

## ESTIMATION OF GENOTYPIC DIVERGENCE IN EUROPEAN COLLECTION OF *PISUM* SPECIES FOR YIELD RELATED TRAITS

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### ABSTRACT

Characterization of the important traits of pea genotypes (yield and yield components) can facilitate the creation of breeding populations for further selection. Research work was conducted on 23 genotypes of peas with the aim of grouping and determination of holders of quantitative traits (yield and yield components) for future breeding process. Cultivar selection was done based on the cultivars presence in large scale production in South-East Europe. Field trial was conducted during two vegetation years, major quantitative traits were measured and major qualitative traits were evaluated. The resulting data were analyzed by basic statistical methods (mean and standard deviation), analysis of variance, principal component analysis and hierarchical cluster analysis. Statistically significant genetic diversity was found for most researched traits. Most of the researched traits were under great influence of the environment, while the environment influence had least impact on the absolute grain mass. In PC analysis first two PC axes explained 79.16% of differences. The strongest association between the traits was obtained for: height of the first pod and total plant height; grain yield and the absolute mass of grains. These two groups were in opposition. The product of the hierarchical cluster analysis was five clear clusters grouped on the basis of nine quantitative traits and three morphological traits.

**Key words:** diversity, *Pisum sativum* L., variability, yield, yield components.

### INTRODUCTION

Center of the origin of pea (*Pisum sativum* L.) has not yet been determined with certainty, despite numerous published papers. One of the most frequently quoted authors is Blix (1974), who in his research on the center of origin of pea states the Mediterranean area, from which it spread to European countries, while the Middle East and Ethiopia is considered as a secondary center. Smartt (1990) pointed out that the pea cultivated in the Mediterranean region, is cultivated primarily as a winter crop. Cousin (1997) mentions Ethiopia and Afghanistan as primer center of origins, with late colonization in Mediterranean area, from where it was spread to other parts of Europe and Asia. Makasheva (1983) brought a theory that is based on the distribution of wild pea subspecies concentrated around areas of North-Western India and Pakistan, extending to Afghanistan and the former Soviet Union. From this source center, peas were spread to moderate

temperature zones of Europe and South-Western Asia.

Field pea (*Pisum sativum* L.) is the main large-grain legume in Europe (FAO, 2008) and an important source of protein. According to the purpose, peas can be divided into grain and fodder peas. Grain pea cultivars are mostly spring crop, while the majority fodder pea cultivars are winter-forms. The average grain yield in Croatia is 2.8 t/ha (FAO, 2008) and is low in comparison to the yield of 4.4 t/ha in France (FAO, 2008). The growing needs for protein, both in human and in animal nutrition, impose peas as an alternative European plant species that will reduce imports of soybean from the United States (Bourin et al., 2002a.), but also to call off the monopoly position of the soybeans producers (Marohnic, 2006).

The increase of the yield and quality and maintenance of grain yield stability are the main peas breeding goals. Knowing the scale and model of pea genetic diversity are very essential for the efficient development of

breeding and conservation of pea's collections (Kenei et al., 2005). Pea can be found around the world in wild forms and local populations that contain valuable genes adapted to different environments (Ceccarelli, 1994). Crossing most different parents is more likely to achieve desirable recombinations. This is evidenced by most researchers using the wild and the local population in order to achieve a wider genetic variability. However, the introduction of wild populations in the breeding program increases the genetic diversity of progeny, and this usually extends the selection cycle because of low agricultural value. Therefore, the choice of parents is particularly delicate, and it is based on the knowledge of the genetic structure and economic value of indigenous populations, old cultivars, spring and winter forms.

Pea yield and yield components are the most studied quantitative traits which, together with divergence estimations, are examined in gene collections. Characterization of the important traits of pea genotypes (yield and yield components) can facilitate the creation of breeding populations for further selection. At the Agricultural Institute Osijek pea germplasm was collected from different European countries with little or no information about the quantitative traits in semi-arid climatic conditions of South-East Europe. Therefore, research was conducted on 23 genotypes of peas with the aim of grouping and determination of holders of quantitative traits (yield and yield components) for further breeding process.

