by multiple individual selection applying.

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Table 1: Distinctive features of the investigated genotypes

Traits	Variety (genotype)			
	Shtambovyi	Pleven 10	Rosacrono	**Pleven 4**
Vine type	short semi erect	Long prostrate	long semi erect	**long semi erect**
Flower position	terminal (with fasciation – Fa, fac)	axial	terminal (with fasciation – fa, Fac)	**axial**
Stipule type	double	normal	double	**normal**
Leaf type	normal	normal	normal	**normal**
Flower color	white	purple	pink	**white**
Specific nodulating ability	0.122*	0.054	0.139	**0.078**
Nodule number per plant	12.500	10.533	22.933	**18.133**
Nodule weight per plant (g)	0.031*	0.009	0.035	**0.017**
Root length (cm)	5.933	6.733	12.100	**9.200**
Root fresh weight (g)	0.172	0.123	0.322	**0.280**
Aboveground fresh weight (g)	7.65	11.14	18.46	**15.39**
Seeds weight per plant (g)	**9.232**	2.348	3.932	3.978

$* p \leq 0.0$

Table 2: Biometrical data of the quantitative traits of the investigated crosses

Hybrids	F_1	F_2	Heterosis F_1 (%)		Depression F_2 (%)	Degrees of dominance	
	x±Sx%	x±Sx%	hypothetical	real		in F_1 (h_{p1})	in F_2 (h_{p2})
Specific nodulating ability							
Shtambovyi x Pleven 10	0.03	0.07	-61.16	-72.02	-92.77	-1.58	**-1.30**
Rosacrono x Pleven 4	0.10	0.06	-8.90	-28.97	43.77	-0.31	**-3.45**
Nodule number per plant							
Shtambovyi x Pleven 10	15.73	20.46	36.60	25.84	-30.07	4.28	**18.16**
Rosacrono x Pleven 4	25.00	21.46	21.75	9.01	14.16	1.86	**0.77**
Nodule weight per plant (g)							
Shtambovyi x Pleven 10	0.01	0.02	-57.38	-72.34	-100.00	-1.06	**-0.55**
Rosacrono x Pleven 4	0.02	0.02	-16.46	-37.74	15.15	-0.48	**-1.70**
Root length (cm)							
Shtambovyi x Pleven 10	9.03	7.57	42.63	34.16	16.24	6.75	**6.17**
Rosacrono x Pleven 4	11.77	12.37	10.49	-2.75	-5.10	0.77	**2.37**
Root fresh weight (g)							
Shtambovyi x Pleven 10	0.22	0.21	51.07	29.59	5.67	3.08	**5.13**
Rosacrono x Pleven 4	0.57	0.49	90.92	78.47	14.85	13.03	**17.94**
Aboveground fresh weight (g)							
Shtambovyi x Pleven 10	33.11	10	252.42	197.22	69.80	13.59	**0.69**
Rosacrono x Pleven 4	40.89*	17.65	141.62	121.53	56.84	15.62	**0.94**
Seeds weight per plant (g)							
Shtambovyi x Pleven 10	5.47	9.37	-5.60	-40.79	-71.39	-0.09	**2.08**
Rosacrono x Pleven 4	**8.69**	**16.49**	**119.62**	**118.35**	**-89.80**	**205.70**	**1089.65**

$* \; p \leq 0.05$

Table 3: Values of the gene parameters for the quantitative traits of the investigated crosses in F$_2$ generation

