

EVALUATION OF GENETIC DIFFERENCES IN VETCH CULTIVARS

Natalia Georgieva^{1*}, Ivelina Nikolova¹, Valentin Kosev¹, Nurettin Tahsin²

¹ Institute of Forage Crops, Department of technology and ecology of forage crops
“General Vladimir Vazov” 89, 5800 Pleven, Bulgaria

²Agricultural University, Faculty of Agronomy, “Mendeleev” 12 Blvd., 4000 Plovdiv, Bulgaria

*Corresponding author: E-mail: imnatalia@abv.bg

ABSTRACT

Genetic variability within common vetch and knowledge of the tested cultivars offer a basis for further improvement and developing novel cultivars. The objective of this research was to determine the genetic distance among vetch cultivars (*Vicia sativa* L., *Vicia villosa* Roth.) and their grouping along some selection valuable traits with a view to their more precise use in the combined selection. The maximum genetic distance was observed between Lorina and Moldovskaia (in cluster B2) which are characterized by pods per plant, pod stem, pod length, seeds per pod and Violeta and Viola (in cluster A1), with long stem and high number of pods per plant. High positive correlations were established between seed weight per plant and seeds per plant, pods per plant and seeds per pod. Number of seeds per plant correlated with pods per plant and seeds per pod. The trait of seed weight per plant had strong influence on seed yield in both vetch species. The traits 1000 seed weight and plant height had high heritability coupled with high genetic advance, which indicates that these traits could be improved through selection procedure.

Key words: cluster analysis, principal component analysis, vetch, correlative dependences.

INTRODUCTION

Vicia L. (*Fabaceae*) is a widespread, moderately large genus. It comprises about 210 species, including recognized 22 sections in two subgenera (*Vicia* and *Vicilla*), widely distributed along regions of Europe, Asia and the American regions (Weber and Schifino-Wittmann, 1999). The Mediterranean area is the principal centre of diversification. In Turkey, 64 species, 22 subspecies and 18 varieties of this genus have been recorded (Inceer and Ayaz, 2005; Emre, 2011). Now there are only two vetch cultivars in the Bulgarian Official Catalogue of Varieties.

Vetch is a forage plant of economic importance and is cultivated for forage or grain production, as a pasture plant, or as a green fertilizer plant. Its green, dry hay is of high quality and is nutritious. The seeds are high in protein and are used as animal feed (Buyukkartal et al., 2013). It is a nitrogen-fixing legume that is widely used as green manure and cover crop (Tenopala et al., 2012).

New and more uniform types have been obtained and widespread, replacing the old landraces, which became threaten of extinction. Given the danger of genetic erosion, scientists started to collect the evolved variability in world collections and to preserve it for future generations, because the genetic variation is of interest to germplasm conservationists and plant breeders (Abdalla, 1976).

Conventional breeding programmers on common vetch (*Vicia sativa* L.) are based on knowledge of relationships between forage yield components and economically important characteristics. Genetic variability within common vetch and knowledge of the tested cultivars offer a basis for further improvement and developing novel cultivars (Mikić et al., 2013). At present time, considerable attention is paid to assessment of individual elements of productivity in this crop, which elements determine its yield in changing environmental conditions (Debelyi et al., 2011). Appropriate plant material is very crucial for any plant breeding program aimed at developing new cultivars (Fırmıciođlu et al., 2009).

One of the most commonly used statistical analysis applied in productivity experiments is principal components analysis (PCA), which primarily gives information on the interaction between genotype and environment. The second method is GGE biplot technique developed by Gabriel (1971) and used for appropriate representation and visualization of received results (Genchev, 2011).

The objective of present study was to determine the genetic distance among vetch cultivars and their grouping along some selection valuable traits with a view to their more precise use in the combined selection.