## MATERIAL AND METHODS

The research included 23 genotypes [cultivars (C) and landraces (L)] of forage peas from the collection of the Agricultural Institute Osijek (Table 1). Cultivar selection was done based on the cultivars presence in large scale production in South-East Europe. Genotypes of pea were studied on the location of Osijek (45°32'N 18°45'E), semi-arid areas (Figure 1), on eutric cambisol (A-(B) VR) during two years (2006-2007) (Table 2).

The trial was sown on March 15<sup>th</sup> 2006 and 2007, in a randomized block system with

four replications. Trial plot size was 12 m<sup>2</sup>, with 130 plants per m<sup>2</sup>. Plot size taken into consideration for measuring the yield was 10 m<sup>2</sup>. From the middle rows of each plot, at technological maturity, 10 randomly selected plants (total of 40 plants per cultivar) were taken for the analysis of quantitative traits: height to the first pod in cm (H1p), total height in cm (H), number of fertile nodes - number (FN), number of pods per plant - number (p/P), number of grains per pod - grain number (G/p), number of grains per plant, - number (G/P), grain weight per plant, g-(W/P), absolute mass of the grain-g (AW), grain yield per area unit - t ha<sup>-1</sup> (Y). Other examined qualitative traits (Table 1) were described for the sample of 40 random plants in 2006.

Table 1. Characteristics of the studied cultivars of forage peas

Cultivar	Origin/ Material	Type	Leaf	Seed colour	Flower colour
Torsz	Hun/C	Spring	Afila	Brown	Rose
Jp-5	Hun/L	Winter/ Spring	Wild	Pigmented	Red
Osječki zeleni	Cro/C	Winter	Wild	Pigmented	Red
Maksimirski ozimi	Cro/C	Winter	Wild	Pigmented	Red
Maksimirski bijeli	Cro/C	Winter/ Spring	Wild	Green	White
NS Pionir	Srb/L	Ozimi	Wild	Pigmented	Red
Poneka	Ger/C	Spring	Wild	Pigmented	Red
Timo	Srb/C	Winter/ Spring	Wild	Pigmented	Red
NS Junior	Srb/C	Spring	Wild	Yellow	White
Moravac	Srb/C	Spring	Wild	Yellow	White
Zekon	Cze/C	Spring	Afila	Yellow	White
Eiffel	Fr/C	Spring	Afila	Yellow	White
Luszany	Cze/C	Spring	Wild	Green	White
Isabella	Nl/C	Spring	Wild	Yellow	White
Adept	Cze/C	Spring	Wild	Yellow	White
Primus	Cze/C	Spring	Wild	Yellow	White
Bohatyr	Cze/C	Spring	Wild	Yellow	White
Erbi	Nl/C	Spring	Wild	Yellow	White
Gold	Cro/C	Spring	Afila	Yellow	White
Baccara	Fr/C	Spring	Afila	Yellow	White
Sponzor	Dk/C	Spring	Afila	Yellow	White
Faktor	Cze/C	Spring	Afila	Yellow	White
Jezero	Srb/C	Spring	Afila	Yellow	White

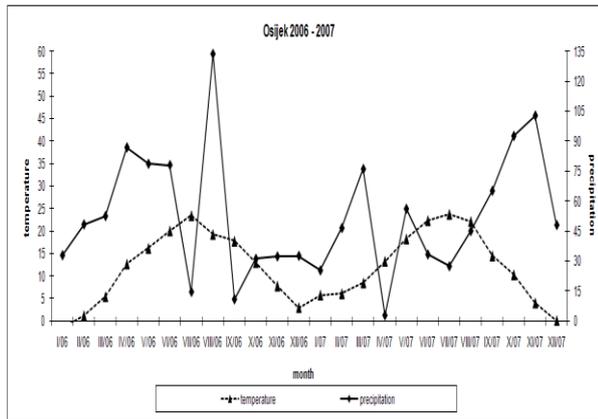


Figure 1. Clime diagram by Walter for Osijek (2006-2007)

