DIVERSITY OF GRASS PEA (*L. SATIVUM*) LANDRACES FOR SUSTAINABLE FIELD GRASS PEA BREEDING IN ALBANIA

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Received December, 2015; Accepted Jaunary, 2016;

ABSTRACT

Fourteen grass peas (*Lathyrus sativum*) landraces collected in three different region of Albania (Korca, Elbasan and Fieri) were used to assess genetic diversity by quantitative morphological traits and molecular markers. The study, carried out in a randomized design with four replications during three growing seasons (2011 - 2013), had the objective of identification and selecting the traits with favourable characteristics for use in grass pea field breeding. ANOVA, principal component analysis and cluster analysis (method ward) used to assess the variability and association among different traits showed significant genetic diversity between collected grass pea landraces. Study identified the traits with more significant weighting on respective PC variances (days to flowering, and to pods, and to maturity, plant height, leaf size, leaflet length, number of pods per plant and seeds per pod, and yield per genotype), which can be used successfully as morphological quantitative markers for evaluation and characterization of the grass pea germplasm. The high amount of genetic variability founded in the present study, available to the breeders, is sufficient for selection of desirable traits and high yield parental lines, and for creation of new favourable gene combinations to sustain field grass pea breeding programs.
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Keywords: genetic variability; grass pea landraces; morphological traits, factorial analysis

INTRODUCTION

The genus *Lathyrus* is a member of the Viciae tribe (family Fabaceae) and consists of about 160 annual and perennial species (Allkin et al. 1986). Kislev (1989) reported that the domestication of Lathyrus began in the Balkan Peninsula as a consequence of the Near East agriculture expansion into the region. Now the cultivation of Lathyrus spread to include marginal lands in Syria, Lebanon, Egypt, Libya, Algeria, Morocco, France and Spain, the Mediterranean basin. Grass pea (*L. sativum*) in many countries despite their limited extend, toxicity to animals and lathyrism[1], is important for human and animal consumption (FAO 1991). Due to the neurotoxin presence, Lathyrus product has been banned in many countries. However, due to the importance of this crops in developing countries, many countries has established of breeding programmes focused on high seed yield and low toxicity genotypes.
The success of a breeding program largely depends on the existence, magnitude and nature of genetic variability present for a specific trait. There are two ways to have a genotype with high seed yield and low toxicity, first is using the genetic engineering to produce transgenic plants (Hanbury et al. 2000), and the second to eliminate the toxic substance by careful selection of high seed yield varieties and through hybridization with low toxin varieties (Qayyum et al. 2001; Ben Brahim et al. 2001). Traditionally, germplasm diversity is assessed by morphological

descriptors, which remain the only legitimate marker type accepted by the International Union for the Protection of New Varieties of Plants (UPOV 2009). Morphological characterization is the first step in the description and classification of the germplasm (Smith, 1989). An understanding of morphological characters facilitate the identification, selection of desirable traits, and designing new populations (Santalla et al. 2001). Many works based on agro-morphological traits have been published (Granati et al. 2003; Lioi et al. 2011; Gixhari et al. 2013), and a variety of molecular techniques have been used to study the diversity of the genus *Lathyrus* (Ben Brahim, 2001; Hossaert et al. 1986; Narayan 1986; Karp et al. 1996; Gixhari et al., 2014). Since many morphological characters (especially quantitative or polygenic characters) are influenced by environmental factors (Simioniuc et al. 2002; Smýkal et al. 2008), and because molecular markers measure genetic diversity at the DNA level (not influenced by the environment), the molecular markers can account for the effective selection in breeding programs.

The Albanian grass pea (*L. sativum*) collection represent a modest valuable group of legumes in continuous extension used mostly for animal consumption. However, little is known about the extent and nature of the variability of these species. The aim of the present study was to identify and evaluate the level of genetic diversity among and within grass pea landraces of Albanian origins, using agro-morphological traits and molecular markers, to aid in the selection of specific traits that can be used more efficiently in the breeding programs.

MATERIALS AND METHODS

The experimental site and design: The study for the assessment of morphological diversity was carried out at the experimental field of Agriculture University of Tirana (latitude: 402405N; longitude: 0194108E; elevation: 40m) during three growing seasons (2011, 2012 and 2013). The plant materials were sowed in a randomized block design with 4 replications of 20 plants per plot and conducted under the same agro-ecological and farming conditions. The analysis was performed on fourteen grass pea landraces collected from Korca (GB001, GB0022, GB0046, GB057, GB0712, GB1131, GB1326 and GB2110), and from Elbasan (GB1421, GB1629, GB1811 and GB2013), and from Fieri county areas (GB2227 and GB2420).