Crosses/ Indicators	T$_n$	N	D	E	H^2	h^2	Pp
Specific nodulating ability							
Shtambovyi x Pleven 10	-0.02	2.13	-1.89	-1.05	0.01	-	-0.08
Rosacrono x Pleven 4	0.00	24.18	-6.68	6.36	0.16*	0.11*	-0.37
Nodule number per plant							
Shtambovyi x Pleven 10	15.47	4.25	1.92	-1.66	0.07	0.03	0.33
Rosacrono x Pleven 4	4.90	14.30	-3.35	3.96	0.11	0.07	0.20
Nodule weight per plant (g)							
Shtambovyi x Pleven 10	0.02	0.26	-0.01	-0.34	0.01	-	0.61
Rosacrono x Pleven 4	-0.01	35.93	-11.13	9.30	0.15**	0.0003**	-1.25
Root length (cm)							
Shtambovyi x Pleven 10	1.66	0.51	1.78	0.37	0.005	-	0.02
Rosacrono x Pleven 4	0.22	8.44	3.45	-2.78	0.001	-	-0.03
Root fresh weight (g)							
Shtambovyi x Pleven 10	0.07	2.17	1.85	-1.06	0.11	0.07	0.23
Rosacrono x Pleven 4	0.22	1.62	2.02	-0.87	0.12	0.08	0.18
Aboveground fresh weight (g)							
Shtambovyi x Pleven 10	2.78	253.25	-20.54	43.42	0.19*	0.14	0.29
Rosacrono x Pleven 4	-0.79	360.76	-28.46	60.84	0.01	-	0.02
Seeds weight per plant (g)							
Shtambovyi x Pleven 10	16.80	1.22	0.72	-0.73	0.42***	0.29***	0.63
Rosacrono x Pleven 4	6.24	24.55	7.29	-6.53	0.35***	0.25***	-0.80

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Table 4: Correlation coefficients (r) among the quantitative traits of *Pisum sativum* L. genotypes

Variables	A	B	C	D	E	F
B	0.407					
C	**0.957*****	0.389				
D	0.153	0.654	0.246			
E	0.147	0.641	0.214	**0.858****		
F	0.177	0.698	0.114	**0.757***	**0.880****	
G	-0.109	0.038	0.125	0.319	0.539	0.112

A - Specific nodulating ability; B - Nodule number per plant; C - Nodule weight per plant (g); D - Root length (cm);
E - Root fresh weight (g); F - Aboveground fresh weight (g); G - Seeds weight per plant (g)
$p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Table 5: Path coefficients for green mass productivity of pea genotypes

Trait	Indirect effects					Total effect
	A	B	C	D	E	
A	**-6.82***	-18.52	7.309	3.82	8.75	**0.11**
B	-0.49	**-0.27***	2.29	5.24	10.83	**8.00**
C	104.42	-1.31	**-5.44***	3.54	1.76	**0.008**
D	0.34	-0.51	-7.53	**2.50***	7.42	**5.40**
E	22.11	-0.31	-15.45	2.08	**4.20***	**0.072**

*- Direct effect; A - Specific nodulating ability; B - Nodule number per plant; C - Nodule weight per plant (g); D - Root length (cm); E - Root fresh weight (g)

Table 6: Path coefficients for seed productivity of pea genotypes

Trait	Indirect effects					Total effect
	A	B	C	D	E	
A	**2.63**	-33.03	9.67	3.67	19.25	**0.58**
B	-0.88	**0.10**	3.04	5.04	23.82	**14**
C	138.26	0.50	**-9.71**	3.4	3.89	**0.024**
D	0.33	0.19	-13.42	**3.31**	16.33	**9.8**
E	48.63	0.12	-27.55	2.76	**4.04**	**0.37**