MATERIAL 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 (43.41° N, 24.61° E), situated in the central part of the Danube hilly plain. Seven vetch cultivars were included in the trial namely Liya, Lorina, Vilena, Moldovskaya, Obrazets 666 (belonging to *Vicia sativa* L.), Violeta and Viola (belonging to *Vicia villosa* Roth.). The origin of Obrazets 666 is Bulgaria and of the rest – Moldova. The trial was set up as a randomised block design method with three replications and with a plot size of 4 m² (1.0 m × 4.0 m). The sowing was done by hand, at the end of March, at a depth of 4 cm and with a rate of 220 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 and length (cm) of the pod; number seeds and pods per plant, number seeds per pod, 1000 seed weight (g) and seed weight per plant (g). For seed yield (kg ha⁻¹) data for each cultivar was recorded on the basis of seed yield per plot at the end of growing season. Seed yield for each cultivar was measured at standard seed humidity (14%).

For all the traits studied an average arithmetical and coefficients of variability (VC, %) were calculated.

The following 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 grouping genotypes based on similarity as a measure of differences (the genetic distance); the Euclidean distance between them was calculated (as a measure for divergence) as the data were standardized preliminary. 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.

All experimental data were processed statistically using the computer software SPSS 13 and Excel for Windows XP.

RESULTS AND DISCUSSION

The meteorological conditions during experimental period differed considerably (Figure 1). The average daily air temperature in 2012 (March – July) was by 1.2 and 1.5°C higher as compared to those in 2013 and 2014, respectively. The high temperature in 2012 coupled with lower sum (with 172.9 and 226.9 mm as compared to 2013 and 2014, respectively) of vegetation rainfalls determined this year as drier and unfavourable for plant development. Considerably greater amount of rainfalls in 2014, their even distribution, as well as lower temperature (not exceeding 24°C) created favourable conditions for plant growth and development. 2013 had an intermediate position.

Knowledge of genetic proximity or distance of genotypes used as starting material is of great interest to plant recombination breeding. Transgressive segregation could be expected with a higher probability when the accessions are genetic-distanced. Combining various valuable genes can be expected in this case (Cowen and Frey, 1987).