Agro-morphological characters: Days to flowering (DF), days to pods (DP), days to maturity (DM), plant height (PH) (cm), Number of primary branches (NPB), leaf size (LS), leaflet length (LLL) and leaflet width (LLW) (cm), pod-bearing position (PBP) (cm), pod-bearing length (PBL), beak length of pod (BLP), pod length (PL), pod width (PW) (cm), number of pods per plant (NPP), number of seeds per pod (NSP), seed size (diameter) (SS) (mm), 1000 seed weight (100SW) (gr) and yield per genotype (YG) (kg), were the quantitative traits estimated using *Lathyrus spp*. descriptors (UPOV 2009).

DNA isolation and markers: DNA was extracted from fresh young leaves of ten randomly plants per landraces and was manually isolated using the Invisorb Plant Genomic DNA Isolation Kit (INVITEK, Germany). DNA concentration was assessed by agarose gel electrophoresis in comparison with a quantitative reference marker. For each primer, the consistent amplified products were recorded. Each RAPD marker was assumed to correspond to a locus with two alleles (presence and absence of the band). The DNA was diluted to 25 ng/μl for Random Amplified Polymorphic DNA (RAPD) analysis. RAPD primers as: OPA02, OPA03, OPA05, OPA10, OPA16, OPA18, and OPD02 (Operon Tech., Alameda, CA, USA) were selected for the present analyses.

Statistical data analysis: An analysis of variance (ANOVA) procedure, a principal component analysis (PCA) and a cluster analysis which generate a dendrogram (ward method) were carried out. RAPD scores converted into binary data by presence (1) or absence (0) of the selected fragment in each genotype were used to assess genetic similarity coefficients on SPSS 12 software 2003.

RESULTS

Analysis of morphological quantitative characters: ANOVA analysis showed the presence of significant differences between pea accessions for the most number of morphological traits analyzed with probability $F < P_{0.05}$ (Tab. 2). High degree of variation was observed for all the morphological characters, except for NPB, LLW and BLP not significant at the probability $P_{0.05}$. PCA on correlations identified the total variance of the principal components (PC) and the proportion of the variances explained by each factor. All quantitative variables contribute to 100% of total

variation. The first three PCs explain 85.97% > 75.0% of the original variation (Tab. 1), acceptable for characterization and evaluation of collections in genebank (Jolliffe 2002).

Table 1. Matrix of eigenvalues of principal components for 14 grass peas and 18 agro-morphological traits.

Principal Components/factor analysis						
PC No.	Eigenvalue	Percent variance	Cumulative Percent	χ^2	df	Prob. > χ^2
1	7.0455	39.142	39.142	941.908	152.158	<.0001*
2	6.7741	37.634	76.775	818.419	147.269	<.0001*
3	1.6553	9.196	85.971	663.948	141.736	<.0001*
4	1.2590	6.994	92.966	563.540	127.346	<.0001*
5	0.4178	2.321	95.278	460.737	113.067	<.0001*

χ^2 – Chi Square, df – degree of freedom; Prob. – probability; *significance level equal to the 0.05 of probability

Relationships among the morphological characters and grass pea genotypes: Relationships between 14 grass pea landraces assessed by agro-morphological traits and genetic similarity coefficients revealed by cluster analyses categorized all grass pea genotypes into three clusters (Fig. 1). Clusters were differentiated by DF, DP. DM, LLL, PBL, PH, LS, NPP, NSP and YG traits significant at the probability $F < P_{0.01}$. Cluster I included five grass pea landraces with similarity between them and which have the highest values for DF, DP, DM, LLL, PBL, PH traits analysed. Cluster II consisted of three grass pea landraces with highest values for LS, NPP, NSP and YG variables analysed. Both I and II clusters had intermediate values for the most variables analysed. Cluster III included six landraces characterized by the lowest or intermediate values for all the variables analysed.

