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The Qualitative Differences for Photosynthetic Content of Common Bean (*Phaseolus vulgaris* L.) Populations in Kosovo

Sali ALIU[1]*, Imer RUSINOVCI[1], Shukri FETAHU[1], Bekim GASHI[2]

[1]*University of Prishtina, Faculty of Agriculture, Department of Crop Science, Prishtina, Kosovo; sali.aliu@uni-pr.edu (*corresponding author)*
[2]*University of Prishtina, Faculty of Natural Science, Department of Biology, Prishtina, Kosovo*

Abstract

Genetic diversity analysis of common bean populations is useful for breeding programs, as it helps to select genetic material to be used for further crossings. Twenty (20) common bean populations were analyzed using qualitative traits, chlorophyll "a" (Chl *'a'*), chlorophyll "b" (Chl *'b'),* total chlorophyll "*a+b*" (Total *Chl)* and carotenoides. The design of the experiment was conducted with leaves of common bean collected from different regions of Kosovo. The experiment was completely randomly with four repetitions. Pigments were extracted by grinding 80-100 mg freshly sampled leaves in 80% (v/v) acetone/water containing $MgCO_3$, at room temperature, preserved in the dark for 24 hours. Concentration of chlorophyll and carotenoid content was measured by spectrophotometer using absorbance recorded at 663 nm, 644 nm and 452.3 nm for maximum absorption of Chl *'a'*, Chl *'b'*, and carotenoids respectively. According to our data the differences between populations for Chl *'a'*, and Chl *'b'* was significantly higher at level of probability LSDp=0.01. The average values for Chl *'a'*, was 1.67 mgg^{-1}, while for Chl *'b'* was 0.74 mgg^{-1}. In addition, the results for carotenoids content between populations were with high differences.

Keywords: bean, carotenoid, chlorophyll, pigments, variation

Introduction

Legumes occupy an important place in human nutrition, being a good source of proteins, dietary fibber, starch, minerals and vitamins. They are characterized by global adaptability, high genotypic and phenotypic diversity, and are used in a wide variety of menus. Among pulses, common bean (*Phaseolus vulgaris* L.) (Lioi and Piergiovanni, 2013) is a worldwide valued crop as both grain legume and fresh vegetable. Dry bean is an important source of protein, participating in human diet all over the world (Singh, 2001). The species *Phaseolus vulgaris L.* has two centers of origin, Mesoamerican and Andean (Singh *et al.,* 1991). Much of the genetic variability of this species in the world has been maintained and conserved ex situ, outside its centers of origin, in gene banks (Borda, 2011). In Kosovo, common bean is cultivated on 7505 ha, with an average yield of 0.9 t/ha, with annual consumption of 11.53 kg per capita. Cultivation of bean for a long time in different agro ecological conditions has created high diversity and variability. The traditional landraces are an important genetic resource for plant breeders, because of their considerable genotypic variation; this variability is maintained by farmers' selection for specific traits (Aliu *et al.*, 2011; Fetahu *et al.* 2010). A great success of developing high yielding of different genotypes, including common bean, has been achieved through breeding programs. Improvement of grain quality is a major objective of most breeding programs (Aliu and Fetahu, 2010.) The photosynthetic process involves CO_2 fixation and consequent production of carbohydrates and O_2 delivery, using an intermediary light energy. Higher plants are classified as C3, C4 and CAM metabolism in relation to CO_2 fixation (Bandyopadhyay *et al.,* 2007). *Phaseolus vulgaris* is an example of a C3 species (Santos *et al.,* 2009), because CO_2 is catalysed into 3-phosphoglyglycerate by the enzyme RUBISCO (ribulose-1,5 bisphosphate carboxylase/ oxygenase) (Furbank and Taylor, 1995). The analysis of chlorophyll *a* fluorescence is appreciated as being a widely used method in research of photosynthetic efficiency in higher plants (Maxwell and Jonson, 2000). The production of dry matter by pasture and crop species has been demonstrated to be ultimately limited by the amount of chlorophyll (Chl) due to the strong relationship of this pigment and the photosynthetic processes (Ciganda *et al.,* 2008). The main objective of this work was to characterize the chlorophyll content of different bean populations according to their geographical origin in Kosovo.

