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GENETIC DIVERSITY ASSESMENT OF PEA (*PISUM SATIVUM* L.) GERMPLASM BASED ON QUANTITATIVE MORPHOLOGICAL AND QUALITATIVE TRAITS.

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ABSTRACT

In order to investigate the genetic diversity present in the pea germplasm stored in Albanian genebank 12 local genotypes were analysed for 23 quantitative morphological traits and 15 qualitative characters. The study was carried out in the regeneration field of Albania genebank, during three years. ANOVA and correlation analysis reveal considerable extent of diversity, and the association among different traits. Most of the quantitative morphological traits showed significant differences and correlation analysis showed high significant positive correlation among different important agro economic traits. Comparisons of means for all pairs using Tukey-Kramer HSD ($q^* = 3.60563$ and $\alpha = 0.05$) show the significant differences between and within genotypes at the $P_{0.05}$ and $P_{0.01}$ levels of the probability. PCA and cluster analysis (Ward's method) carried out separately for morphological data and qualitative data divide the whole germplasm into three groups in respect of genetic diversity and similarity and identifying traits with agronomic interest which account for genetic diversity and the demarcation of distinguishable morphological groups will facilitate the maintenance and agronomic evaluation of the collections.

Keywords: Pea, *Pisum sativum* genotypes, quantitative-qualitative traits, cluster analysis.

INTRODUCTION

Legume crops are an important food source for human and animal consumption in Albania. The Albanian germplasm of the pea collection is a valuable group of legumes species for animal production. The legume collection in Albania Gene Bank (AGB) contains more than 200 local forms and breeding lines or cultivars with known or unknown origin. In general, little is known about the extent and nature of the variability of these species. In Albania, legumes such as garden pea (*Pisum sativum*) used mostly for human consumption and grass pea (*Lathyrus sativum*) used mostly for animal forage are also planted to a limited extend. Despite their limited extend both, garden and grass peas, are important for human and animal consumption (FAO. 1991; FAOSTA, 2011; Ellis et al., 2011, Graham et al., 2003). The introduction of improved varieties into a given farming system often considered as a threat that leads to loss of genetic variability (Frankel, 1970; Walters, 2003) and the presence of modern varieties in farming systems is usually taken as *prime facie* evidence of genetic erosion (Brush, 1986; Van De Wouw et al., 2009).

To know genetic diversity of the underutilized forage legume plants including garden pea, AGB launched a series of field and laboratory tests to identify, characterize, and evaluate the underutilized forage legume plants and their potential for further breeding. The main goal of the study is to characterize and evaluate the major characteristics of garden pea (*Pisum sativum*) accessions and (ii) to analyse morphological genetic diversity and identify the genotypes with forage value within the Albanian garden pea collection and with potential for further genetic programs.

MATERIALS AND METHODS

Plant Materials: The garden pea accessions that are part of the AGB forage legume collection contains more than 30 local and unknown garden peas (*Pisum spp*) genotypes. Initially, through the field and laboratory tests it was evaluated the entire forage collection of garden peas in order to reduce the collection to a manageable number of the most promising genotypes. This resulted in a set of 12 garden pea (*Pisum sativum*) genotypes. Three garden pea

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genotypes (V10-B25, V11-B29, V12-B30) were from Albania, one from Russia (V7-B9), one from Germany (V8-B11), one from Sweden (V9-B18) and six other ((V1-B2, V2-B4, V3-B5, V4-B6, V5-B7, V6-B8) were signed with unknown origin. All 12 garden peas planted in the second year and further analyzed through field tests for the most important quantitative and qualitative characters used for characterization and evaluation of garden pea forms (UPOV 2009; Upadhyaya et al., 2011).

Crop management of experimental plots: *The experimental site* was carried out in the AGB regeneration field, situated in northern part of Tirana (Valias: latitude: 402405N; longitude: 0194108E; elevation: 40m) during three growing seasons (2010 and 2012). Soils were loamy sand, well drained, with a pH ranging from 6.0-7.0 and the land surface flat (0 - 0.5%). Growing conditions were the same for each genotype and consistent with established farming practices of the area and with the variety used.