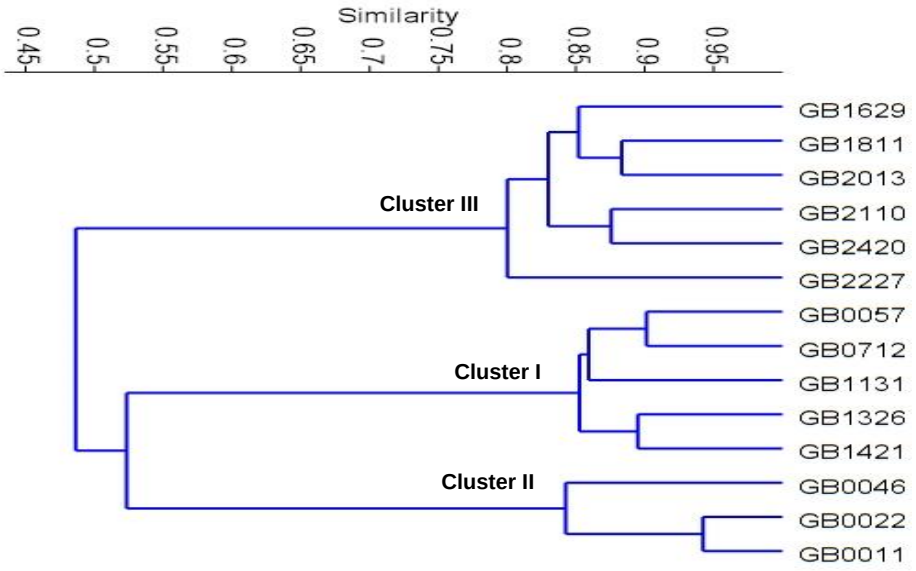

Figure 1. Dendrogram of relationships among 14 grass pea landraces

The maximum information from agro-morphological data was received using ordination methods in combination with cluster analyses (Messmer et al. 1993; **J**olliffe 2002). Three-dimensional scaling of relationships (accessions x traits) that accounts for the larger proportion of the total variance in PC1, PC2 and PC3 revealed by PCA indicate that the contribution of each grass pea accession and of each quantitative agro-morphological trait on the total of variation is not equal. There were five grass peas included in PC1 that account for 39.14% of total variation, and three grass peas in PC2 which contribute with 37.63% on the total variation. Six other grass peas landraces included in PC3 account for 9.19% on the total variation (Tab. 1; Fig. 2).

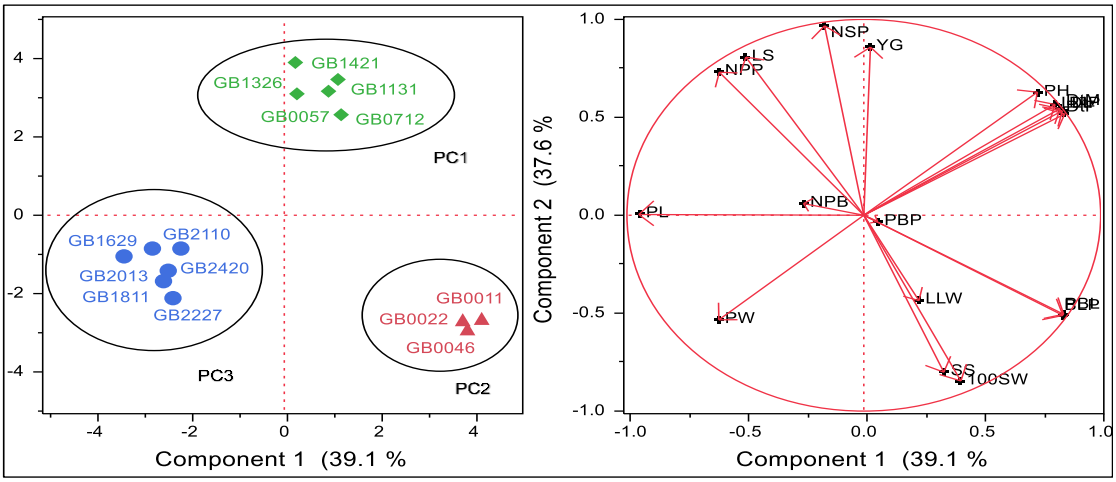

Figure 2. Relationships among 14 pea genotypes based on morphological quantitative traits revealed by PCA.