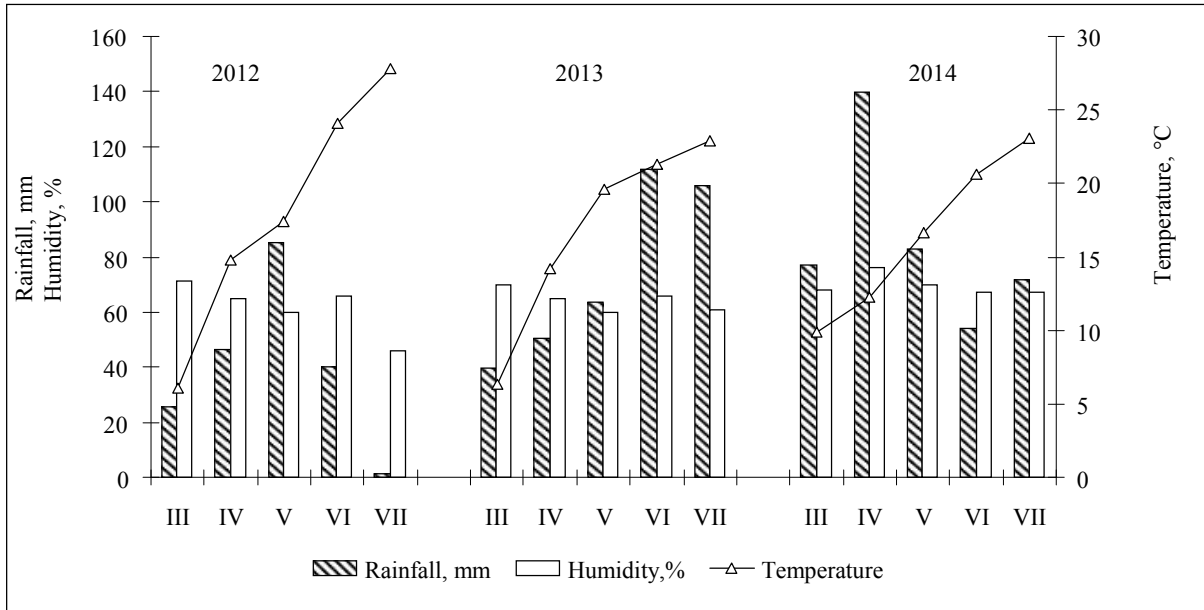


Figure 1. Climatic characterization of the experimental period

Principal component analysis

The genetic diversity among vetch accessions was estimated through applying factor analysis by the method of principal components (PC analysis) on the basis of studied quantitative traits (Table 1). There were two eigenvalues which determined the choice of two principal components (Table 2). The first component explained 87.34% and the second one – 9.16% of the total variance.

The main principal components (F1 and F2) determined 96.50% of the total variance

of genotype \times trait. The first component was mainly related to the following traits: 1000 seed weight, seeds per pod and pod length. Seeds per plant and pods per plant were traits participating in the formation of the second component. Liya, Vilena and Lorina were characterized by positive values of both principal components (first quadrant, Figure 2). The Bulgarian cultivar Obrazets 666 was phenotypically very similar to the Moldavian cultivar Moldovskaya and they were located in the second quadrant.

Table 1. Distinctive features of the investigated cultivars

Cultivar	Plant height (cm)	Pods per plant	Pod stem (cm)	Pod length (cm)	Seeds per pod	Seeds per plant	Seed weight per plant (g)	1000 seed weight (g)	Seed yield (kg ha ⁻¹)
Liya	80.43	8	0.33	5.12	5	42	2.50***	57.20**	1491.8***
Lorina	80.37	7	0.29	5.16	5	37	2.08**	56.91**	985.3
Vilena	83.63	7	0.29	5.35*	5	40	2.26**	57.47**	1226.0**
Moldovskaya	82.40	7	0.36	5.23*	5	32	1.71	56.15*	920.3
Obrazets 666	69.25	7	0.29	4.71	5	30	1.44	52.24	744.5
Violeta	129.30***	12**	0.20	2.47	2	27	0.64	26.56	281.7
Viola	132.17***	12**	0.21	2.49	2	29	0.65	33.76	315.1
*LSD _{0.05}	20.57	3.01	0.15	0.51	1.03	13.46	0.42	3.14	264.6
**LSD _{0.01}	28.84	4.23	0.21	0.72	1.45	18.87	0.58	4.41	370.9
***LSD _{0.001}	40.77	5.98	0.29	1.02	2.05	26.68	0.83	6.23	524.3

Table 2. The eigenvalues and vectors of the correlation matrix for 9 traits of vetch genotypes

Traits	Parameters					
	Influence of factor		Correlations between variables and factors		Eigenvectors	
	F1	F2	F1	F2	F1	F2
Plant height	0.861	0.090	-0.928	0.300	-0.331	0.330
Pods per plant	0.826	0.154	-0.909	0.393	-0.324	0.433
Pod stem	0.780	0.052	0.883	-0.228	0.315	-0.252
Pod length,	0.976	0.013	0.988	-0.114	0.352	-0.125
Seeds per pod	0.972	0.000	0.986	0.006	0.352	0.006
Seeds per plant	0.672	0.324	0.820	0.569	0.292	0.627
Seed weight per plant	0.921	0.076	0.960	0.275	0.342	0.303
1000 seed weight	0.979	0.009	0.989	-0.093	0.353	-0.102
Seed yield	0.873	0.107	0.934	0.327	0.333	0.360
Eigenvalue	7.860	0.824	-0.928	0.300	-0.331	0.330
Variability (%)	87.336	9.159	-0.909	0.393	-0.324	0.433
Cumulative %	87.336	96.495	0.883	-0.228	0.315	-0.252

