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Analysis of variability in qualitative traits of (Lathyrus sativum) accessions in Albanian genebank

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Abstract

Assessment of genetic diversity of grass pea (Lathyrus sativum) genotypes stored in Albanian genebank, was carried out in the Experimental field of Agricultural University of Tirana, during two growing seasons. Twelve local forms of grass pea's collection, evaluating 15 qualitative traits with high inheritance degree, were used for the assessment of variability between qualitative traits and genetic diversity of *Lathyrus sativum* genotypes. The grass pea collection in genebank is a modest valuable group of legumes species for animal production, but, little is known about the extent and nature of the variability of the species. The aim of the study was the evaluation of the major qualitative traits, important for characterizing the grass pea genotypes, and determining the potential of this forage species in agriculture. Principal Components Analysis (PCA) and cluster analysis (ward method) identified the variances of the principal components (PC) and the proportion of the total variance each factor accounts for and range grass pea genotypes into three different cluster groups. Study identified the qualitative 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: Cluster analysis, grass pea genotypes, principal components.

1. Introduction

Lathyrus gender is part of the family Fabaceae and comprises more than 190 species and subspecies. Most widespread of *Lathyrus sativus L*. is with 2n = 14 chromosomes. Other important economic species include *Lathyrus cicera* and *L.Tingitanus* , for cereals, *L. ochrus, L.* l*atifolius* and *L. sylvestris* as forage species. [16] describes two separate centers of origin of gender *Lathyrus*. One center is Central Asia that includes north-western India, Afghanistan, Tajikistan and Uzbekistan, while Abyssinia known as the second center. In addition Vavilov notes the tendency of similarity in diversity of gender Lathyrus those of legumes others like lentil (*Lens culinaris*), beans (*Vicia faba*), in the form of small seeds are found in South Asia and South-West, while around Mediterranean region, most of them were cultivated forms with large seeds and white flowers [10]. Lathyrus is very old crop [15], and it was found in India about 2000-1500 BC years. Combination of the data phytogeographic with those archeobotanic show that the origin of cultivation of *L.sativus* is in the Balkan Peninsula in the early Neolithic period, which dates back to the early 6th millennium BC [13]. Plant genetic resources have the main contribution to developing of agriculture for agri-food products increasing. Today the preservation of genetic resources is considered vital and necessary for human society. Genetics banks provide the main means of preservation of Genetics resources and serve as safe reserve base materials that are required in the genetic improving of plants [2].

The legume base collections in Albania genebank contains more than 200 local forms and breeding lines or cultivars with known or unknown origin, but were recorded in the bank as accessions.

To prevent genetic erosion, the AGB has launched a national initiative to inventory, regeneration and evaluate the genetic variability of the underutilized legume plants and the wild species including grass peas, garden pea and forage. As a result, a series of field and laboratory tests to identify, characterize, and evaluate the underutilized legume plants and their genetic potential with interest to use the structures of forage for further breeding, were realized.

2. Materials and methods

Plant Materials: The grass pea collection (*Lathyrus sativum* L.) stored in Albania genebank contains more than 30

local and unknown grass peas genotypes. Initially, through the field and laboratory tests it was

evaluated the entire forage collection for resistance to diseases of grass peas in order to reduce the collection to a manageable number of the most promising genotypes. All the local forms or varieties that were susceptible to most common diseases and pests [6] were eliminated. From remaining grass pea genotypes, tested on laboratory for germination energy and germination capacity [2,9], amount of seeds per plant were preserved [7] for the second year field and laboratory tests [14]. This resulted in a set of 12 grass peas (*Lathyrus sativum*) genotypes planted in the second year and further analyzed through field and laboratory tests for the most important quantitative and qualitative characters used for characterization and evaluation of grass pea forms [8]. Of the 12 genotypes, 7 grass peas (*Lathyrus sativum*) genotypes (GB804, GB1628, GB1629, GB1632, GB633, GB1634 and GB1638) have registered with the origin "unknown", the while 5 others genotypes (GB806, GB807, GB1630, GB1636 and GB1637) marked originating from “gene banks”.