Experimental design, were carried out in a Randomized Block Design in four replication per genotype and 10 m^2 each plot. Plants belonging to species other than legumes planted around the entire field served as a buffer protective zone. Field observations and measurements were realized on 20 plants selected from the two middle rows of each plot.

Quantitative plant descriptors analysed were: *Stem length (cm) (STL)(cm), Stem number of nodes up to and including first fertile node (STNoNod), Leaf maximum number of leaflets (LMxNoLL), Leaflet size (LLS), Leaflet length (LLL), Leaflet width (LLW), Leaflet position of broadest part (LLPBP), Stipule length (StL), Stipule width (StW), Stipule size (StS), Stipule length from axil to tip (StLax-T), Stipule length of lobe below axil (StLlo-ax), Petiole length from axil to first leaflet or tendril (PetLax-1T), Petiole length from axil to last tendril (PetLax-lsT), Peduncle length of spur (PedLsp), Peduncle length from stem to first pod (PedLST-1P), Peduncle length between first and second pods (PedL1P-2P), Pod length (PodL), Pod width (PodW), Pod number of seed per pod (PodNoS), Weight Seeds per plant (WSpPL), Weight (gr) of 1000 seeds (W100-S)(gr), Yield per genotype (YpG)kg/ha*$^{-1}$, measured using (*Pisum sativum L.*) UPOV methodology (UPOV, 2009; CPVO, 2010).

Qualitative plant (or coded) *characters* observed were *Plant anthocyanin (PLA), Stem Anthocyanin (STA), Plant Habitat (PLHb), Leaf shape (LLsh), Foliage color (Fcol), Foliage intensity color (Ficol), Leaf-leaflets (LLfL), shape of leaflets (LfLsh), Leaflet position (LLP), Leaflet dentation (LLD), Stipule flecking density (StFD), Color of wing (Wcol), Shape base of flower (FLsh), Shape of apex (Apsh), Pod curvature (Pcurv)* evaluated using UPOV methodology (UPOV, 2009; CPVO, 2010).

Statistical Analysis: The differences between garden pea genotypes for the mean values of the biometric field observations and measurements were compared using one-way ANOVA analysis.

Principal Components Analysis (PCA) on correlation and classification of accessions according to characterization (agro-morphological characters) and evaluation data (Hofer et al., 2009; Sagan et al., 1994; Smykal et al., 2011; Tar'an et al., 2005), were used to identify garden pea genotypes of relatively similar characteristics. The number of principal components to be retained in the analysis was determined using the minimum eigenvalue criterion proposed by Kaiser (1960), and the *scree* test proposed (first) by Cattell (1966).

The distances and similarity between garden pea genotypes were determined based on cluster analysis of quantitative and qualitative (or coded) traits. For cluster analysis, the Ward's the minimum variance method is used. The method reduces the within cluster sum of squares over all partitions and merges the cluster to maximize the likelihood at each level of the hierarchy. Ward's method was selected because is more suitable for clusters with a small and approximately the same number of observations (Milligan, 1980). All the statistical analysis was conducted in SAS JMP Statistical Discovery (2012).

RESULTS AND DISCUSSION

Analysis of morphological characters: ANOVA analysis shows the presence of an important variability in the study materials and the F ratio values, significant at the $P_{0.05}$ and $P_{0.01}$ levels of the probability, proved the presence of significant differences between garden pea genotypes. Comparisons of means for all pairs using Tukey-Kramer HSD ($q^* = 3.60563$ and $\alpha = 0.05$) show the significant differences between and within garden pea genotypes at the $P_{0.05}$ and $P_{0.01}$ levels of the probability (Table 1). There were significant differences between *pea genotypes* related to *stem number nods, leaflets characters (number, length, width and position) petiole length from axils to first and last tendril, peduncle length from stem to first pod and from first and second pods, pod characters (length, width, seeds per pod), weight of 1000 seeds and yield per genotype.* All these characters were significant at the probability $0.0001^* < 0.005$. There were also significant differences between *pea genotypes* related to *stem length, leaflet size, and stipule width* (significant at the respectively probabilities 0.0013*, 0.0476*, 0.0008*, < 0.005).

