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Evaluation of Phenotypic Diversity in Common Bean (*Phaseolus vulgaris* L.) Using Specific Descriptors

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ABSTRACT

Normally, the bean (*Phaseolus vulgaris* L.) is considered a self-pollinated plant. The aim of this study was to present the effects of cross-pollination within a bean germplasm collection (over 450 accessions) in climatic conditions from South-eastern part of Romania. This paper presents the study of two climbing bean accessions seeds sown. For each accession sown, there were taken into study nine new biotypes harvested. The seeds sown represented the control variant for the analysis of the harvested biotypes. The seeds were evaluated in what it concerns quantitative (100 seed weight, length, thickness and width) and qualitative characteristics (shape, main and secondar seed color, shape of secondar color, intensity and color of hilum ring). The mean weight of 100 seeds at V_1 was 39.41 g (higher value than the control variant - 38.82 g) and at V_2 the mean weight (43.81 g) was smaller than control variant (46.95 g). The coefficient of variability for this parameter had a medium value (V_1 11.42% and V_2 16.72%), the other analyzed parameters had a small variability. All maximum values at V_2 were recorded at 2.1. (weight - 57.67 g, length - 16.30 mm, width - 9.25 mm and thickness - 6.41 mm) and the minimum at 2.5. (weight - 31.15 g, length - 12.39 mm, width - 7.41 mm and thickness - 5.30 mm). The main colors of the control seeds were brown (V_1 - medium and V_2 - dark). At V_1 there were white seeds (1.5.), and at V_2 khaki (2.9.). In 2020, a high percentage of allogamy was found in cultivated accessions.

Keywords: *Phaseolus vulgaris* L., germplasm collection, qualitative characteristics, quantitative characteristics.

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is one of the most important legume crops in all continents in the world except Antarctica, because of its high protein, fiber, and complex carbohydrate content. Beans (*Phaseolus* spp.) are extremely diverse crops in terms of cultivation, methods, in diverse environments and elevations ranging from sea level to 3.000 m height, morphological variability, and utilities (dry as pulse and green as vegetable). The tremendous variability for plant types and growth behavior makes beans part of the most diverse production systems of the world (Pathania et al., 2014).

In South and South-East Romania there are favorable conditions for common bean production, as well as a great tradition in cultivation (Ruşti and Munteanu, 2008). Collecting and conserving biodiversity, in what it concerns bean, represents one of the main activity objectives in any breeding program (Nechifor et al., 2011; Szilagyi et al., 2011). Thus, in 2010 Vegetable Research and Development Station (VRDS) Buzău, Romania started the collecting of local bean populations from the main vegetable areas in Romania. Bean germplasm collection of this station contains now over 450 accessions. Similar researches were made in Romania by Rădulescu (1940), Munteanu (1985), Stan et al. (1993, 1995), Leonte et al. (2004), Riviș and Nedelea (2008) Giurcă and Murariu (2009), Danci et al. (2010) and Madoșa et al. (2010, 2011).

In Romania, normally, the bean (*Phaseolus vulgaris* L.) is considered a self-pollinated plant. Ali et al. (2020) also has the same opinion. Olaru (1982) and Ciofu et al. (2013) agree with Ali et al. (2020), but they do not exclude cross-pollination. In a normal crop Bliss (1980) and Ruști and Munteanu (2008) estimate that bean is highly self-pollinated, and only 0.2-0.5% cross-pollinated. In Puerto Rico, Vakili (1976), quoted by Bliss (1980), reports that carpenter bees (*Xylocopa*

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brasilianorum L.) were responsible for levels as high as 15 to 20% cross-pollination.

Over time, in what it concerns some accessions, the phenotypic instability phenomenon has been more intensive because of some particularities concerning flower morphology (Debouck and Hidalgo, 1986).

Studying flower aspect at accessions that presented a high variability percent there was observed that stigma and anthers were no longer protected by the keel. Under the pressure of different insects (especially bees) the stigma get out of the keel and have contact with the full of pollen bees feet from another flower or it comes off and leaves free the stigma and the anthers. The style turning over inside the keel is foreclosed by the brush of hairs near the stigma and also because of the style scrolling degree. At the proveniences that present such flower morphology, the pollination is allogamy and can be made by insects - entomophily and by wind - anemophily (Teodorescu, 2016).

The aim of this paper was to present the effects of cross-pollination within a bean germplasm collection in climatic conditions from South-eastern part of Romania.

MATERIAL AND METHODS

The study was carried out in Buzău, Romania (45°9′N and 26°49′E) in 2020. The biological material (climbing accessions) was cultivated according to the common production technology recommended for this area by the specialty literature (Munteanu et al., 1989; Ciofu et al., 2003; Ruşti and Munteanu, 2008). The determination of plants, pods and seeds characteristics was made according to U.P.O.V. guideline (2015), C.P.V.O. protocol (2009) and color scale (Genchev and Kiryakov, 2005).