Table 2. Chemical characteristics of eutric cambisol soil at Osijek

Location/ soil profile	pH		Humus %	AL (mg/100g)	
	KCl	H <sub>2</sub> O		P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
Osijek / A-(B)v-R	6.41	7.00	2.01	39.7	37.7

The resulting data were analyzed by basic statistical methods (mean and standard deviation), analysis of variance, principal component analysis (PC) using computer software SAS 9.1 (SAS Institute, 2003) and NTSYS pc 2.20b (Rohlf, 2005). Traits were measured by different units, so the average values of the traits were standardized before the PC analysis to eliminate differences in values. Hierarchical analysis was made on the basis of qualitative and quantitative (12 properties) data using the Ward model (Ward, 1963).

## RESULTS

Statistically significant genetic diversity was found for most researched traits at  $P < 0.01$  (Table 3). Researched traits (height, height to first pod, number of fertile nods, number of grains per plant, number of pods per plant, number of grains per pod, grain weight per plant and grain yield) were under great influence of the environment, while the environment had least impact on the absolute grain mass. Tall cultivars (Pionir NS, Timo) had a larger number of fertile nods per plant, and thus more grain per plant. Number of

pods per plant, as a major component of yield, was highest in cultivar Erbi, higher than in NS Pionir cultivar. Number of grains per pod did not vary much according to cultivar, and the largest number of grains per pod was found in cultivar Poneka, while the maximum absolute grain weight was found in cultivar Primus. Genotype Bohatir achieved the highest overall grain yield per area unit, while Moravac genotype had the highest grain weight per plant. The first three components with eigenvalues  $>1$  explained 91.33% of variability between 23 genotypes for 9 quantitative traits (Table 4). Traits that mostly contributed to PC1 axis were: plant height, height to the first pod, grain yield, absolute mass, fertile nods and the number of grains per pod. PC2 axis was defined by the number of pods and weight of grains per plant. Number of grains per plant contributed maximum to PC3 axis. The first two PC axes explained 79.16% of differences. Relations between genotypes and traits (biplot) are shown in figure 2.

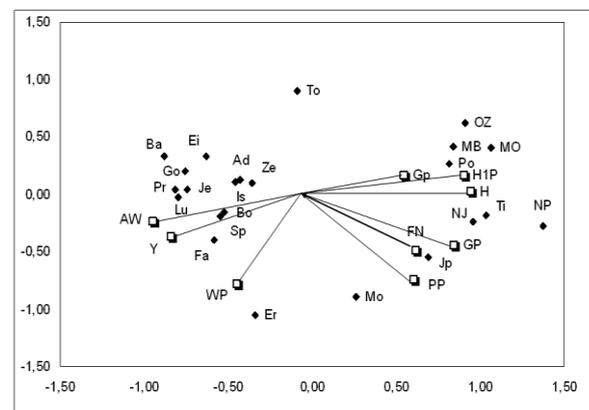


Figure 2. Analysis of the major components for 9 agronomic traits and 23 pea cultivars

Genotypes Moravac, Erbi and Torsz were separated from the two main groups of genotypes that were grouped into two opposing sides. On the left side genotypes with reduced height with very large grains were grouped, while opposite to them were tall genotypes with a large number of fertile nods. The strongest association between the traits was obtained for: height of the first pod and total plant height; grain yield and the absolute mass of grains. These groups were in opposition to each other.