There were not significant differences between *pea genotypes* related to *stipule length, stipule length from axils to tip, and weight seeds per plant* (significant at the respectively probabilities 0.3662, 0.1268, 0.1373 > 0.005).

Table 1. Garden pea genotypes and all pair means comparisons using Tukey-Kramer HSD test.

Genotype	STL (cm)	STNoNod	LMxNoLL	LLL (cm)	LLW (cm)	LLPBP	StL (cm)
V1-B2	70.55±10.10^{abc}	18.05±0.05^{cd}	42.77±0.67^{b}	5.51±1.43^{b}	3.39±0.33^{b}	1.64±0.02^{bc}	5.77±1.80^{a}
V2-B4	39.3±1.33^{c}	10.07±1.00^{h}	26.93±1.60^{h}	3.61±0.23^{b}	1.92±0.41^{c}	1.14±0.01^{d}	3.69±0.53^{a}
V3-B5	78.8±20.19^{ab}	15.90±0.35^{ef}	43.35±0.30^{b}	4.94±1.45^{b}	3.03±0.51^{bc}	1.73±0.04^{b}	5.29±1.91^{a}
V4-B6	57.78±12.85^{abc}	19.23±1.07^{bc}	42.02±0.19^{bc}	4.95±1.67^{b}	2.89±0.62^{bc}	1.56±0.10^{c}	5.65±2.04^{a}
V5-B7	75.05±4.66^{ab}	14.03±0.06^{g}	34.58±0.40^{g}	9.46±0.36^{a}	4.89±0.02^{a}	1.94±0.05^{a}	8.53±0.28^{a}
V6-B8	74.43±19.15^{ab}	21.15±0.65^{a}	37.62±0.76^{ef}	4.97±1.89^{b}	3.06±0.91^{bc}	1.59±0.03^{c}	6.43±3.18^{a}
V7-B9	86.23±12.36^{a}	16.87±0.23^{de}	40.02±0.72^{cd}	5.13±1.10^{b}	3.34±0.08^{b}	1.56±0.02^{c}	5.35±1.91^{a}
V8-B11	58.75±6.39^{abc}	19.02±0.03^{bc}	36.78±0.68^{fg}	5.15±0.97^{b}	3.29±0.08^{b}	1.60±0.05^{c}	5.46±1.86^{a}
V9-B18	73.57±6.51^{ab}	19.67±0.50^{ab}	39.20±1.04^{de}	4.93±0.35^{b}	2.93±0.13^{bc}	1.63±0.03^{bc}	4.96±0.51^{a}
V10-B25	72.45±6.93^{ab}	18.35±0.30b^{cd}	38.85±0.91^{def}	4.53±1.07^{b}	3.09±0.03^{bc}	1.60±0.05^{c}	6.17±2.54^{a}
V11-B29	52.28±6.02^{bc}	19.67±0.59^{ab}	41.75±0.65^{bc}	4.51±0.81^{b}	2.61±0.13^{bc}	1.59±0.03^{c}	5.41±1.94^{a}
V12-B30	68.18±3.85^{abc}	15.05±0.25^{fg}	45.98±0.69^{a}	4.71±0.85^{b}	2.30±0.47^{bc}	1.61±0.03^{bc}	5.03±1.06^{a}

Levels not connected by same letter are significantly different.

Table 1 (Continued)

