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Evaluation of genetic divergence and heritability in pea (*Pisum sativum* L.)

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ABSTRACT

An experiment on genetic evaluation of five genotypes of forage pea (Glyans, Svit, Kamerton, Modus, Pleven 4) was conducted during 2012-2014 period. Analysis of variance showed significant differences among genotypes for the traits pod width, seeds per plant, seed weight per plant and 1000 seed weight. The estimates of genetic parameters of five varieties of *Pisum sativum* L. indicated a good amount of genetic variation in the experimental materials under investigation. Moderate phenotypic and genotypic coefficients of variation were observed for most of traits except pod length and pod width. For the traits studied seeds per plant, seed weight per plant and plant height were found high heritability along with high genetic gain indicating preponderance of additive effects. Therefore, selection programme based on these characters would be more effective in improving yield parameters of forage pea. The seed yield was positively and significantly correlated with 1000 seed weight and pod stem, which suggested the possibilities of improving seed yield by simultaneous improvement of these traits.

Key words: heritability, *Pisum sativum*, trait association, variability

Introduction

Field pea (*Pisum sativum* L.) is one of the world's oldest domesticated crops. Its area of origin and initial domestication lies in the Mediterranean, primarily in the Middle East. The pea (*Pisum sativum* L.) is an important vegetable crop due to its high nutritive value. Its improvement is based mainly on exploiting the natural sources of germplasm by means of selection or hybridization followed by selection (Zohary & Hopf, 2000).

Important genetic diversity collections of *Pisum* with over 2000 accessions are found in national genebanks in at least 15 countries, with many other smaller collections worldwide (Coyne et al., 2011; Smykal et al., 2011; Smýkal et al., 2012)

Genetic variability is considered as an important factor which is essential prerequisite for crop improvement program for obtaining high yielding progenies (Tiwari & Lavanya, 2012). The evaluation of genetic variability is important to know the source of genes for a particular trait within the available germplasm. There was no common criterion for selection of genotypes on the basis of specific objectives among the plant materials (Chakraborty & Haque, 2000).

The most important tasks for a pea breeding are development of high yielding varieties with stable productivity, with sufficiently good resistance to disease and unfavorable environmental conditions, increases in protein content essential amino acids and favorable ration among them (Tiwari et al., 2001).

An insight into the magnitude of variability present in a crop provides the basis for effective selection. Heritability is the portion of phenotypic variation which is transmitted from parent to progeny. The higher the heritable variation, the greater will be the possibility of fixing the character by selection methods (Sharma & Bora, 2013).

Lack of sufficient genetic variability for economically important character is one of the reasons attributed for in significant progress in crop improvement. The natural selection over years operated towards increasing the potentiality for survival and wider adoption at the cost of yield traits. Further the ones contributing for higher yield seem to be scattered in the natural population. A great extent of variability has been observed in different agronomic

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characters of pea with respect to plant height, days to flowering, pod length, and seed weight (Pallavi et al., 2013).

The research work in this study aims at studying genetic variability and heritability of some traits in forage pea (*Pisum sativum*) which may help to select suitable genotypes for future breeding programs.

Materials and Methods

A small-plot field trial was carried out from 2012 to 2014 at the Second Experimental Field of the Institute of Forage Crops, Pleven (43.41ºN, 24.61ºE), situated in the central part of the Danube hilly plain. Five spring pea (*Pisum sativum* L.) cultivars were included in the trial namely Glyans, Svit, Kamerton and Modus from the Ukraine and Pleven 4 from Bulgaria. The trial was set up as a randomised block design method with three replications and with a plot size of 4 m^2 (1.0 m x 4.0 m). The sowing was done by hand, at a depth of 5 cm and with a rate of 120 viable seeds m^{-2}. The plants were cultivated in conditions of organic farming (without use of any fertilizers and pesticides). Weeds were controlled mechanically during growth period. Ten randomly selected plants from each unit were marked and used to estimate yield components – plant height (cm); stem (mm), length (cm) and width (mm) of the pod; number seeds and pods per plant, 1000 seed weight (g) and seed weight per plant (g). For seed yield (kg da^{-1}) data for each cultivar was recorded on the basis of seed yield per plot at the end of growing season. Seed yield in each cultivar was measured at a standard seed humidity of 14%. For all the traits studied an average arithmetical and coefficients of variability (VC, %) were calculated. The next statistical methods were used to process the experimental data: factor analysis by the method of principal components (Vandev, 2003); hierarchical cluster analysis by the method of Ward (1963) – 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. Genetic advance in absolute unit (GA) and Genetic Gain (GG), assuming selection of the superior 5% of the genotypes, were estimated in accordance with the methods illustrated by Johnson et al, (1995) using the software GENES. The experimental data were processed statistically using the computer software SPSS 13 and Excel for Windows XP.