Factorial analysis identified highest weighting of PC1 and PC2 on the total variation was explained especially by grass pea landraces collected in Korca region of Cluster I and II. The higher level of variation showed by grass peas of these two clusters, can be explained by the ‘’wild nature” of variation source between and within grass pea landraces collected in Korca region. This suggests most of these landraces could be used as parents or as possible reserve of desirable traits (genes) for breeding schemes, as “no any” breeding activity was carried out with these grass pea landraces. Similar results and interpretations reported Lioi et al. (2011); Infantino et al. (1994). The lowest weighting of total variation was showed by grass pea landraces collected in Elbasan and Fieri County areas, which were more uniform and similar among them (Cluster III). Uniformity and similarity among these grass pea landraces could be ascribed to relative similarity of climatic and growing conditions among two nearness collecting sites (Elbasan and Fieri areas), and to possible common parent origin in their pedigree (Gixhari et al. 2014).

Relationships among the agro-morphological traits: Dimensional scaling for relationships among the agro-morphological traits, showed the PC1 that explained 43.1% of the variation was positively related to the seven morphological traits (DM, DP, DF, PH, LLL, PBL and BLP) with eigenvectors more than 0.25 (Tab. 2; Fig. 3).

Table 2. F ratios and eigenvectors of three principal components for morphological traits in grass pea.

Morphological			ANOVA			Eigenvectors
Quantitative Traits		*F* Ratio	Prob > *F*	PC1	PC2	PC3
Days to flowering	DF	1850.328	<.0001*	0.3122	0.2089	0.0612
Days to pods	DP	1685.228	<.0001*	0.3194	0.1986	0.0429
Days to maturity	DM	7834.159	<.0001*	0.3157	0.2048	0.0448
Plant height	PH	372.6318	<.0001*	0.2761	0.2515	0.1022
No. of primary branches	NPB	0.8672	0.5912	-0.0957	0.0229	0.6919
Leaf size	LS	28.1515	<.0001*	-0.1876	0.3102	0.1651
Leaflet length	LLL	61.9905	<.0001*	0.3042	0.2173	0.0807
Leaflet width	LLW	1.3038	0.2491	0.0877	-0.1674	0.5727
Pod-bearing position	PBP	2.1713	0.0291*	0.0218	-0.0120	0.1222
Pod-bearing length	PBL	23.0454	<.0001*	0.3178	-0.1942	-0.0347
No. of pods per plant	NPP	15.9580	<.0001*	-0.2298	0.2806	0.0559
Beak length of pod	BLP	18.6165	0.0508	0.3168	-0.1964	-0.0534
Pod length	PL	9.6813	<.0001*	-0.3578	0.0014	0.0396
Pod width	PW	3.4997	0.0010*	-0.2306	-0.2051	0.1966
No. of seeds per pod	NSP	37.8573	<.0001*	-0.0624	0.3716	0.0122
Seed size (diameter)	SS	3.3226	0.0016*	0.1273	-0.3078	0.1397
1000 seed weight	100SW	13.2197	<.0001*	0.1544	-0.3250	0.1686
Yield per genotype	YG	4.3312	0.0001*	0.0106	0.3298	0.1763

F – ANOVA *F*-ratio; *significance level equal to the 0.05 of probability; in bold eigenvectors > 0.25.