Genotype	StW (cm)	StS	StLlo-ax (cm)	PetLax-1T	PetLax-lsT	PedLsp	PedLST-1P
V1-B2	3.31±0.27^{ab}	15.37±0.72^{b}	0.81±0.03^{ab}	3.75±0.02^{f}	8.15±0.13^{c}	1.18±0.04^{e}	28.40±0.72^{f}
V2-B4	1.82±0.16^{c}	6.27±0.53^{h}	0.47±0.03^{cd}	3.15±0.04^{h}	5.23±0.12^{g}	1.01±0.06^{f}	12.87±0.64g
V3-B5	3.03±0.41^{bc}	11.50±0.35^{ef}	0.70±0.22^{abcd}	4.75±0.08^{d}	8.05±0.15^{c}	1.26±0.04e	35.63±1.19^{cd}
V4-B6	2.78±0.71^{bc}	10.39±0.37^{fg}	0.61±0.03^{bcd}	4.22±0.05^{e}	7.13±0.12^{d}	1.74±0.03a	49.70±2.41^{ab}
V5-B7	4.600.10^{a}	23.42±1.15^{a}	0.46±0.04^{d}	5.80±0.02^{a}	5.66±0.07^{f}	1.44±0.02bc	33.35±0.59^{de}
V6-B8	3.65±0.78^{ab}	14.47±0.49^{bc}	0.66±0.02^{abcd}	4.12±0.03^{e}	8.09±0.03^{c}	1.40±0.09cd	33.54±0.60^{de}
V7-B9	3.15±0.09^{abc}	13.06±0.16^{cd}	0.77±0.21^{abc}	3.62±0.08^{fg}	6.47±0.18^{e}	1.04±0.06f	35.35±1.00^{cd}
V8-B11	3.30±0.17^{ab}	14.0±0.06^{bcd}	0.70±0.03^{abcd}	3.58±0.05^{g}	8.73±0.24^{ab}	1.54±0.03b	37.25±0.66^{c}
V9-B18	2.70±0.18^{bc}	12.88±0.19^{de}	0.71±0.08^{abcd}	5.00±0.07^{b}	8.41±0.11^{bc}	1.49±0.03bc	31.56±1.18^{ef}
V10-B25	3.53±1.18^{ab}	13.21±0.31^{cd}	0.96±0.17^{a}	5.85±0.07^{a}	8.67±0.06^{ab}	1.30±0.05de	52.20±1.73^{a}
V11-B29	2.95±0.09^{bc}	12.54±0.47^{de}	0.65±0.04^{abcd}	4.91±0.02^{bc}	8.81±0.02^{a}	1.21±0.02e	46.55±0.80^{b}
V12-B30	2.37±0.33^{bc}	9.32±0.48^{g}	0.53±0.01^{bcd}	4.83±0.04^{cd}	6.63±0.17^{e}	1.23±0.03e	37.62±1.08^{c}

Levels not connected by same letter are significantly different.

Table 1 (Continued)

Genotype	PedL1P-2P	PodL (cm)	PodW (cm)	PodNoS	W100-S (gr)	YpG (kv/ha)
V1-B2	4.84±0.05^{c}	5.10±0.49^{bc}	0.90±0.17^{bcd}	4.91±0.04^{b}	121.6±5.72^{ab}	16.36±43.6^{a}
V2-B4	6.15±0.13^{b}	3.50±0.30^{d}	1.14±0.13^{ab}	2.69±0.20^{d}	130.13±0.23^{a}	41.74±12.43^{d}
V3-B5	6.12±0.09^{b}	4.18±0.38^{cd}	0.86±0.08^{bcd}	4.11±0.08^{c}	114.17±3.33^{ab}	78.51±24.9^{bcd}
V4-B6	5.90±0.08^{b}	5.13±0.86^{bc}	1.13±0.07^{ab}	5.85±0.08^{a}	130.37±2.06^{a}	11.02±68.5^{abc}
V5-B7	6.14±0.26^{b}	7.00±0.32^{a}	1.35±0.01^{a}	4.71±0.02^{b}	148.40±20.27^{a}	9.29±45.1^{bcd}
V6-B8	6.83±0.27^{a}	5.74±0.57^{ab}	1.05±0.14^{bc}	5.82±0.31^{a}	140.50±16.89^{a}	7.54±54.5^{bcd}
V7-B9	7.31±0.33^{a}	4.44±0.15^{bcd}	0.77±0.03^{cd}	4.89±0.18^{b}	120.83±10.25^{ab}	11.07±63.0^{abc}
V8-B11	7.24±0.37^{a}	5.07±0.40^{bc}	1.10±0.04^{ab}	4.59±0.20^{bc}	126.83±20.50^{a}	12.12±39.1^{ab}
V9-B18	5.90±0.20^{b}	4.51±0.44^{bcd}	0.91±0.00^{bc}	5.89±0.18^{a}	117.30±6.67^{ab}	9.39±1.49^{bcd}
V10-B25	6.03±0.15^{b}	4.33±0.56^{bcd}	0.63±0.10^{d}	4.37±0.22^{bc}	83.33±11.55^{bc}	6.91±34.8^{bcd}
V11-B29	6.95±0.25^{a}	4.46±0.57^{bcd}	1.03±0.06^{bc}	4.68±0.21^{b}	70.00±17.32^{c}	5.97±278.7^{cd}
V12-B30	5.84±0.23^{b}	5.36±0.51^{bc}	0.98±0.13^{bc}	4.47±0.23^{bc}	83.50±22.95^{bc}	6.59±20.8^{bcd}