Results

Genetic diversity of germplasm determines the limit of selection in crop improvement. Furthermore knowledge of genetic associations among agronomic traits is regarded to support considerable help to maintain genetic improved to breeding programme. Crop improvement with heritable characters, estimation of genetic parameters and their association is prime importance in breeding (Ajmal et al., 2009; Bozokalfa et al., 2010; Esiyok et al., 2011).

ANOVA of quantitative traits. The analysis of variance (Table 1) indicated the existence of significant variability for the characters pod width, seeds per plant, seed weight per plant and 1000 seed weight. The difference between the varieties included in this study for plant height, pods per plant, pod stem, pod length and seed yield was statistically non-significant.

Estimation of genotypic (GCV) and phenotypic coefficient of variation (PCV). The comparison of characters as regards to the extent of genetic variation could be better judged by the estimation of genotypic coefficient of variation (GCV) in relation to their respective phenotypic coefficient of variation (PCV) (Chakraborty & Chakraborty, 2010). The results in Table 2 showed that PCV was higher than GCV for plant height, pods per plant, pod stem, pod length and seed yield indicating the importance of environmental influence on their expression. On the other hand, large difference between GCV and PCV was observed for plant height, pods per plant, pod stem and 1000 seed weight.

Table 1. *Analysis of variation for quantitative traits in pea (Pisum sativum).*

Sources of variation	df	Plant height	Pods per plant	Pod stem	Pod length	Pod width	Seeds per plant	Seed weight per plant	1000 seed weight	Seed yield
Years	2	1230.854	15.268	0.016	0.118	0.0318	112.224	5.7617	19.999	20504.96
Varieties	4	198.307^{ns}	1.354^{ns}	0.011^{ns}	0.063^{ns}	0.013*	29.459*	1.514*	2525.669**	3510.668^{ns}
Residual	8	89.035	0.825	0.007	0.030	0.002	5.650	0.352	118.825	1373.567
CV (%)		12.93	15.63	11.92	3.23	4.93	11.26	14.53	5.49	16.17

*; ** significant at 5% and 1% level of probability; ns - non significant

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Table 2. *Genetic component of variation for quantitative traits in pea (Pisum sativum).*

Traits / Parameters	Plant height	Pods per plant	Pod stem	Pod length	Pod width	Seeds per plant	Seed weight per plant	1000 seed weight	Seed yield
Minimum	51.20	3.00	0.58	4.84	0.77	12.90	2.63	151.00	164.00
Maximum	111.30	9.20	0.89	5.72	1.18	30.7	6.48	235.77	366.70
Mean	72.98	5.813	0.725	5.394	0.979	21.11	4.085	198.63	229.244
Sd	8.13	0.672	0.062	0.146	0.068	3.134	0.71	29.015	34.209
GCV (%)	8.27	7.22	5.07	1.95	6.35	13.35	15.23	14.26	11.64
PCV (%)	12.92	15.70	11.79	3.20	4.92	11.22	14.50	5.48	16.17
$\sigma^2 g$	36.424	0.1763	0.0013	0.0111	0.0039	7.9365	0.3872	802.2814	712.3672
$\sigma^2 p$	89.0357	0.8253	0.0075	0.0303	0.0023	5.6503	0.3524	118.8256	1373.567
$\sigma^2 e$	52.612	0.649	0.006	0.019	-0.002	-2.286	-0.035	-683.456	661.200
GA	7.952	0.400	0.031	0.131	0.168	6.878	1.344	151.614	39.596
GG (%)	26.635	32.194	24.607	6.648	10.091	23.196	29.936	11.305	33.304
H^2 (%)	55	39	35	52	83	81	77	95	61

Sd - std. deviation; GCV - genotypic coefficient of variation; PCV - phenotypic coefficient of variation; $\sigma^2 g$ - genotypic variances; $\sigma^2 p$ - phenotypic variances; $\sigma^2 e$ - environmental variance; GA-genetic advance; GG - genetic gain; H^2 - broad sense heritability (%)

Among the investigated characters very small difference between GCV and PCV was observed for the traits like pod width, seeds per plant and seed weight per plant. It indicates that the observed variations for the trait were mostly due to genetic factors. However, the environment played a little role on the expression of this trait. The genotypic coefficient of variances (GCV) varied from 1.95% (pod length) to 15.23% (seed weight per plant). The estimates of GCV were found to be highest for seed weight per plant followed by 1000 seed weight and seeds per plant respectively. The higher GCV for these traits indicated further selection could improve the genotypes. The remaining characteristics had lower estimates of GCA.