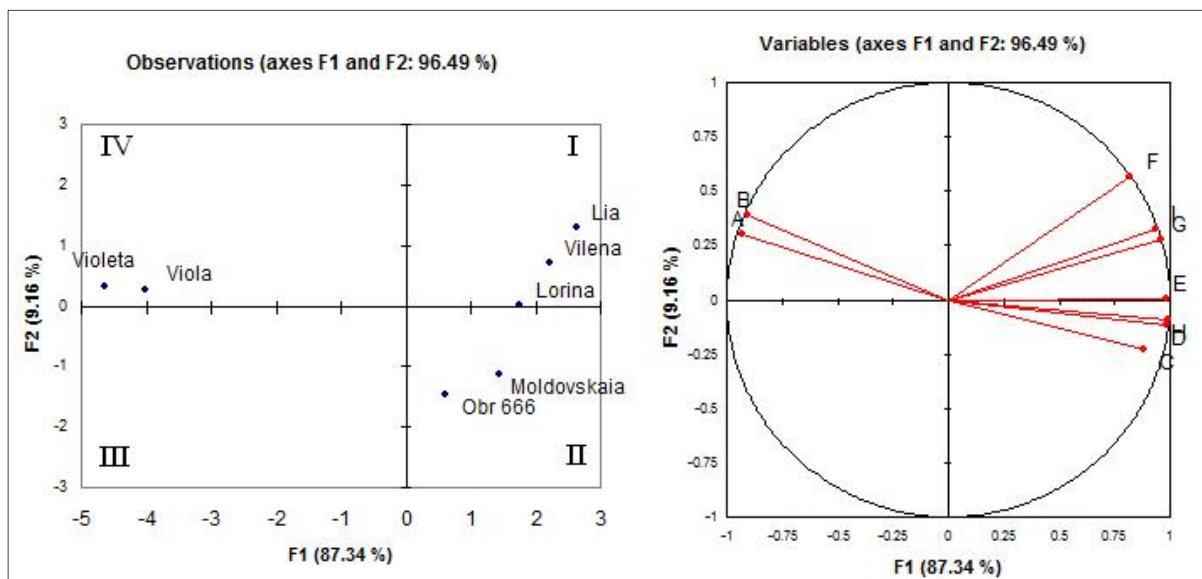


Figure 2. Principal component analysis of quantitative traits of vetch genotypes

A – plant height; B – pods per plant; C – pod stem; D – pod length; E – seeds per pod; F – seeds per plant; G – seed weight per plant; H – 1000 seed weight; I – seed yield.

Cultivars which were situated in the upper left quadrant of the coordinate system (Violeta and Viola) had positive values only in regard to component F2. Their close position showed that they had similar phenotype in relatively large number of traits. The location of varieties in bipolar plane showed that determinative meaning had following traits: 1000 seed weight, pod length and seeds per pod. They were vectors with greatest length and defined the level of diversity. The correlation of plant height and

pods per plant with principal component F1 was negative.

Hierarchical cluster analysis

Hierarchical cluster analysis of the vetch cultivars was done on the basis of the values of studied quantitative traits. The Euclidean distance was used as a measure for genetic distance. Results of the carried out analysis were presented in the form of a dendrogram (Figure 3). They indicated that during the three experimental years the cultivars were

clustered into two main clusters (A and B). Their location by years showed that one part

of them changed its cluster or moved from one cluster to other.