Experimental design: Experiments were carried out in a randomized block design replicates four times per genotype. Field services are carried out based on agro technical extensive product recommended for forage crop. Observations and qualitative assessments of morphological traits are carried out on all plants of each variant in all repetitions

The site of experiment: The study to identifying and assessing of the genetic diversity of grass pea genotypes was carried out in the field regeneration of Genebank at the experimental didactic (EDE) of AUT, Valias (width: 402405N; length: 0194108E, the height above sea level: 40m) during three years (Figure 1).

Figure 1. The location in EDE, Valias Tirane

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The land: The land where it is carried out the experiment is alluvium-sand type of meadows and gray coffee, with pH 6.0 to 7.0 and sloping 0% to 0.5% of the surface.

Qualitative morphological traits observed were: plant growth habit (PLH), nodes number (NN), stem colour (STC), anthocyanin pigmentation (PLA), number of primary branches (BN), kind of leaflets per leaf (LLN), leafleat colour (LLC), leaflet shape (LLSH), flower colour (FlC), plant nodes (PN), pod shape (PSH), pod curvature (Pcur), pod beak shape (BeSh), immature pod colour (Pcol) and seed shape (SSH) that were evaluated using Lathyrus spp coded descriptors methodology [8].

Statistical analysis

The differences between grass pea genotypes for the values observed in the field and laboratory analysis were analysed by ANOVA. To identify similarities of the grass pea genotypes and morphological triats was conducted analysis of the main components analysis (PCA) on correlation and classification of genotypes according to agro-bio-characteristics and morphological features with high quality heritage. The number of principal components to be retained in the analysis was determined using the minimum eigenvalue (mineigen) criterion proposed by [12]. Distances and similarities between grass pea genotypes were determined by cluster analysis, Ward method for qualitative traits. All statistical analyzes were performed with SAS JMP [11].

3. Results and Discussions

Analysis of qualitative morphological traits

ANOVA analysis shows the presence of an important variability in the study materials and the **F** ratio values, significant at the P0.01 and P0.05 levels of the probability, proved the presence of significant differences between grass pea genotypes connected with plant growth habit, plant nodes, stem colour,

antocianin pigmentation, number, colour and leaf shape, flower colour, the number of pod, the pod curvature, immature pod colour and seed shape.

Analysis of correlation coefficients

There are very positive correlation between plant growth habit (PLH), with nodes number NN, PLA,LLC, LLSH and PN (r ranged from 0.51 to 0.71), about the very strong positive correlation between NN, with PLA, LLC and LLSH, PN and Pcur where the correlation coefficient (r varies from 063 up to 0.85).Traits (STC) stem colour shows strong positive correlation with the number of pod (Pcol) and very strong positive correlation with seed shape (SSH), (r respectively 0.56 and 0.84), a very strong positive correlation between anthocyanin pigmentation (PLA) with leaflet shape (LLSH) r 0.95 and strong positive with kind of leaflets per leaf (LLN) and plant nodes (PN), (r respectively from 0.50 to 0.60). There were strong positive to very strong positive correlation between number of primary branches (BN) with the pod shape (PSH) between number of primary branches (BN) with pod beak shape (BeSh) (r varies from 0.59 to 0.85). Also the color and leaf shape (LLC) shows very strong positive correlation with the leaflet shape (LLSH) and plant nodes (PN) (r goes from 0.78 to 0.92). Some of the qualitative morphological characteristics show strong correlations between them such as the plant shape, pod beak shape, colour and leaf shape. The high values of autocorrelations create the possibility for more efficient plant evaluation of grass pea genotypes through the main components analysis (PCA) and enable groups of genotypes with the similarities between their traits.

Principal Components Analysis on Correlations

The principal components analysis on the correlations identified the presence and proportion of variance of the main components that contribute to the total variance. Eigenvalues and variances percentages of each the main feature is given in Table 1.