Estimation of genotypic and phenotypic variances. The estimates of genetic variances of quantitative traits were smaller than their respective phenotypic variances for plant height, pods per plant, pod stem and pod length. However, the estimates of genetic variances were higher than those of environmental variances for pod width, seeds per plant, seed weight per plant, 1000 seed weight and seed yield. The value for genotypic variances ranged from 0.0013 to 802.281. Genotypic variance was the highest for 1000 seed weight (802.281) followed by seed yield (712.376) and plant height (36.424) indicating the greater magnitude of genetic variability for these traits. Phenotypic variance values ranged from 0.0075 – 1373.567. The highest phenotypic variance was that of seed yield. Minimum phenotypic variance was observed for pod stem. Higher environmental variance in seed yield (661.2) and plant height (52.612) while the minimum was obtained for other traits. This indicated that both seed yield and plant height were highly influenced by environment while the other traits were less influenced.

The genetic parameters discussed here are functions of environmental variability, so estimates may differ in other environment. The environmental variance was greater than the genetic variance for temporal data for plant height, pods per plant, pod stem and pod length indicating polygenic traits.

Estimation of heritability of broad sense. The heritable variation with heritability (broad sense) estimates would give reliable indication of expected improvement through selection (Johnson et al., 1955). High estimates of broad sense of heritability (Table 2) were recorded for 1000 seed weight (95%), pod width (83%), seeds per plant (81%), seed weight per plant (77%) and seed yield (61%) whereas remaining characters revealed moderate heritability. Heritability estimate provides guide for the selection procedure to be followed by the breeder for improvement of these traits under a given environment (Sharma & Bora, 2013). Nawab et al., (2008) also reported for high heritability for 1000 seed weight.

Estimation of genetic advance. The heritability estimate provides information on the magnitude of the inheritance of quantitative traits, but provides no indication of the amount

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of genetic progress that would result from selecting the best individuals (Hefny, 2013). The genetic heritability couple with high genetic advance in percent mean observed in 1000 seed weight, seed yield and seeds per plant suggested that effective selection may be done for these traits (Table 2).

The high genetic advance as percent of mean along with high heritability was obtained for 1000 seed weight (151.61), seed yield (39.59), plant height (7.952), and seeds per plant (6.878) while the lowest for pod stem, pod length and pod width indicating that these characters are least affected by environment. High heritability along with high genetic advance reveals that additive type of heritability is there. Therefore, these traits can be improved by selection and genotypic variability so realized can be maintained in subsequent generation.

Based on the moderate heritability and high genetic advance shown by the different characters, especially plant height, it could conclude that the determinant genetic effects of the phenotypic expression of this character are fundamentally of additive type and selection may be effective to improve the trait. High heritability coupled with low genetic advance for pod length (52%; 0.131), pod width (83%; 0.168) and seed weight per plant (76%; 1.344), indicated that the involvement of non-additive gene action (dominance and epistasis) and the high value of heritability is being exhibited due to the favorable influence of the environment than the genotype.

Principal component analysis. In order to assess the patterns of variation, principal component analysis (PCA) was done by considering all the nine variables simultaneously. There are two eigenvalues greater than 1 which determined the choice of the two main components. The first component explained 51% and the second - 34.34% of the total variance. The main principal components (F1 and F2) accounted more than 85% of the total variation in the field pea varieties (Table 3).

Out of the 9 traits considered, half of them exerted strong and half weak effects on these components. In the formation of the first component among those traits having greater influence were included: pod stem, pod length, 1000 seed weigh and seed yield. Whereas pods per plant, pod width, seeds per plant and seed weight per plant were among the traits exerting positive influence on the second component. While plant height, pod stem, 1000 seed weight and pod length were among those traits which influenced slightly. The third principal component (F3) accounted for 11% of the total variation and was with positive relation only with plant height. Varieties were phenotypically very different between them (Figure 1). Glyans was characterized by positive values of the both components, Svit, Kamerton and Modus were arranged in the second quadrant. Pleven 4 was situated in fourth quadrant showing negative values of component F2. Position of the varieties in the first, second and fourth quadrant showed that together they are phenotypically similar in a small number of traits). From the location of the varieties in bipolar plane it was found that from the characteristics studied H, C and I were with the greatest length and determining the level of diversity.