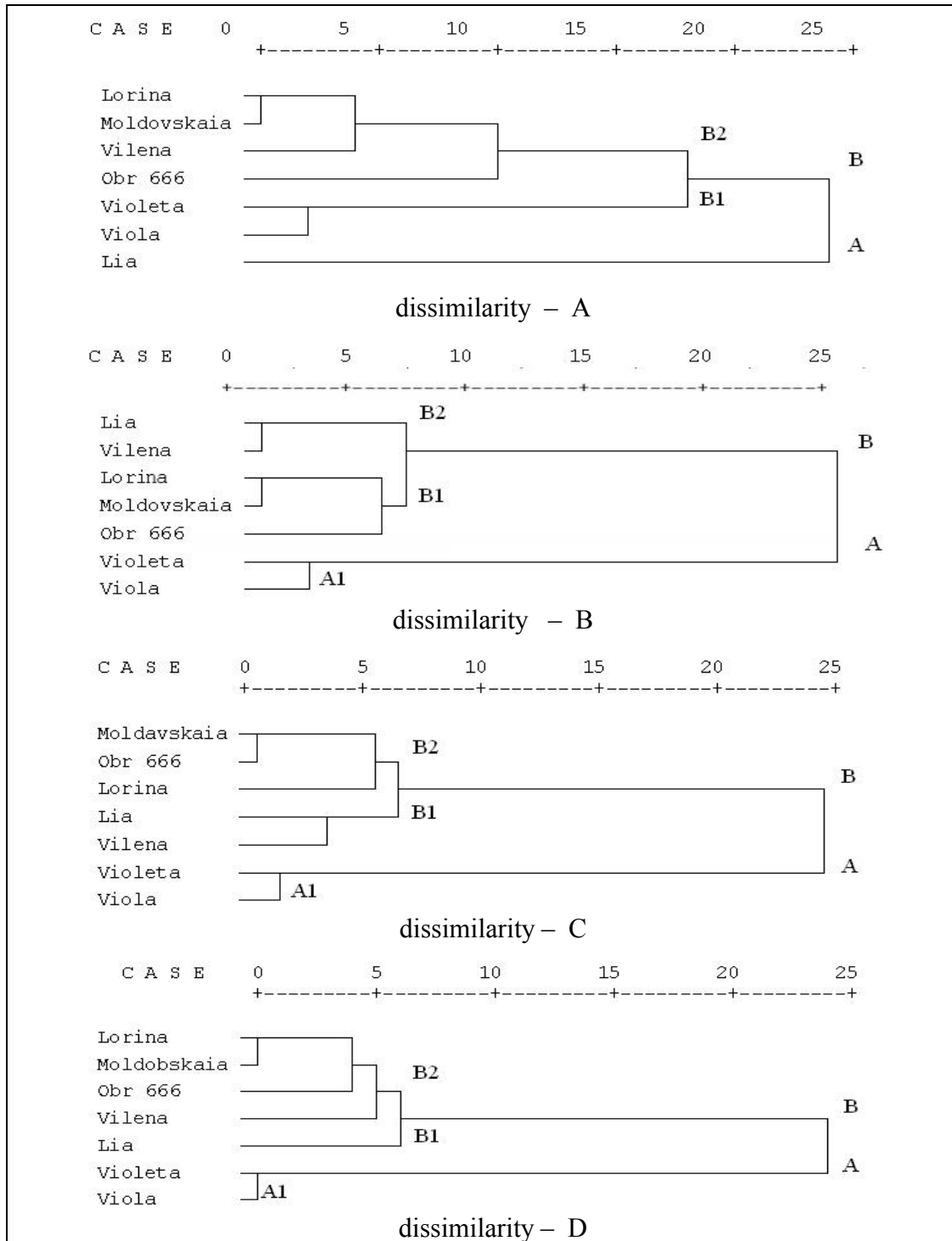


Figure 3. Dendrograms of vetch cultivars (A – 2012; B – 2013; C – 2014; D – 2012-2014)

Environmental conditions influenced manifestation of genetic similarity in result of redetermination of genetic formulas controlling the characteristics (Dragavtsev and Averynova, 1983). During 2012 in cluster “A” was the high-productive cultivar Liya that showed

presence of significant genetic distance among it and the other cultivars. Cluster “B” was divided in two subgroups - “B1” and “B2”. The first one included cultivars Violeta and Viola which were distinguished by long stem and a large number of pods per plant. The second

subgroup combined cultivars Lorina, Moldovskaya and Vilena. The Bulgarian cultivar Obrazets 666 could be accepted as relatively similar to them because of similarities in some traits (pods per plant, pod stem, pod length, seeds per pod) but it separated itself.