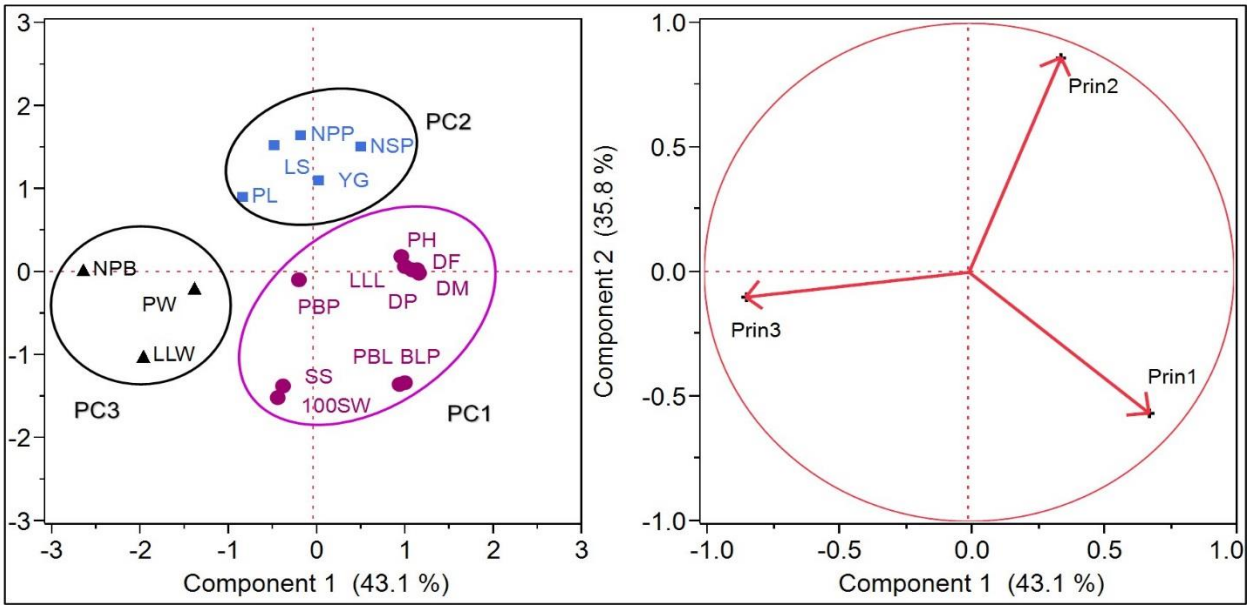

Figure 3. Dimensional relationships among agro-morphological traits revealed by principal component analyses

Significant positive correlation was found among DM, DP, DF, PH, LLL traits (coefficient of correlation r range from 0.81 to 0.98). PL trait showed a substantially negative weight on PC1 (eigenvector = -0.3578) and negative correlation (r range from -0.66 to -0.78). The PC2, that explained 35.8% of the variation, was positively related to LS, NPP, NSP and YG traits. The correlation among these traits range from 0.45 to 0.72. In this study PH trait account for PC2 the part of variance that was not account for in PC1. Traits as DM, DP, DF, LS, and LLL showed significant differences but their contribution on PC2 variance was not high. Seed size (SS) and 100SW traits showed positive correlation with LLW, PBL and BLP (r range from 0.52 to 0.75). The third component (PC3) was positively related to NPB, LLW, LS, PW, 100SW and YG characters (Tab. 2). Yield (YG) is correlated positively with DF, DP, DM, and PH, LS, LLL and NSP traits (r range from 0.45 to 0.88). The significant correlations among quantitative morphological traits can be useful for breeders to use, and to set up the grass pea ideotype.

Genetic similarity/distances assessed by morphological data: Genetic similarity/dissimilarity evaluated by combination of quantitative agro-morphological traits using cluster analysis method showed the presence of similarity/distances between grass pea landraces GB0057, GB0712, GB1131, and GB1326 collected in Korca areas and GB1421 collected in Elbasan (included in PC1) (Fig. 2). Presence of similarity was also found among grass pea landraces GB0011, GB0022 and GB0046 collected in Korca (included in PC2). The coefficient of genetic similarity ranged from 0.47 to 0.93, indicating that a high level of genetic diversity existed among the 14 grass pea landraces collected in three different areas of Albania. Highest similarity was found between GB0011 and GB0022 (coefficient of similarity 0.93), and minimal similarity or maximal distance (11.468524505) was found among accession GB0011 collected in Korca and GB1629 collected in Elbasan areas. The higher estimated genetic distance (diversity) could be ascribed to differences between grass pea landraces of different origin. This diversity can be utilized for genetic improvement without losing genetic diversity in grass pea.

Genetic similarity/distances assessed by RAPD data: Genetic similarity assessed by RAPD markers, using the Jaccard index of similarity (Nei 1978; Reif et al. 2005) show the presence of similarity and differences between local grass pea landraces. In comparison with morphological analysis the cluster analysis based on molecular data generates a dendrogram with higher number of clusters (Fig. 4). Study results show presence of similarity among GB0057 and GB0712 (cluster I), and between GB0011 and GB0022 (cluster II). In this study high genetic diversity was found between the landraces collected in Korca with grass pea landraces collected in Elbasan and Fieri areas. Granati et al. (2003); Chaudhary et al. (2003) reported that morphological, biochemical and molecular markers revealed a large genetic diversity among accessions collected in distant geographic areas. Grass peas collected in Elbasan and Fieri areas (relatively nearest areas) showed less genetic diversity among them (cluster III).