Table 3. *The Eigen values and vectors of the correlation matrix for 9 traits of pea genotypes.*

	F1	F2	F3
Varieties			
Glyans	0.165	0.831	0.002
Svit	0.683	0.179	0.000
Kamerton	0.042	0.310	0.569
Modus	0.001	0.447	0.498
Pleven 4	0.979	0.013	0.001
Traits			
Plant height	0.440	0.007	0.448
Pods per plant	0.044	0.827	0.071
Pod stem	0.884	0.025	0.057
Pod length	0.562	0.152	0.261
Pod width	0.219	0.661	0.057
Seeds per plant	0.344	0.553	0.069
Seed weight per plant	0.362	0.629	0.001
1000 seed weight	0.916	0.065	0.018
Seed yield	0.820	0.172	0.008
Parameter			
Eigenvalue	4.590	3.090	0.990
Variability (%)	51.002	34.337	10.997
Cumulative %	51.002	85.339	96.337

F1; F2; F3 = principal component 1, 2 and 3 respectively

Cluster analysis. Hierarchical cluster analysis of the varieties was done on the basis of the quantitative traits studied. The Euclidean distance was used for as a measure for genetic distance. Results shown as a dendrogram (Figure 2) indicated the different grouping of the varieties by similarity and difference. Samples were clustered into three main clusters.

Cluster "I" was composed of one variety – Pleven 4 which was characterized by relatively higher values of plant

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height and number seeds per plant. The varieties falling in cluster "II" were Modus and Kamerton which were genetically better in seed yield than Pleven 4. The varieties in cluster "III" – Svit and Glyans – were associated with the highest values of pods per plant, 1000 seed weight, seed weight per plant and seed yield. To obtain more pronounced transgressive forms in hybrid combinations should be genotypes from different groups included to expect better combination of the favorable genes in one genotype. The hierarchical cluster analysis can be used in the selection to plan the initial parent combinations (Dragavtsev & Averyanova, 1983).

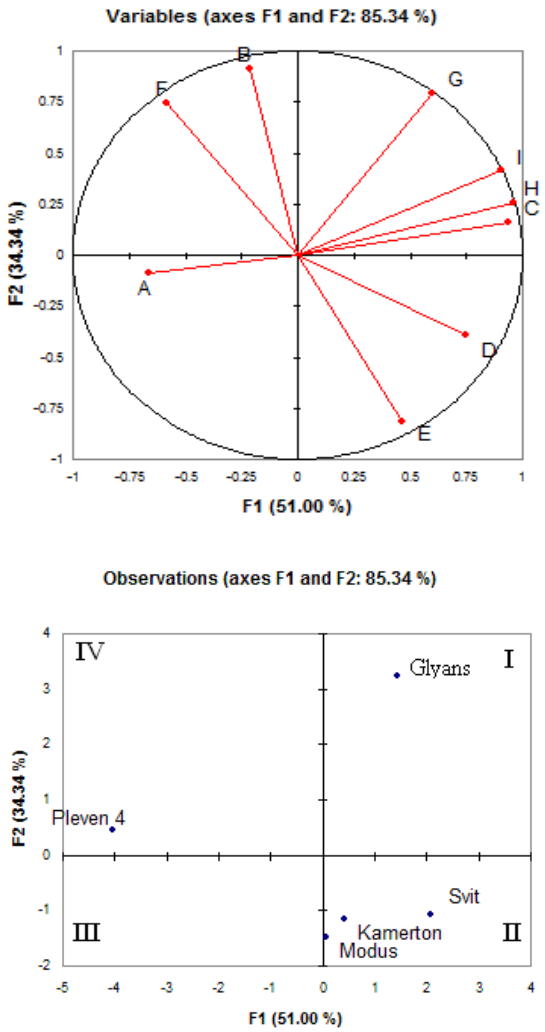


Figure 1. *Principal component analysis of quantitative traits of spring pea genotypes. A- Plant height; B- Pods per plant; C- Pod stem; D- Pod length; E- Pod width; F- Seeds per plant; G- Seed weight per plant; H-1000 seed weight; I- Seed yield.*