## ROMANIAN AGRICULTURAL RESEARCH

Table 3. Average values (Avr.) and  $\pm$  standard deviation (S.D) for traits of 23 pea cultivars in two years of research

Cultivars	H		H1p		FN		p/P		G/P		G/p		W/P		AW		Y	
	Avr.	S.D	Avr.	S.D	Avr.	S.D	Avr.	S.D	Avr.	S.D	Avr.	S.D	Avr.	S.D	Avr.	S.D	Avr.	S.D
Torsz	110.2	7.9	88.52	6.9	3.4	0.7	4.9	0.7	18.9	5.0	3.8	0.5	3.5	1.1	189.6	15.0	3.11	0.6
Jp-5	149.7	39.3	93.4	25.9	5.8	1.1	5.8	1.6	34.3	10.3	4.0	0.6	3.2	2.0	181.2	15.2	3.76	0.9
Osječki zeleni	137.4	43.4	90.5	33.9	4.8	1.0	6.7	1.6	29.6	8.2	4.4	0.5	2.8	1.1	90.8	13.6	1.46	0.7
Maksimirski Ozimi	146.2	56.1	93.7	37.9	5.2	1.9	7.1	2.8	31.8	10.3	4.6	0.3	3.4	1.0	108.6	10.8	1.89	0.6
Maksimirski Bijeli	129.6	47.4	83.5	37.6	5.0	0.7	7.4	1.8	29.9	8.7	4.0	0.3	3.0	1.0	100.4	17.8	1.78	0.4
NS Pionir	147.9	41.0	89.6	29.1	6.5	1.2	9.2	2.1	39.9	10.4	4.3	0.6	3.8	0.9	97.2	10.3	1.97	0.6
Poneka	159.4	58.9	105.9	40.4	5.0	1.6	6.4	2.3	31.7	12.3	5.0	0.5	4.2	1.6	131.4	10.0	4.49	0.5
Timo	152.9	46.8	94.1	34.9	6.5	1.4	8.9	2.1	31.5	10.0	3.5	0.5	3.7	1.0	119.8	10.0	2.37	0.5
NS Junior	170.5	47.4	111.5	32.2	5.5	1.2	8.7	1.8	39.9	10.8	4.5	0.6	6.3	1.8	156.6	15.6	3.06	1.2
Moravac	120.5	37.0	73.2	18.4	5.3	2.0	8.5	2.9	36.3	11.3	4.3	0.5	8.3	3.0	225.1	12.8	4.65	0.5
Zekon	74.3	30.1	43.5	16.9	3.9	2.0	6.4	3.4	25.2	13.2	4.0	0.2	5.7	3.1	222.5	23.8	3.71	0.8
Eiffel	75.0	5.2	52.3	6.1	3.8	0.2	5.6	1.5	20.0	7.2	3.5	0.4	4.8	1.4	250.7	20.2	4.43	0.9
Luszanyi	65.5	19.2	36.5	10.6	4.1	1.6	6.0	1.9	21.4	5.5	3.7	0.3	5.7	1.3	266.0	13.5	5.50	0.6
Isabela	72.2	12.9	46.2	9.8	4.4	0.8	6.4	0.6	22.6	5.4	3.5	0.8	4.9	0.8	223.1	12.1	4.40	1.0
Adept	82.3	6.9	52.1	7.4	4.6	0.4	6.3	1.0	20.1	2.7	3.7	0.8	5.0	1.0	222.5	24.2	4.75	1.9
Primus	81.3	6.7	54.2	8.9	4.0	0.7	6.6	2.0	18.2	7.3	2.7	0.2	5.1	1.4	299.8	25.9	4.70	1.8
Bohatyr	79.1	30.2	44.1	16.4	4.6	2.1	7.2	3.2	22.7	7.2	3.4	0.5	5.3	1.4	238.1	20.6	5.70	1.1
Erbi	63.6	18.4	30.9	7.0	5.8	2.5	9.4	4.1	30.1	6.9	3.5	0.8	7.3	1.7	241.5	9.1	5.52	1.1
Gold	49.8	12.5	25.6	7.3	3.8	1.0	5.6	1.4	22.6	3.6	4.2	0.5	5.3	0.7	236.3	13.4	5.24	1.1
Baccara	60.7	14.1	35.2	7.8	3.3	1.1	5.2	1.6	20.0	4.7	4.0	0.6	5.3	1.2	268.1	12.3	5.16	1.3
Sponzor	76.8	27.4	44.9	15.4	4.1	1.8	6.8	2.9	25.0	7.2	3.9	0.6	6.0	2.0	239.6	16.8	5.51	1.0
Faktor	76.3	25.8	40.7	13.2	5.0	2.1	7.2	3.3	23.3	7.4	3.5	0.7	6.4	2.2	272.8	9.1	5.16	1.3
Jezero	66.9	15.0	39.2	7.6	3.4	1.4	5.7	2.7	24.0	9.2	4.4	0.5	6.5	2.2	277.2	20.3	5.28	1.1
MS (year)	81405**		36249**		113.5**		172.4**		721.4**		16.6**		77.6**		19500**		21.9**	
MS (genotype)	9019**		4513**		5.3**		10.4**		264.9**		1.5**		11.7**		25958**		11.5**	
MS (y x g)	1560**		730**		3.7**		12.8**		144.0**		0.3**		4.7**		4789**		3.6**	