Tab. 1. Geographical origin of different common bean populations

Code	Genus	Longitude	Latitude	Elevation	Locality
PMF-01	*Phaseolus spp.*	21°24'21"	43°19'53"	557	Cernic-Gjilan
PMF-02	*Phaseolus spp.*	21°33'49"	42°26'03"	454	Pogragj-Gjilan
PMF-03	*Phaseolus spp.*	21°02'13"	42°32'38"	549	Dobraj e madhe-Lipjan
PMF-04	*Phaseolus spp.*	21°05'54"	42°30'31"	572	Hallaq i madh-Lipjan
PMF-05	*Phaseolus spp.*	21°05'37"	42°51'27"	633	Lupq i eperm-Podujevë
PMF-06	*Phaseolus spp.*	21°10'18"	42°50'27"	575	Penuh-Podujevë
PMF-07	*Phaseolus spp.*	20°53'34"	42°42'44"	652	Qirez-Skenderaj
PMF-08	*Phaseolus spp.*	20°46'30"	42°43'42"	603	Llaushë-Skenderaj
PMF-09	*Phaseolus spp.*	20°58'01"	42°43'29"	534	Dervar-Vushtrri
PMF-10	*Phaseolus spp.*	20°58'11"	42°45'04"	529	Mihaliq-Vushtrri
PMF-11	*Phaseolus spp.*	20°43'59"	42°16'49"	356	Velezh-Prizren
PMF-12	*Phaseolus spp.*	20°42'29"	42°14'26"	372	Atmaxh-Prizren
PMF-13	*Phaseolus spp.*	20°43'02"	42°07'56"	1141	Zgatar-Dragash
PMF-14	*Phaseolus spp.*	20°45'40"	42°05'31"	1000	Bellobrad-Dragash
PMF-15	*Phaseolus spp.*	20°46'08"	42°05'06"	351	Studenqan-Suhareke
PMF-16	*Phaseolus spp.*	20°49'48"	42°20'25"	415	Sopi-Suharek
PMF-17	*Phaseolus spp.*	20°50'34"	42°20'36"	564	Carabreg-Deqan
PMF-18	*Phaseolus spp.*	20°16'38"	42°33'24"	581	Isniq-Deqan
PMF-19	*Phaseolus spp.*	20°18'09"	42°33'26"	490	Vitomiric-Pejë
PMF-20	*Phaseolus spp.*	20°18'41"	42°37'01"	487	Raushiq-Pejë

Materials and methods

Twenty (20) common bean populations were collected from various agro-climatic regions of Kosovo, thus representing different geographical origin (Tab. 1).

Methods of pigment extraction

Pigments were extracted by grinding 80-100 mg of freshly sampled leaves in 80% (v/v) acetone/water containing $MgCO_3$ (0.5% w/v) at room temperature, kept for 24 h in the dark. Photosynthetic pigments of all samples were extracted in triplicate to minimize experimental errors. Concentration of chlorophyll and carotenoid contents were measured by using absorbance recorded at 663 nm, 644 nm and 452.5 nm for maximum absorption of chlorophyll "*a*" (Chl '*a*'), chlorophyll "*b*" (Chl '*b*') and carotenoids, respectively. The extinction coefficients were determined by a UV-Vis spectrophotometer. Pigment contents were calculated in mg·g−1 dry leaf weight (DW) by applying the absorption coefficient equations described by Lichtenthaler (1986), Aliu *et al.* (2013), Gashi *et al.* (2012).

$$Chl'a'(mg.g-1DW)=\frac{[10.3(OD663)-0.918(OD644)]\times V\times 100}{FW\times DW}$$

$$Chl'b'(mg.g-1DW)=\frac{[19.7(OD663)-3.87(OD663)]\times V\times 100}{FW\times DW}$$

$$Carotenoids(mg.g-1DW)=\frac{[4.75(OD452.5)-0.226(Chl'a'+Chl'b')]\times V\times 100}{FW\times DW}$$

where: DW- Dry leaf weight,
FW- Fresh leaf weight,
OD- Optical density,
V- Volume of sample.