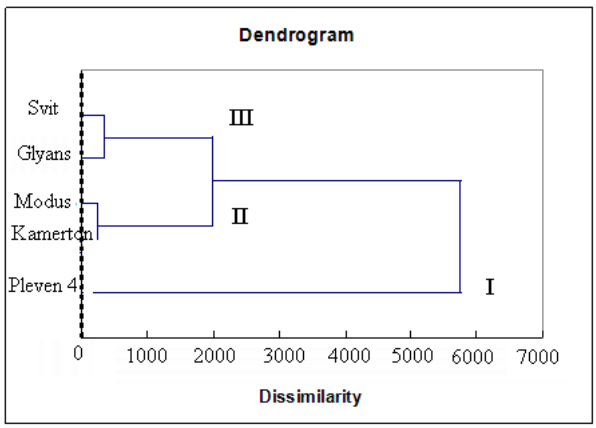


Figure 2. *Dendrogram of spring pea varieties (2012-2014).*

Association of the investigated traits. Table 4 reveals that seed yield had positive and significant correlations with 1000 seed weight (r=0.96) and pod stem (r=0.942) whereas, negative association but non-significant showed the seed yield with plant height (r=-0.569) and seeds per plant (r=-0.202). The negative correlation observed between seed yield and plant height indicates that tall plants supporting many leaves could increase total biomass instead seed weight. A positive association exists between pod stem with 1000 seed weight (r=0.916) and pods per plant with seeds per plant (r=0.914). This strong positive correlation of 1000 seed weight and pod stem with seed yield shows that they should be used as selection criteria for maximizing seed yield.

Discussion

For observed high heritability coupled with moderate genetic gain in number of pods per plant, seeds per pod, plant height and 1000 seed weight reported Mahanta et al., (2001), Chaudhary & Sharma (2003) and Kumari et al. (2008). These results indicated that an effective selection for this trait could be done.

Similar results also were reported by Mathura et al. (2006), Raikumar & Singh (2001), Habtamu & Million (2013). Therefore, even if heritability estimates provide basis for phenotypic performance, the estimate of heritability and genetic advance should always be considered simultaneously, as high heritability is not always associated with high genetic advance (Johnson et al., 1995).

Sing & Sing (2006) reported high heritability for plant height, pod per plant, 100 seed weight and seed yield per plant couple with high expected genetic advance in pea.

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Sureja & Sharma (2000), Kalloo et al., (2005) and Sharma & Bora (2013) also reported high estimates of genotypic variability for yield and its contributing traits.

Our results in regard to phenotypic coefficients were in line with the results obtained by Sardana et al., (2007) and Fikreselassie (2012). They found that the phenotypic coefficients of variation were close to genotypic coefficients of variation except plant height, seed per pod, 1000 seed weight and seed yield per plant.

On the basis of the obtained results, the following conclusions may be drawn and used for further research on the quantitative traits on forage pea and its application in breeding and the development of new varieties:

The analysis of variance showed significant differences among genotypes for the traits pod width, seeds per plant, seed weight per plant and 1000 seed weight.

Table 4. *Correlation coefficients (r) among the quantitative traits of spring pea varieties*

	Plant height	Pods per plant	Pod stem	Pod length	Pod width	Seeds per plant	Seed weight per plant	1000 seed weight
Pods per plant	0.163							
Pod stem	-0.418	-0.036						
Pod length	-0.173	-0.339	0.736					
Pod width	-0.162	-0.715	0.322	0.830				
Seeds per plant	0.440	0.914*	-0.406	-0.566	-0.769			
Seed weight per plant	-0.461	0.563	0.699	0.109	-0.394	0.211		
1000 seed weight	-0.732	-0.016	0.916*	0.543	0.198	-0.415	0.786	
Seed yield	-0.569	0.206	0.942*	0.561	0.105	-0.202	0.872	0.960*

* significant at 5% level of probability

The results of coefficient of variation analysis indicated that the phenotypic coefficient of variation was higher than the genotypic coefficient of variation for plant height, pods per plant, pod stem, pod length and seed yield. Moderate phenotypic and genotypic coefficients of variation were observed for most of traits except pod length and pod width.

For the traits studied seeds per plant (81%; 23.196%), seed weight per plant (77%; 39.936%) and plant height (55%; 26.635%) were found high heritability along with high genetic gain indicating preponderance of additive effects. High heritability coupled with low genetic gain for 1000 seed weight (95%; 11.305), pod width (83%; 10.091) and pod length (52%; 6.648) indicated the involvement of dominance and epistasis.

The seed yield was positively and significantly correlated with 1000 seed weight (r=0.96) and pod stem (0.942), which suggested possibilities for improving the seed yield by simultaneous improvement of these traits.

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