During second experimental year (2013) Liya changed its main cluster and passed to subgroup "B2" of cluster "B", where there was another high-productive cultivar Vilena. From cluster "B" (subgroup "B1") cultivars Violeta and Viola (which were the least productive, with 1000 seed weight under 34 g) formed cluster "A". Moldovskaya and Lorina showed similarity in the studied traits. They, together with Obrazets 666, maintained their main cluster but changed their subgroup.

The cultivars did not change their clusters during 2014, but the most of them changed their places in the subgroups. Exceptions to this were Violeta and Viola which did not change their cluster and preserved their place in the same subgroup as in 2013.

The cluster analysis, carried out on data for the three years allowed more genes to participate in the genotype grouping. In this way the phenotypic stability was reported to a greater degree. A significant change in the grouping of varieties, based on the average data for three years relative to 2012 and 2014, was not established. The accessions from main cluster "A" were distinguished by higher values of some traits: plant height and number of pods per plant. Liya was referred to subgroup "B1" – this variety had the highest yield and 1000 seed weight above 57 g. Lorina, Moldovskaya and Obrazets 666 formed subgroup "B2". In the same subgroup, but forming second branch, was cultivar Vilena, which is characterized by high yield, long pods and large number of seeds per plant.

Analysis of the results showed that the cultivars were clustered into three main clusters. In the first cluster were included Violeta and Viola, which were genetically similar to each other. Lorina, Moldovskaya, Vilena and Obrazets 666 were genetically distant from them (Violeta, Viola) and they formed second subgroup according to similarity. The third subgroup was composed of one cultivar – Liya.

It could be characterized as comparatively closer to the last subgroup based on its genetic similarity in some traits. To obtain more pronounced transgressive forms in hybrid combinations genotypes from different groups should be included, to obtain a better combination of favourable genes in one genotype. The hierarchical cluster analysis can be used in the selection to plan the initial parent combinations (Dragavtsev & Averyanova, 1983).

Estimation of genetic advance and heritability (broad sense)

The consequence of heritability coupled with genetic advance showed that 1000 seed weight and plant height had high heritability as well as high genetic advance, indicating that these characters are least affected by environment (Table 3). Thus, these characters may be more efficiently improved through selection.

The trait pod stem showed lower heritability coupled with low genetic advance, indicating the involvement of non-additive gene action (dominance and epistasis) along to the stronger influence of the environment. So selection would be less effective for them, and other breeding methods should be taken for improvement of these traits.

The varieties were investigated in regard to variability of studied traits by coefficient of variation (CV, %) which characterized the phenotypic diversity (Figure 4). The coefficient varied in different ranges for every trait. The variability was least pronounced for pod length, strong for length of pod stem and weak to middle for the rest of traits.

The variability level of plant height, seeds per pod and seed weight per plant was least pronounced in variety Obrazets 666. Only Lorina from Moldavian varieties showed similar variation of the same traits. The foreign common vetch cultivars demonstrated very good stability in regard to pod stem, 1000 seed weight and seed yield. Large range of coefficient of variation was found in hairy vetch varieties for most traits, especially seed yield, seed weight per plant and seeds per plant. That indicated a greater genetic instability.

Table 3. Genetic component of variation for quantitative traits in vetch

Traits Parameters	Plant height	Pods per plant	Pod stem	Pod stem	Pod width	Seeds per plant	Seed weight per plant	1000 seed weight	Seed yield
GCV (%)	26.29	25.46	11.35	29.60	32.02	12.22	45.45	26.44	51.43
GCV/ PCV	2.14	1.30	0.37	4.44	2.30	0.54	3.10	7.27	2.95
σ^2_g	609.96	4.83	0.00	1.67	1.80	17.01	0.54	165.25	1920.76
σ^2_p	133.76	2.88	0.01	0.08	0.34	57.29	0.06	3.13	221.23
σ^2_e	-476.2	-1.95	0.01	-1.59	-1.46	40.28	-0.48	-162.12	-1699.53
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 (%)	93.19	83.44	29.57	98.34	94.08	47.11	96.66	99.37	96.30

GCV – genotypic coefficient of variation; PCV – phenotypic coefficient of variation; σ^2_g – genotypic variances; σ^2_p – phenotypic variances; σ^2_e – environmental variance; GA – genetic advance; GG – genetic gain; H² – broad sense heritability (%).