Levels not connected by same letter are significantly different.

Principal Components Analysis on Correlations: Principal Components Analysis on Correlations identified the variances of the principal components and the proportion of the total variance each factor accounts for. Eigenvalues and percent of variances each factor accounts for are given in table 2. Based on the mineigen criterion (Kaiser, 1960), and the *scree* test (Cattell, 1966), five principal components, that account for 81.5% of the total variation, are retained for further analysis. The six PC components with Eigenvalue 0.92 (very clear less of one) and account for only 4.03% on the total variance, are not retained in our study (Table 2). PCA results show that the major sources of variation in the measurements is given by the first five PCs. Twenty three quantitative variables contribute in the total source 100% of variance. Overall, the first five PCs account for a substantial proportion of total variation, 81.5%. The percentages of total variation accounted for by each of the first five PCs are 36.07%, 20.5% 10.49,

8.32% and 6.56%, respectively (Table 2). The proportion of total variation more than 75% is acceptable in this kind of studies (Cadima et al., 2001; Jolliffe 2002).

Table 2. Principal Components on Correlations of garden pea characteristics.

Number	Eigenvalue	% variance	Percent	Cum Percent	ChiSquare	DF	Prob>ChiSq
1	8.2975	36.076		36.076	1154.89	247.358	<.0001*
2	4.6116	20.051		56.127	945.747	240.429	<.0001*
3	2.4139	10.495		66.622	791.532	226.241	<.0001*
4	1.9146	8.324		74.946	697.972	208.723	<.0001*
5	1.5098	6.564		81.511	608.475	190.960	<.0001*
6	0.9263	4.027		85.538	522.286	173.266	<.0001*
7	0.7803	3.392		88.930	464.676	155.886	<.0001*
8	0.6844	2.976		91.906	407.565	139.025	<.0001*
9	0.5208	2.264		94.170	345.362	123.127	<.0001*
10	0.3412	1.483		95.653	287.807	107.926	<.0001*
11	0.2899	1.261		96.914	245.898	93.637	<.0001*
12	0.1858	0.808		97.722	201.278	80.165	<.0001*
13	0.1534	0.667		98.389	170.512	67.676	<.0001*
14	0.1005	0.437		98.826	139.423	56.309	<.0001*
15	0.0898	0.391		99.216	118.129	45.825	<.0001*
16	0.0826	0.359		99.575	92.868	36.591	<.0001*
17	0.0335	0.146		99.721	54.712	28.352	0.0021*
18	0.0267	0.116		99.837	40.864	21.078	0.0060*
19	0.0154	0.067		99.904	25.722	14.761	0.0376*
20	0.0110	0.048		99.952	17.153	9.570	0.0595
21	0.0070	0.030		99.982	9.111	5.392	0.1274
22	0.0027	0.012		99.994	1.329	2.342	0.5938
23	0.0014	0.006		100.000	0.000	.	.

For PC1 (36.07% of total variation) *leaflet width, length and size*, *stipule width and length from axil to tip* were characters with significant weighting (Table 4). Three variables *leaflet width and size*, and *stipule width* with nearly the same value of eigenvectors, are the same important to the PC1.Variation in Component 2 (20.5% of total variation) was mainly result of differences in *petiole length from axil to first leaflet*, *stem number of nodes*, *leaf maximum number of leaflets* and *peduncle length from stem to first pod* (Table 3). In PC3 there are *peduncle length of spur*, *weight (gr) of 1000 seeds* and *number of seed per pod* that account for 10.49% of the total of variation. In addition, *peduncle length of spur* showed greater weighting.