Data analyses

The experiment was performed in a randomized design with five (5) replicates. Differences among pigments were tested using SPSS 19 and MINITAB-14 statistical program. Mean separation within traits are done by Duncan's Multiple Range test. Pearson's correlation coefficients were computed to express the relationship among characteristics. Mean values of the physiological traits for local bean populations were standardized and used for computing Euclidean distances between them.

Results and discussion

Significant differences among the Local Bean Populations (LBP's) for chlorophyll and carotenoids content are given in Tab. 2. Statistical analysis showed significant differences among the studied LBP's for physiological parameters. The results of chlorophyll *a* in our study showed that local bean populations had a large variation among genotypes. The LBP's (PMF-14) produced maximum Chl '*a*' on value 2.24 $mg.g^{-1}$. The lowest Chl '*a*' (0.82 $mg.g^{-1}$) was determined for the PMF-01. The differences between these LBP's were + 1.42 $mg.g^{-1}$, or with genetic variation was 85.02% (Tab. 2).

The twenty (20) LBP's of the experiment revealed significant differences among genotypes for Chl '*b*' (Tab. 2). The overall average value for Chl '*b*' in our study was 0.742 $mg.g^{-1}$. The LBP's (PMF-12) had the highest content of Chl '*b*' (0.91 $mg.g^{-1}$) which was significantly higher from other populations, while the other LBP's (PMF-02) had the lowest content of Chl '*b*' (0.41 $mg.g^{-1}$). The difference between them was +0.50 $mg.g^{-1}$ or 67.38% with genetic variation. Lobato *et al.*, (2009) reported different results of photosynthetic pigment content in some different bean populations, with higher genetic variation. For total chlorophyll (Chl '*a+b*') the highest Chl '*a+b*' (3.05 $mg.g^{-1}$) was recorded for the PMF-14, while the lowest Chl '*a+b*' was recorded for the PMF-01 (1.57 $mg.g^{-1}$). The difference between them was +1.48 $mg.g^{-1}$ or 61.15% (Tab. 2). The highest carotenoids concentrations was at LBP's coded as PMF-14 (0.88 $mg{\cdot}g^{-1}$), whereas the lowest was recorded at LBP's 04 on value 0.43 (1.26 $mg{\cdot}g^{-1}$). The differences between LBP's were +0.45 $mg{\cdot}g^{-1}$ or with genetic variation 70.31%. Results are presented in Tab. 2.

Tab. 2. The chlorophyll (photosynthetic) pigment content in local bean populations