Genotypes Erbi and Moravac were related to the trait of grain weight per plant, while the genotype Torzs in opposition to them had a small number of pods per plant. In the researched material carriers of higher yield were cultivars: Bohatyr, Luszanyi and Sponzor, while the carriers of higher seed mass were cultivars Primus and Jezero. On the contrary, cultivars Poneka and NS Junior were carriers of genes for the plant height, while the total variability in the number of grains per plant was most strongly influenced by

genotypes JP-5, NS Junior, Timo and NS Pionir. Hierarchical cluster analysis produced 5 clear clusters (Figure 3) grouped on the basis of 9 quantitative traits and 3 morphological traits. Cluster 1 included one exotic cultivar (Torsz), different from all others by morphological and agronomic traits. Clusters 2 and 3 included the highest diversity among the studied cultivars, whereas in clusters 4 and 5 diversity between cultivars was minimal. Grain weight per plant significantly correlated with the number of

pods per plant,  $r = 0.61$  (Table 5). Grain yield per unit area was in strong, positive and statistically significant correlation with the absolute mass of grain ( $r = 0.79$ ) and grain weight per plant ( $r = 0.56$ ).

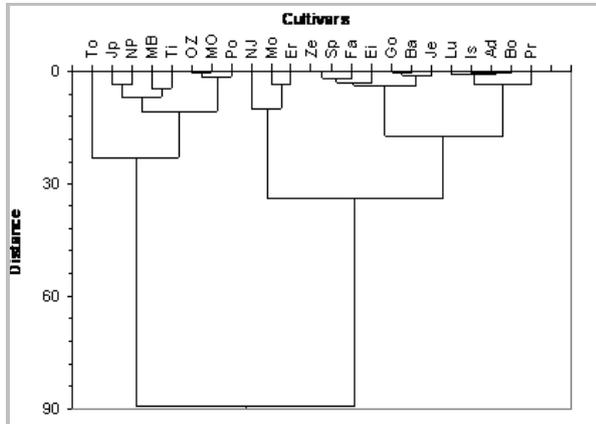


Figure 3. Dendrogram based on the morpho-agronomic traits of 23 accession of *Pisum sativum* by Ward's method