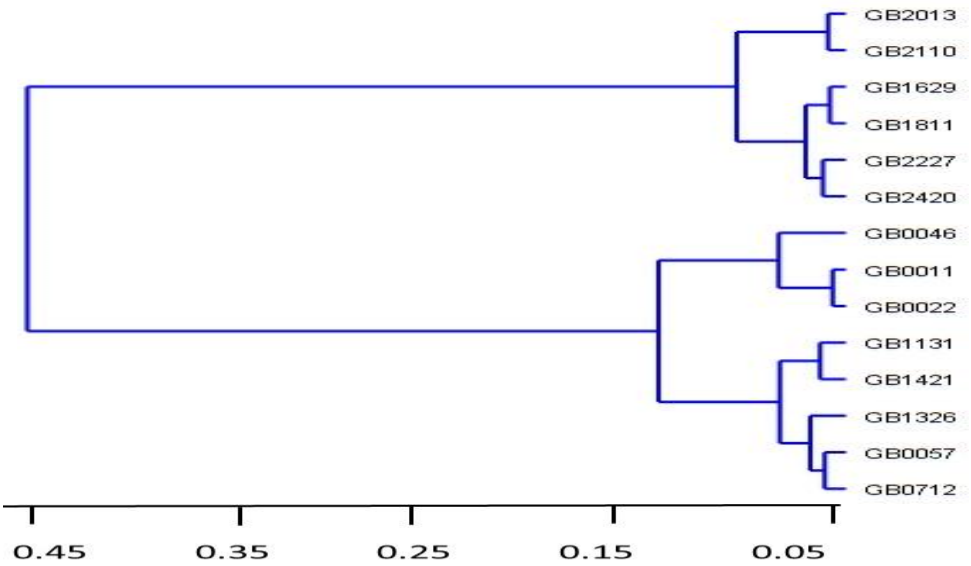

Figure 4. Dendrogram constructed on the basis of RAPD data in 14 genotypes of grass pea

The genetic similarity coefficient among 14 local grass pea accessions evaluated by RAPD markers varied from 0.05 to 0.45 indicating high amount of genetic diversity existed among the 14 grass pea genotypes (Fig. 4). Coefficient of correlation for molecular markers (0.72) was higher than coefficient of correlation estimated by conventional morphological methods (0.63). Similar studies reported genetic distances from 0.0 to 0.66 (Tar´an et al. 2005), from 0.24 to 0.84 (Cupic et al. 2009), from 0.05 to 0.48 (Ford et al. 2002).

In this study the relationship between morphological and molecular analysis was 58%. Observed differences between molecular and morphological methods were attributed to sampling errors and probably to difficulties in obtaining and inaccurate field morphological data. Lucia et al. (2010) reported a low correspondence between the data obtained using agronomic traits or molecular markers, and Baranger et al. (2004); Simioniuc et al. (2002); Hoey et al. (1996); Tar´an et al. (2005) reported low to medium correlations among molecular and morphological data. The grass pea landraces evaluated here, were mainly constituted of a mix large seeds (GB0011, GB022, GB046 and GB1811) and of small seeds (all other grass pea genotypes). The grass pea landraces GB057, GB1131 and GB1421 were the genotypes with highest seed yield. According to Qayyum et al., (2001), Ben Brahim et al., (2001) and Hanbury et al. (2000) the high yield and large seed genotypes can be used to select low toxin varieties.

Good understanding of the most important agro-morphological traits can facilitate identification of any individual landraces and selection of desirable traits (genes), increasing the information of grass pea germplasm. The traits with more significant weighting on respective PC variance can be utilised successfully as morphological quantitative markers for evaluation and characterization of the grass pea germplasm. Results of this study, congruent with (Yunus et al., 1991; Infantino et al., 1994; Granati et al., 2003; Lioi et al., 2011), suggests considerable level of variability is available to the breeders for sustainable field grass pea breeding programs.

CONCLUSION

The field trials and factorial analysis permitted the evaluation of genetic variability of 14 grass pea landraces and identification of the most important agro-morphological traits with potential for sustainable the future grass pea breeding programs.

The traits with more significant weighting on PC1 variance (DM, DP, DF, PH and LLL) and on PC2 variance (LS, NPP, NSP and YG) can be utilised successfully as morphological markers for evaluation and characterization of the grass pea germplasm.

The significant correlations among quantitative morphological traits (DM, DP, DF, PH, LLL with r from 0.81 to 0.98, and LS, NPP, NSP, YG with r from 0.45 to 0.72) can be useful for breeders to use, among the most correlated traits, the easier to record, select and to set up high yield and low toxin grass pea varieties.

The high amount of genetic variability founded in the present study suggest the collected grass pea landraces have considerable level of variability available to the breeders and it could be sufficient for the creation of new favourable gene combinations to sustain field grass pea breeding programs.

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