Therefore, the experimental variants were the following:

V_1				**V_2**			
1 Mt	47 BBA	1.5.	47 BBAH	2 Mt	79 BB	2.5.	79 BBH
1.1.	47 BBAC	1.6.	47 BBAJ	2.1.	79 BBB	2.6.	79 BBI
1.2.	47 BBAD	1.7.	47 BBAK	2.2.	79 BBD	2.7.	79 BBJ
1.3.	47 BBAE	1.8.	47 BBAM	2.3.	79 BBE	2.8.	79 BBK
1.4.	47 BBAG	1.9.	47 BBAN	2.4.	79 BBF	2.9.	79 BBM

For characterization, there were used 10 seeds from each accession, according to *Handbook on evaluation of Phaseolus germplasm* (De la Cuadra et al., 2001).

Length determination was made in mm measured in parallel with the hilum, width was measured from the hilum to the opposite side and thickness was measured perpendicular on width in cross section (Figure 1).

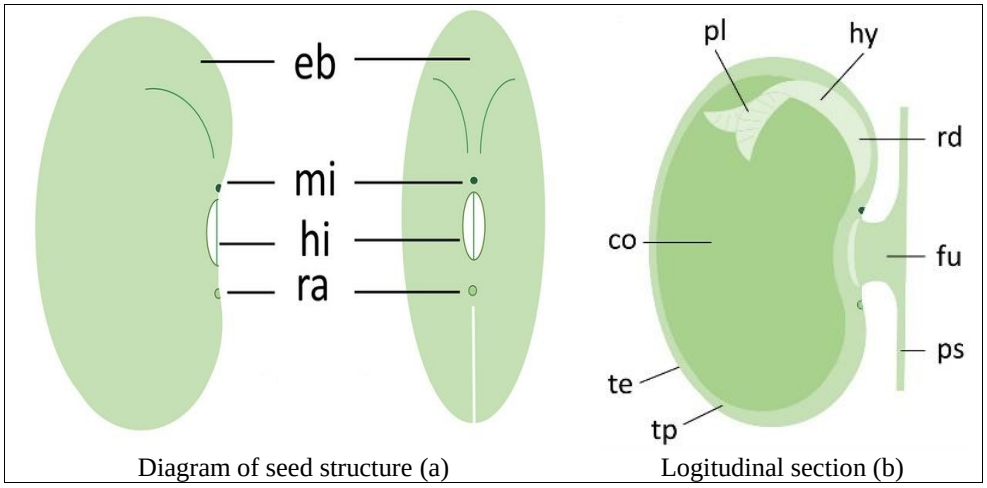

Figure 1. Seed structure of a dicotyledon **(a)**: eb - embryo bulge; mi - micropyle; hi - hilum; ra - raphe; **(b)**: pl - plumule; hy - hypocotyl; rd - radicle; co - cotyledon; fu - funicle; ps - pod; te - testa; tp - testa perenchyma (Chen et al., 2021)

A more detailed overview of the plumule - radicle complex includes epicotyl and primary leaves (Debouck and Hidalgo, 1986).

Rădulescu (1940) quoted by Olaru (1982) pays particular attention to the coloring area around hilum, otherwise the hilum is always white, the edge surrounding the hilum may have a different coloration with various intensity (absent, weak, medium and strong) to the rest of testa and also it is interrupted at the micropyle and caruncle. Caruncle (raphe) represents a heart - shaped lift having the tip facing the end of the seed. The stripe of the caruncle represents a narrow band which leaves from the top of caruncle, it extends to the end of the seed - were it ends like a swallow`s tail, and it has a variable length (Figure 2).

Figure 2. Aspects of the embryo bulge, micropyle, hilum, caruncle and the stripe of the caruncle (Teodorescu, 2024)

According to Descriptor for *Phaseolus vulgaris* (IBPGR, 1982), van Schoonhoven and Pastor-Corrales (1994) and Debouck (2009) 100 seeds mass were randomly chosen for measuring seed mass (small - less than 25 g, medium - 25 to 40 g and large - more than 40 g).

In order to determine seeds weight there was used an electronic balance reading to 0.0001 g (Partner WAS220/x).

The experimental design was a randomized block with 3 replicates for each variant. The seeds sown represented the control variant for the analysis of the harvested biotypes. In order to analyze the results analysis of variance and multiple comparison method Duncan's test were used (Harter, 1960; Săulescu and Săulescu, 1966).