Table 4. The main components of quantitative traits for forage peas

Traits	PCA 1	PCA 2	PCA 3
Eigenvalues	5.29	1.84	1.09
Percent (%)	58.73	20.43	12.17
Total percent	58.73	79.16	91.33
Eigenvectors			
Height (H)	0.95	0.02	0.09
Height from 1 <sup>st</sup> pod (H1p)	0.90	0.16	0.10
Fertile node (FN)	0.62	-0.49	-0.45
No. of pods per plant (p/P)	0.60	-0.74	-0.19
No. of grains per pod (G/p)	0.84	-0.44	0.25
No. of grains per plant (G/P)	0.55	0.16	0.75
Grain weights per plant (W/P)	-0.44	-0.77	0.39
Absolute weight (AW)	-0.94	-0.23	0.06
Yield (Y)	-0.84	-0.36	0.20

Table 5. Correlation coefficients (below the diagonal) and significance (above the diagonal)

	H1p	H	FN	p/P	G/P	G/p	W/P	AW	Y
H1p	<u>1.00</u>	0.01	0.01	0.01	0.09	0.61	0.62	0.01	0.01
H	0.97	<u>1.00</u>	0.01	0.01	0.01	0.59	0.08	0.01	0.01
FN	0.62	0.48	<u>1.00</u>	0.01	0.05	0.01	0.01	0.01	0.31
p/P	0.47	0.32	0.93	<u>1.00</u>	0.01	0.02	0.01	0.01	0.84
G/P	0.53	0.39	0.79	0.84	<u>1.00</u>	0.01	0.01	0.01	0.03
G/p	0.04	0.05	-0.23	-0.20	0.29	<u>1.00</u>	0.16	0.01	0.01
W/P	-0.04	-0.15	0.45	0.61	0.53	-0.12	<u>1.00</u>	0.01	0.01
AW	-0.59	-0.56	-0.31	-0.24	-0.44	-0.39	0.46	<u>1.00</u>	0.01
Y	-0.48	-0.50	-0.08	-0.01	-0.19	-0.30	0.56	0.79	<u>1.00</u>

## DISCUSSION

Description of the important agronomic traits is a basic prerequisite for the efficient use of germplasm in breeding programs (Duvick, 1984). It is important to identify and select genotypes that have important agronomic properties and use them in direct hybridization. Pea germplasm showed little genetic variability (Baranger, 2004; Simioniuc, 2002). That is problem in pea breeding since breeding program depends on the amount of genetic variability in the basic breeding population. Explaining variance in each of the components helps to make better use and planning resources for improvement of economically important characteristics. Pea genotypes used in this study were divergent for the most studied traits. This information could be used in the selection process for the purpose of breeding. The studied genotypes showed statistically significant variation for the average number of nods per plant between years. Significant differences between years were the result of the dry period in 2007 at the beginning of plant generative development. Popovic and Stjepanovic (1992) state that the lacks of humidity and high temperatures result in pea flowers abortion and poor growth of the pods. The results obtained in the conducted survey coincide with the results obtained by Stelling and Ebmeyer (1990) and Bourion et al. (2002b) with average number of 4.8 to 4.9 fertile nods. Based on the results it can be concluded that the number of pods per plant is closely related to grain yield and is sensitive to drought stress conditions at flowering stage. Tekeli and Ates (2003) found that average values of the number of pods per plant in wild pea genotypes varied during their four-year study from 9.3 to 16.5 pods.

Uzun et al. (2004) report a variation of the numbers of pods per plant from 7.4 to 12 pods. It is evident that the values obtained by us are lower than those found by the aforementioned researchers. This can be interpreted as the influence of environmental factors in different geographical areas (the environment).