Table 3. Eigenvectors value for PC1, PC2, PC3, PC4 and PC5.

PC1	Eigenvectors	PC2	Eigenvectors	PC3	Eigenvectors
Characters	Axis 1	Characters	Axis 2	Characters	Axis 3
Leaflet-width	0,3197	Petiole-length	0,3873	Length of spur	0,4358
Leaflet-size	0,3197	No. of nodes	0,3801	Weight 100-seeds	0,3753
Stipule-width	0,3167	Max no. leaflets	0,3449	No. seed/pod	0,3271
Leaflet-length	0,3165	Peduncle-length	0,3356		
Stipule-length	0,3084				
from axil to tip	0,3042				

PC4	Eigenvectors	PC5	Eigenvectors
Characters	Axis 1	Characters	Axis 2
Yield per genotype	0,4804	Peduncle length between 1st - 2nd pods	0,6374
Weight per plant	0,3536		
Stip.length of lobe below axils	0,3356.		

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In PC4 there are *yield per genotype* and *stipule length of lobe below axil* that account for 8.32% of the total of variation. *Yield per genotype* showed greater weighting than other. In PC5 there is only *peduncle length between first and second pods* that account for 6.56% of the total of variation (Table 3).

Analysis of distances: Study results for distances between garden pea genotypes using hierarchical clustering Ward method, range pea genotypes into four different cluster groups. There are 5 garden peas genotypes included into the 1st cluster group (V1-B2, V4-B6, V6-B8, V9-B18 and V8-B11), and 5 other garden pea genotypes into the 2nd cluster group (V3-B5, V11-B29, V7-B9, V10-B25 and V12-B30), and one pea genotype (V5-B7) into the 3rd cluster and one other pea genotype (V2-B4) is included into the 4th cluster (Figura 1).

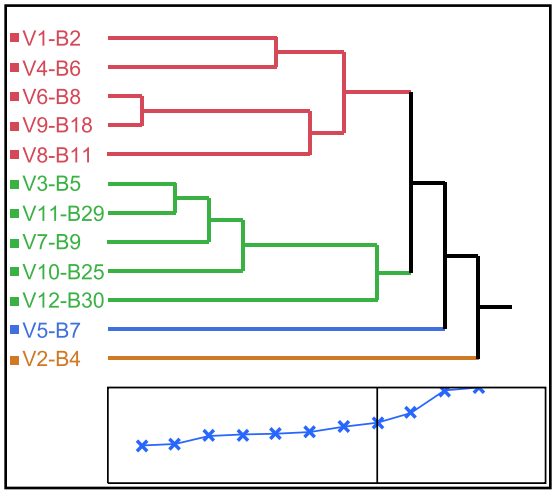

Fig. 1 Dendrogram by cluster analysis of different garden pea genotypes for all characters analyzed.

Cluster analysis allows the identification of pea genotypes with 'unknown' origin or 'donation' from other countries, without specific origin. Cluster dendrogram ranged one 'unknown' garden pea genotype (V3-B5) and one from Russia (V7-B9) into the 2nd cluster group. Having not significant differences between them these two 'unknown' genotypes show similarity in the most biometric characteristics measured between them and garden pea genotypes originated from Albania. Two pea genotypes one from Germany (V8-B11) and one from Sweden (V9-B18) and three 'unknown' garden pea genotypes (V4-B6, V6-B8, V1-B2) included into the 1st cluster group show similarity in the most biometric characteristics measured between. These pea genotypes show different characteristics and significant differences with pea genotypes originated from Albania.

CONCLUSION

The field test accomplished in this study permitted the first characterization of the most important morphological diversity and determination of the patterns or variation of garden pea genotypes with high forage value.
PCA results show that the first **five** PCs account for a substantial proportion of total variation, 81.5%. The percentages of total variation accounted for by each of the first **five** PCs are 36.07%, 20.5% 10.49, 8.32% and 6.56%, respectively
The results of the study clearly range garden pea genotypes into two different cluster groups. Cluster analysis allows the identification of some pea genotypes with 'unknown' origin or 'donation' from other countries, without specific origin.

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