Table 1. Analysis of the main components PC1, PC2 and PC3(grass pea genotypes x 15 qualitative traits)

Number	*Eigenvalue*	*Percent*	*Cum Percent*	*ChiSquare*	*DF*	*Prob>ChiSq*
1	**6.6170**	**44.113**	**44.113**	**1143.81**	**104.606**	**<.0001***
2	**4.8328**	**32.219**	**76.332**	**991.058**	**101.728**	**<.0001***
3	**2.0094**	**13.396**	**89.728**	**831.692**	**95.135**	**<.0001***
4	0.8633	5.755	95.483	687.559	84.335	<.0001*
5	0.3463	2.308	97.792	555.935	72.477	<.0001*

Table 2. Eigenvectors value for PC1, PC2 and PC3

Traits		**Eigenvectors**		
		PC1	**PC2**	**PC3**
Plant growth habit	PLH	**0.27854**	-0.14306	**0.29652**
Plant nodes	NN	**0.32163**	0.09197	0.26792
Stem colour	STC	-0.10783	**0.38446**	-0.22367
Anthocyanin pigmentation	PLA	**0.37442**	-0.10296	-0.06275
Number of primary branches	BN	-0.25456	-0.12430	**0.39231**
Kind of leaflets per leaf	LLN	0.11711	-0.40735	-0.08859
leaf colour	LLC	**0.36916**	0.04257	-0.03947
leaflet shape	LLSH	**0.37442**	-0.10296	-0.06275
Flower colour	FlC	-0.06374	-0.37855	0.11392
Pods class per peduncle	PN	**0.29678**	0.22778	0.26930
Pod shape	PSH	-0.23208	-0.21974	**0.41665**
Pod curvature	Pcur	0.14528	**0.34170**	**0.36922**
Pod beak shape	BeSH	-0.37442	0.10296	0.06275
Immature pod colour	Pcol	-0.06972	**0.37142**	**0.37493**
Seed shape	SSH	0.00373	**0.33179**	-0.28131

Mineigen based on criteria [12] and scree test [1] three principal components are retained for further analysis. PCA analysis results show that the major source of the total variation is given by the first three components of PC1, PC2 and PC3. Fifteen qualitative traits of 12 grass pea genotypes provide 100% of the total variance, while the first three components (PC1, PC2 and PC3) account 89.7% of the total variance. The percentages of variations accounted by PC1, PC2 and PC3 were respectively 44.1%, 32.2% and 13.4%.

In PC1 (44.1% of total variance) traits as PLA, LLSH, LLC, NN, PN and PH are the traits with

greater weight in determining variance of component. PLA, LLSH and LLC traits with the approximations eigenvectors have nearly the same weight in the size of the variance in PC1.

In PC2 (32.2% of total variance) there are STC, Pcol, Pcur and SSH traits who have the most weight in the variance of second size component (PC2). Traits as LLN and FlC have shown negative influence in the PC2 variance. **In PC3** (13.4% of total variance) there are PSH, BN, Pcol and Pcur traits that have the most weight in the variance of the third component (PC2). In PC3 traits as PSH and Pcol have contributed in the variances not accomplished by the traits of the second component PC2.