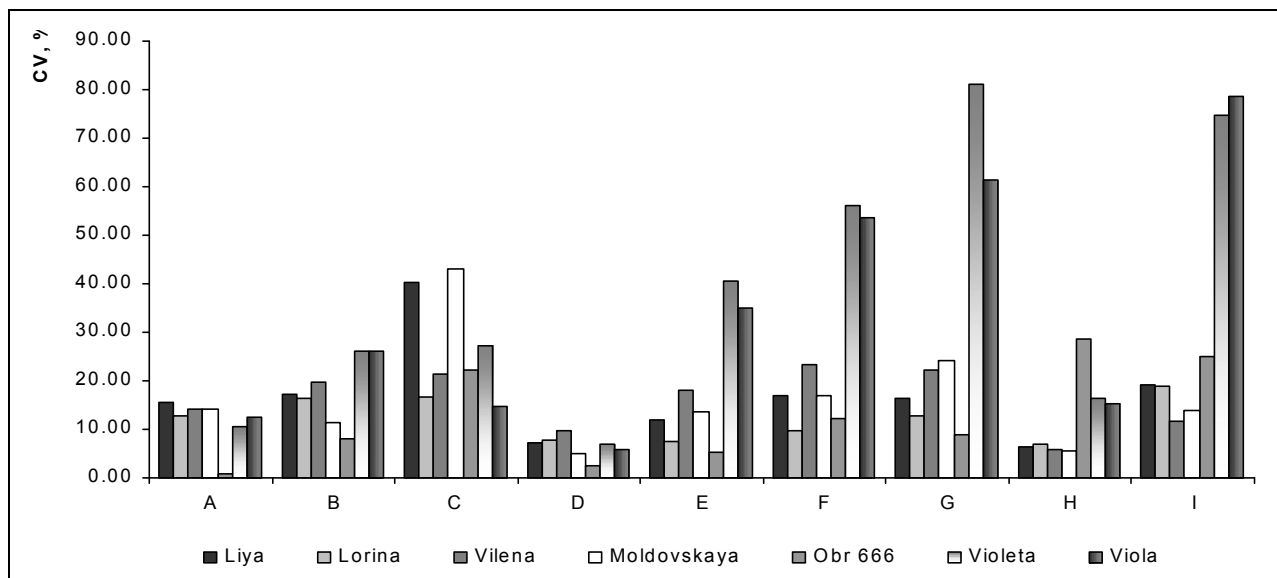


Figure 4. Coefficients of variability (CV, %) in vetch varieties

Correlations between the studied traits and seed yield in common vetch were estimated (Table 4).

Seed weight per plant and seeds per plant had the most essential meaning and very high significance among the yield components determining productivity. A high positive association was observed between seed weight per plant and seeds per plant ($r=0.85$), pods per plant ($r=0.69$), plant height ($r=0.512$), seeds per pod ($r=0.499$) and between seeds per plant and pods per plant ($r=0.765$) and seeds per pod ($r=0.643$). The trait of pod length showed a moderate correlation with 1000 seed weight ($r=0.384$), seeds per pod

($r=0.345$) and seeds per plant ($r=0.326$). The seed yield was in positive interaction with seed weight per plant ($r=0.433$) and 1000 seed weight ($r=0.411$).

Correlative dependences among traits in hairy vetch cultivars showed some analogy with common vetch cultivars but in most cases the correlative coefficients had higher values (Table 5).