*- Direct effect; A - Specific nodulating ability; B - Nodule number per plant; C - Nodule weight per plant (g); D - Root length (cm); E - Root fresh weight (g)
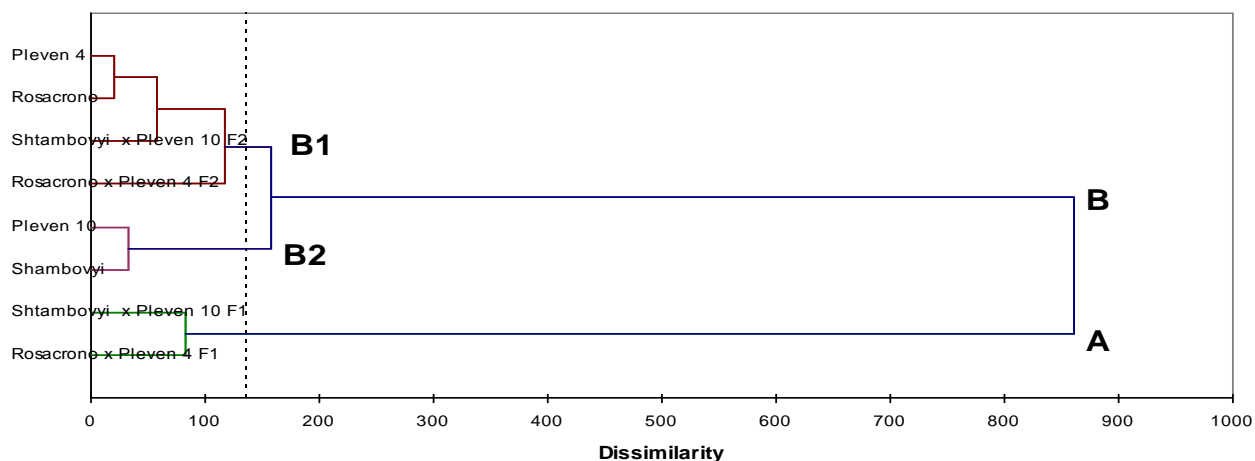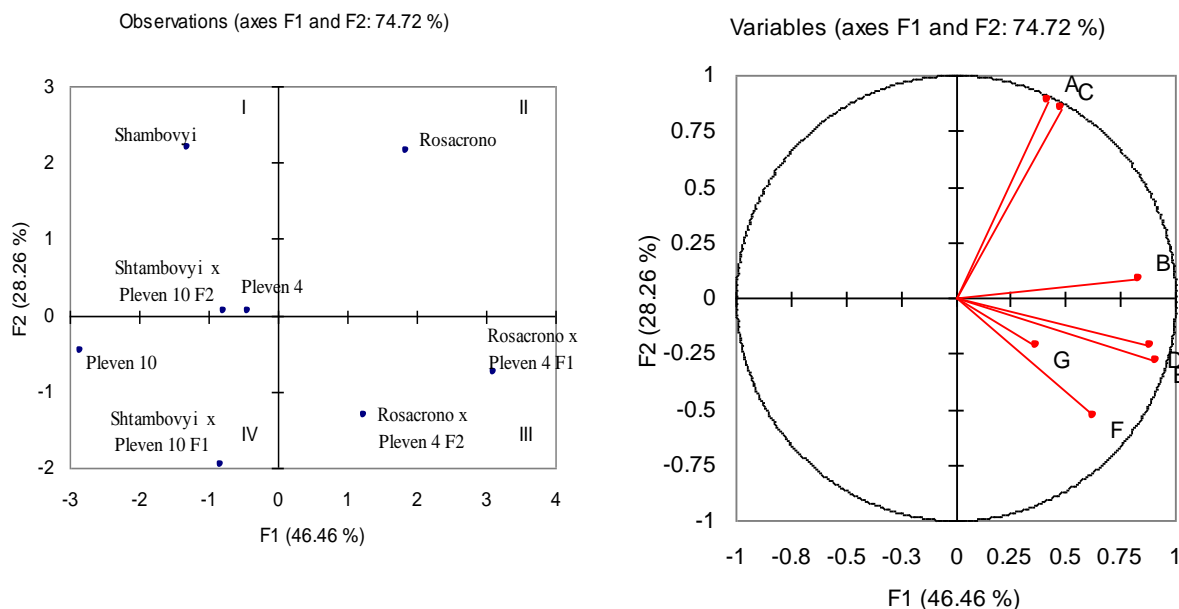
Dendrogram

Fig. 1: Dendrogram of forage pea varieties and hybrids

Fig. 2: Principal Component Analysis of quantitative traits of *Pisum sativum* genotypes
A - Specific nodulating ability; B - Nodule number per plant; C - Nodule weight per plant (g); D - Root length (cm);
E - Root fresh weight (g); F - Aboveground fresh weight (g); G - Seeds weight per plant (g)