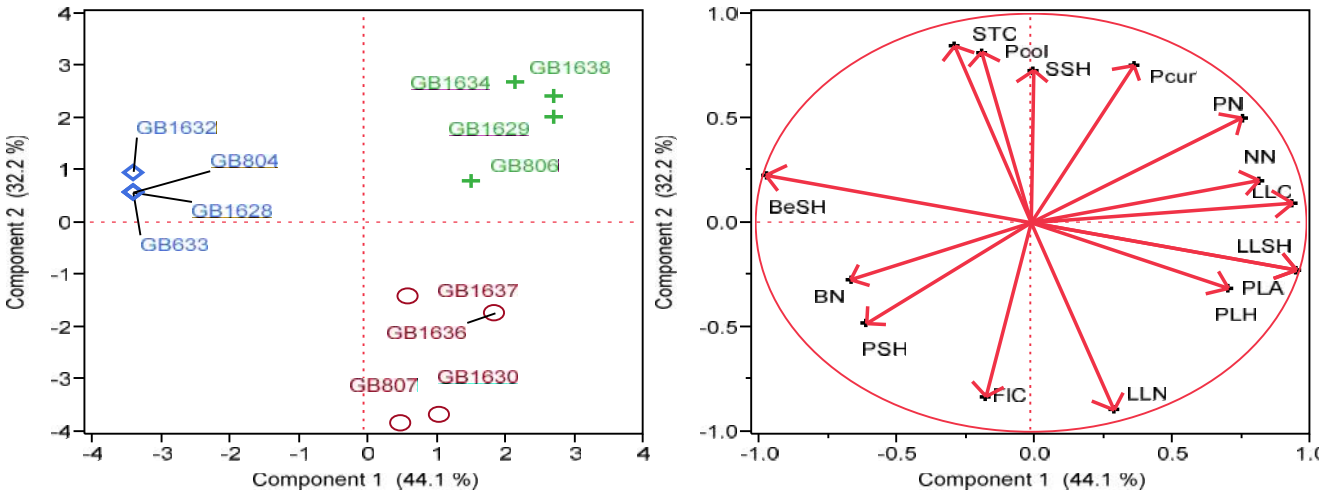

Figure 2. Relationships between grass pea genotypes x 15 morphological qualitative traits

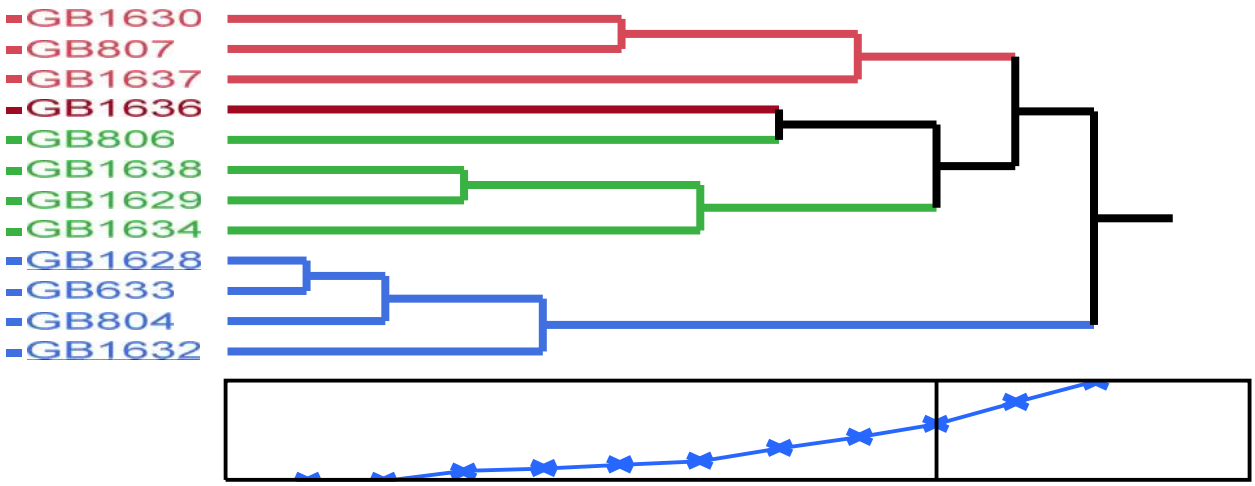

Figure 3 Dendrogram by cluster analysis of different grass pea genotypes for all characters analyzed

Analysis of genetic distances

Cluster analysis for the assessment of the Euclidian genetic distances (method Ward) grouped grass pea genotypes into three different cluster groups. The first cluster group included 4 grass pea genotypes (GB1630, GB1636, GB807, and GB1637), similarities between them in the second cluster group were included four grass pea genotypes as: GB806, GB1638, GB1629, GB1634, and in the third group were included GB1628, GB633, GB804, and GB1632. Results of the study show that all grass pea genotypes are selected forms not or not improved most of them represent the local forms with wide variability in the content of the wild genes.