The variation of the number of grains per plant in studied genotypes, in the two years of research showed that the trait was under a strong influence of environmental factors, but also showed variation among genotypes. Uzun et al. (2004) obtained similar values for the number of grains per plant, which varied from 24.5 to 43.1. Stelling and Embeyer (1990) indicated average values of the number of grains per pods from 2.3 to 4.5 and the absolute mass from 178 to 278 g. They also found a high estimated heredity for both traits and a negative correlation between them ( $r = -0.34$ ). Uzun et al. (2004) reported an average variation in the number of grains per pod from 4 to 4.7, but without significance, and a significant variation of absolute grain weight from 160 to 203 g, while Simioniuc et al. (2002) found the variation of absolute grain mass from 134.6 to 351.3 g. It is important to note that the spring genotypes had a significantly higher absolute mass of grains and that all genotypes, except for Poneka and NS Junior, differed only slightly. Absolute grain weight was conditioned by the influence of genotype and less by influence of the particular year, while other traits were significantly influenced by the particular year. Čupić et al. (2005), based on path analysis, found that the yield was in direct positive correlation with the absolute grain weight, and that this trait represented the primary selection criterion in breeding peas.

Multivariate analysis of traits estimated with principal components analysis (PCA), allows the identification of genotypes that differ by agronomical value. This kind of analysis was used in many different crops, such as alfalfa (Tucak et al., 2009), chickpea (Ozgun et al., 2004; Naghavi et al. 2005), blackgram (Ghafoor et al., 2001) mungbean (Siddique et al., 2006) and peas (Bourion et al., 2002b) for the purpose of grouping and identification of important properties carriers. Bourion et al. (2002a) grouped 8 peas' genotypes on the basis of 7 quantitative traits. Ghafoor et al. (2003), in their research based on 11 quantitative traits, found that the first three PCA axes explained 88.58% of the variance between 62 chickpea genotypes. Our results are consistent with the results of Tar'an

et al. (2005), based on morphological and physiological data, where four components explained 90.6% of variability for 65 cultivars of peas.

Dividing genetic variability in smaller parts provides better grouping of genotypes and planning of appropriate genotypes for traits improvement. Genotypes with the largest number of pods per plant achieved highest yield per plant. High grain yields per plant were not equally manifested in the yield per area unit, which was influenced by pods breaking resistance and by plant lodging resistance. These disagreements are probably the result of non-compliance properties, and inability to make up the yield from other properties in mechanized harvesting. Cluster analysis reveals grouping based on the most important traits and provides the characteristics of each group and the share of genetic diversity in the researched material. Similar conclusions about the usefulness of cluster analysis in small differences complex situations, reached Acikgoz et al. (2009) in their research studies on grain yield of peas in different Mediterranean locations. On the basis of cluster analysis we have tried to find the links within each cluster. Cluster 2, including three Croatian cultivars, consisted mainly of local populations and old cultivars, which marked the period of selection and breeding. Cluster 3 had the highest number of pods and grains per plant, as a result of the stability of these cultivars in semi-arid conditions. Linkage within the Cluster 4 is the origin of cultivar Dick Trom which is the predecessor of all the new modern semi-leafless cultivars (Simioniuc et al., 2002). Cluster 5 included mostly Czech cultivars, indicating a relatively narrow choice of parents from the rich Czech gene collections.

Based on the research of 23 cultivars of forage peas, we confirmed the existence of significant differences between cultivars and years for the all nine researched traits. The absolute grain mass was under greater influence of genotypes, while other properties were under stronger influence of the year. Researched material was divided in two height groups; also it was divided in accordance with technological purposes.

A wide range of differences was determined for most of the investigated traits and germplasm with specific characteristics was identified. This enables the selection based on grouping by cluster groups. Grain weight per plant was in medium strong positive correlation with the numbers of pods per plant, while the yield per area unit was strongly positively correlated with the absolute grain mass, which is the main criterion for the future selection.

### CONCLUSIONS

The present investigation revealed that most of the studied traits were highly variable.

Principal components analysis with first three components (eigenvalues > 1) explained 91.33% of variability between 23 genotypes. Nine quantitative traits separated the majority of the cultivars into two main groups.

Cluster analysis revealed grouping based on the most important traits and provided the characteristics of each group and the share of genetic diversity in the researched material. Positive traits for breeding were found in all clusters.

This indicates that promising materials exist, which could be used for future pea breeding programmes.

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