Seed weight per plant was in high positive correlation with seeds per plant ($r=0.985$), pods per plant ($r=0.907$) and seeds per pod ($r=0.846$); seeds per plant – with pods per plant ($r=0.854$) and seeds per pod ($r=0.928$). The greatest influence on seed yield

in winter vetches had following traits: seeds per plant ($r=0.732$), seed weight per plant ($r=0.709$), seeds per plant ($r=0.664$) and pods

per plant ($r=0.633$). Plant height correlated negatively with all studied traits with exception of 1000 seed weight.

Table 4. Correlation coefficient among the quantitative traits of common vetch cultivars

Traits	Plant height	Pods per plant	Pod stem	Pod length	Seeds per pod	Seeds per plant	Seed weight per plant	1000 seed weight
Pods per plant	0.371*							
Pod stem	0.043	0.222						
Pod length	-0.172	0.137	0.450**					
Seeds per pod	0.009	0.011	0.092	0.345*				
Seeds per plant	0.279	0.765**	0.237	0.326*	0.643**			
Seed weight per plant	0.512**	0.690**	0.141	0.155	0.499**	0.850**		
1000 seed weight	0.14	-0.03	-0.037	0.384**	0.299*	0.177	0.273	
Seed yield	0.295*	0.185	-0.298*	0.004	0.228	0.283	0.436**	0.411**

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

Table 5. Correlation coefficients among the quantitative traits of hairy vetch cultivars

Traits	Plant height	Pods per plant	Pod stem	Pod length	Seeds per pod	Seeds per plant	Seed weight per plant	1000 seed weight
Pods per plant	-0.626**							
Pod stem	-0.339	0.205						
Pod length	-0.746**	0.372	0.658**					
Seeds per pod	-0.690**	0.607**	0.744**	0.727**				
Seeds per plant	-0.703**	0.854**	0.583**	0.614**	0.928**			
Seed weight per plant	-0.652**	0.846**	0.535**	0.583**	0.907**	0.985**		
1000 seed weight	0.397*	-0.409*	-0.065	-0.020	-0.302	-0.377	-0.334	
Seed yield	-0.633**	0.633**	0.324	0.339	0.664**	0.732**	0.709**	-0.536**

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

Similarly to the results obtained in this trial with large-flowered wild populations, in numerous trials in various regions of Turkey, it was found that seed yield was significantly and positively correlated to both number of pods per plant and number of seeds per plant (Cakmakci and Acikgoz, 1994; Cakmakci et al., 2003).

Further, the large variation in 1000 seed weight could also be useful in selecting populations with higher seedling viability as larger seeded populations have greater viability (Fıncıoğlu et al., 2009).

Genetic diversity is an important factor in any crop improvement programme for obtaining high yielding variety. The cluster analysis indicated that the geographic and

genetic diversity are not necessarily related, viz. germplasm accessions collected from the same geographic region fell in different genetic clusters (Bedassa et al., 2013).

It is worthy to note that in calculating cluster mean, the superiority of a particular accession with respect to a given character could get diluted by other accessions that are grouped in the same cluster but are inferior or intermediate for the character in question (Million, 2012). Hence apart from selecting genotypes from the clusters which have higher inter-cluster distance for hybridisation one can also think of selecting parents based on the extent of divergence with respect to a character of interest (Gemechu et al., 2005; Fikreselassie et al., 2012).

CONCLUSIONS

On the basis of the obtained results, the following conclusions may be drawn and used for further research on the quantitative traits on vetch and its application in breeding programs. The maximum genetic distance was observed between Lorina and Modovskaia (in cluster B2), which are characterized by pods per plant, pod stem, pod length, seeds per pod and Violeta and Viola (in cluster A1) with long stem and great number of pods per plant. High positive correlations were established between seed weight per plant and seeds per plant, pods per plant and seeds per pod. Number of seeds per plant correlated with pods per plant and seeds per pod. The trait of seed weight per plant had strong influence on seed yield in both vetch species. The traits 1000 seed weight and plant height had high heritability coupled with high genetic advance, indicated that these traits could be improved through selection.

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