4. Conclusions

The field and laboratory tests enabled the characterization and evaluation of accessions of grass pea and identified their genetic potential with interest for use in fodder structures and the improvements of the species programs. Analysis of the main components identified the presence and proportion of variance of the three main components (PC1, PC2 and PC3) that contribute to the total variance with 89.7%.

Traits as PLA, LLSH, LLC, NN, PN and PH were with greater weight in determining variance of PC1 component, and in PC2 there were STC, Pcol, Pcur and SSH traits who have the most weight in the variance of this component. Cluster analysis and PCA clearly ranged the pea genotypes in three cluster groups distinct between them. Results of the study show that all pea genotypes are local forms (not selected) with wide genetic variability.

5. References

1. Cattell R. B. **The scree test for the number of factors**. *Multivariate Behavioral Research,* 1996, *1,*245-276.
2. Engels J.M.M., Visser L. **''A guide to effective management of germplasm collections''**. 2003, *IPGRI. Rome*.
3. Gixhari B, Doko A, Hobdari V, Vrapi H **Diversity of Grass Pea (*L.sativum*) Landraces For Sustainable Field Grass Pea Breeding In Albania**. International Journal of Ecosystems and Ecology Sciences (IJEES). 2016, 6 (1): 81-88.
4. Gixhari B, Ismaili H, Vrapi H, Hodaj B, Hobdari H, Sevo R., **Genetic diversity assesment of pes (Pissum sativum L.) germplasm based on quantitative morphological and qualitative traits**. International Conference of Ecosystems (ICE*)*. 4068-2-8, 2013, 997-1003
5. Hobdari V, Gixhari B, Elezi F, Bode D, Dias S, Libohova Z, **Characterization of Grass Pea (*Lathyrus sativum*.). Genotypes stored in Albanian gene Bank**. Journal of International Environmental Application & Science. 2012, 7 (4). 2012, 709-714
6. Hagedorn D.J., **Compendium of Pea Diseases**. The American Phytopathological Society, Minnesota, LISA, US. 1984,
7. Hong T.D., Ellis, R.H. **A protocol to determine seed storage behavior**, 1996, 23-44.
8. IPGRI. **Descriptors for *Lathyrus* spp**. 2000 International Plant Genetic Resources Institute, Rome, Italy.
9. ISTA. **International Rules for Seed Testing. Edition** International Seed Testing Association, 2005 Bassersdorf, Switzerland.
10. Jackson M.T. and Yunus, A.G. **Variation in the grasspea (*Lathyrus sativus* L.) and wild species**. Euphatica, 1984,37: 549-559. 68
11. JMP SAS **Statistical Discovery** 2012.
12. Kaiser H. F., **The application of electronic computers to factor analysis**. *Educational and Psychological Measurement,* 1960, *20,* 141-151.
13. Kislev M.E. **Origin of the cultivation of Lathyrus sativus and L. cicera (Fabaceae)**. Econ. Bot., 1989, 43262–270.
14. Rao NK, Hanson J, Dulloo ME, Ghosh K, Nowell D and Larinde M.. **Manual of seed handling in genebanks.Handbooks for Genebanks No. 8**. Bioversity International, Rome, Italy. 2006, (103-117).
15. Saraswat K.S. **The ancient remains of the crop plants at Atranjikera**. J. Ind. Bot. Soc. 1980, 59:306-319.
16. Vavilov N.I. **The origin, variation, immunity and breeding of cultivated plants** Chron. Botan. 1951, 13: 1-36.
17. Kramer C.Y. "**Extension of multiple range tests to group means with unequal numbers of replications''**. *Biometrics*, 1956, 12, 309–310.
18. Milligan G.W. "**An Examination of the Effect of Six Types of Error Perturbation on Fifteen Clustering Algorithms,"** *Psychometrika*, 1980, 45, 325–342.
19. Tukey J., **"A problem of multiple comparisons**," *Dittoed manuscript of 396 pages, Princeton University.* 1953,