Code	Chl 'a'	Chl 'b'	Chl 'a+b'	Carotenoids
PMF-01	0.82^{d}	0.74^{abc}	1.57^{d}	0.56^{bcde}
PMF-02	1.24^{cd}	0.41^{c}	1.66^{cd}	0.48^{cde}
PMF-03	1.47^{bcd}	0.57^{abc}	2.05^{abcd}	0.54^{cde}
PMF-04	1.69^{abc}	0.83^{ab}	2.53^{abcd}	0.43^{d}
PMF-05	1.81^{abc}	0.71^{abc}	2.53^{abcd}	0.63^{abcde}
PMF-06	2.18^{ab}	0.82^{ab}	3.01^{a}	0.82^{ab}
PMF-07	1.19^{cd}	0.65^{abc}	1.83^{bcd}	0.49^{cde}
PMF-08	1.55^{abcd}	0.75^{abc}	2.30^{abcd}	0.66^{abcde}
PMF-09	1.85^{abc}	0.88^{a}	2.73^{ab}	0.69^{abcde}
PMF-10	1.76^{abc}	0.84^{a}	2.61^{abc}	0.66^{abcde}
PMF-11	1.76^{abc}	0.67^{abc}	2.44^{abcd}	0.70^{abcde}
PMF-12	1.94^{abc}	0.91^{a}	2.85^{ab}	0.76^{abc}
PMF-13	1.93^{abc}	0.85^{a}	2.78^{ab}	0.84^{ab}
PMF-14	2.24^{a}	0.81^{ab}	3.05^{a}	0.88^{a}
PMF-15	2.17^{ab}	0.80^{ab}	2.97^{a}	0.84^{a}
PMF-16	1.93^{abc}	0.85^{a}	2.78^{ab}	0.71^{abcd}
PMF-17	1.35^{cd}	0.85^{a}	2.25^{abcd}	0.53^{cde}
PMF-18	1.44^{bcd}	0.841^{ab}	2.25^{abcd}	0.51^{cde}
PMF-19	1.21^{cd}	0.46^{bc}	1.68^{cd}	0.45^{de}
PMF-20	1.92^{abc}	0.61^{abc}	2.53^{abcd}	0.72^{abcd}
Average	1.672	0.7425	2.42	0.645

*Means followed by the same letter are not significantly different at p=0.05 according to Ducan's multiple range test.

The knowledge of correlation coefficients between different traits helps the breeder to find out the nature and magnitude of the association between particular traits which are mostly used to attain better yield of the crops. Values of Pearson's correlation coefficient estimated for all pairs of studied characteristics are presented in Tab. 3.

Tab. 3. Pearson's correlation among the investigated parameters

Traits	Chl 'a'	Chl 'b'	Chl 'a+b'	Carotenoids
Chl 'a'	1	0.527^{**}	0.959^{**}	0.837^{**}
Chl 'b'	0.527^{**}	1	0.746^{**}	0.352^{**}
Chl 'a+b'	0.959^{**}	0.746^{**}	1	0.773^{**}
Carotenoids	0.837^{**}	0.352^{**}	0.773^{**}	1

*0.05 significant, ** 0.01 significant

The results showed that significant and positive correlation coefficients were found between Chl *'a'* and Chl *'b'* (r=0.52**), Chl *'a'* and carotenoids (r=0.983*). Also, Chl *'b'* with all traits showed higher significant correlations.

The cluster analysis reported here differentiates between local bean populations on the basis of their similarity (Fig. 1). The 20 local bean populations were classified into two main groups. The first group had only one population, with number PFM-01, which is different from the rest of the analysed populations. The second group is the largest, with different subgroups and at the same time different from the first group for physiological traits.

Conclusions

The results of the analysis of variance for physiological parameters of local bean populations confirmed the existence of a significant variability of the characteristics,

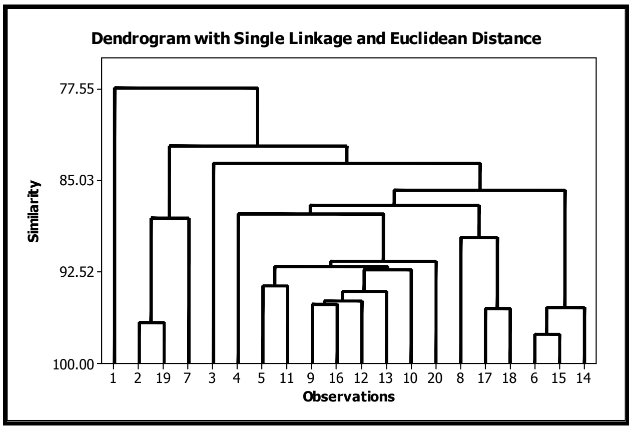

Fig. 1.Genetic grouping of local bean populations by cluster analysis

depending on the base of inheritance and the effect of ecological factors, in this case the sea level (elevation). The field and laboratory investigations suggest that some of the local bean populations studied represent a highly physiological resource, as valuable genetic material that could be successively used for further breeding programs and can be a good base for the development of a pure line.

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