There was used coefficient of variation (CV) because it allows the direct comparison of data sets variation (Ireland, 2010). The coefficient of variation in a single sample with observations is defined as CV=s/*m*, where *m* is mean and *s* is standard deviation (Forkman, 2009). Variability appreciation according to the CV values (Munteanu and Fălticeanu, 2008; Giurcă and Murariu, 2009) was made this way: low variability (CV<10%), mean (CV=10-20%) and high (CV>20%).

RESULTS AND DISCUSSION

Most of the accessions cultivated presented a high degree of instability. The appearance of a large number of biotypes represents the result of the presence of numerous insects in the crop (Figure 3).

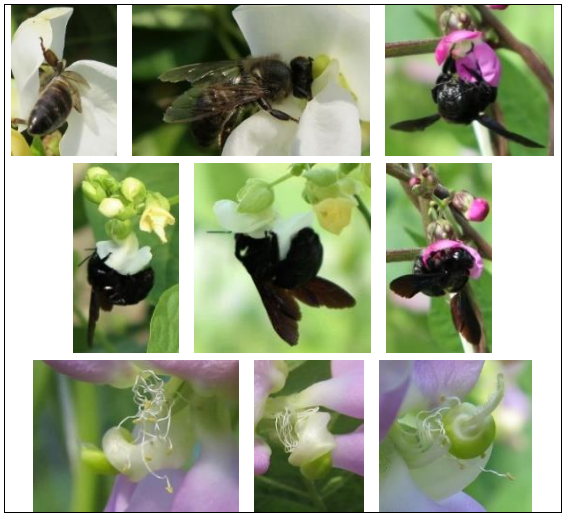

Figure 3. Insects present in the crop and flowers after their visit (Teodorescu, 2024)

This paper presents quantitative and qualitative characteristics of seeds harvested to 9 biotypes in comparation with seeds sown.

Seeds weight varied at V_1 from 32.25 g (1.8.) to 48.52 g (1.6.) with a mean value 39.41 g and a CV medium (11.42%). Seeds length registered the maximum value at 1.4. (13.977 mm) and the minimum value at 1.8. (11.47 mm) with a mean value 12.68 mm. Except weight, the CV in what it concerns all quantitative characteristics had a value between 6.19% (length) and 9.86% (length/width); this shows a low variability. The largest width (8.8 mm) and smallest thickness (5.24 mm) from V_1 were found at 1.1. (Table 1).

Table 1. Main quantitative characteristics of the seeds V_1

V_1	100 seeds mass (g)	Seeds dimensions (mm)			Length/ Width	Width/ Thickness
		Length	Width	Thickness		
1 Mt	38.82 cd*	13.32 abc	8.27 b	5.96 ab	1.61	1.39
1.1.	38.73 bc	13.56 abc	8.82 a	5.24 c	1.54	1.68
1.2.	36.24 cd	12.82 cde	7.78 cd	5.93 ab	1.65	1.31
1.3.	41.45 bc	13.68 ab	8.76 a	5.92 ab	1.56	1.48
1.4.	38.63 bc	13.97 a	7.08 e	5.39 bc	1.97	1.31
1.5.	37.21 cd	12.17 def	7.61 d	5.80 bc	1.60	1.31
1.6.	48.52 a	12.53 de	8.82 a	6.51 a	1.42	1.36
1.7.	43.19 b	12.11 ef	8.44 ab	6.47 a	1.44	1.30
1.8.	32.25 d	11.47 f	7.75 cd	5.98 ab	1.48	1.30
1.9.	41.53 bc	12.93 bcd	8.14 bc	6.46 a	1.59	1.26
Mean	**39.41**	**12.86**	**8.15**	**5.97**	**1.59**	**1.37**
Stand. var.	4.50	0.80	0.59	0.43	0.16	0.13
CV%	**11.42**	**6.19**	**7.21**	**7.25**	**9.86**	**9.21**
Max	48.52	13.97	8.82	6.51	1.97	1.68
Min	32.25	11.47	7.08	5.24	1.42	1.26
	LSD 5% = 4.7465 LSD 1% = 6.5095 LSD 0.1% = 8.8601	LSD 5% = 0.7195 LSD 1% = 0.9868 LSD 0.1% =1.3431	LSD 5% = 0.4251 LSD 1% = 0.5829 LSD 0.1% = 0.7935	LSD 5% = 0.5372 LSD 1% = 0.7367 LSD 0.1% = 1.0028		

* Within a column, the means followed by the same letter are not significantly different (Duncan test, α=0.05)

Before harvesting, the pods from V_1 were different (Figure 4a). During harvesting it was found that the funicle had various colors (Figure 4b and 4c), pod's degree of curvature was different (Figure 4d and 4e) and a dehiscent pod had two types of seeds inside (Figure 4f).

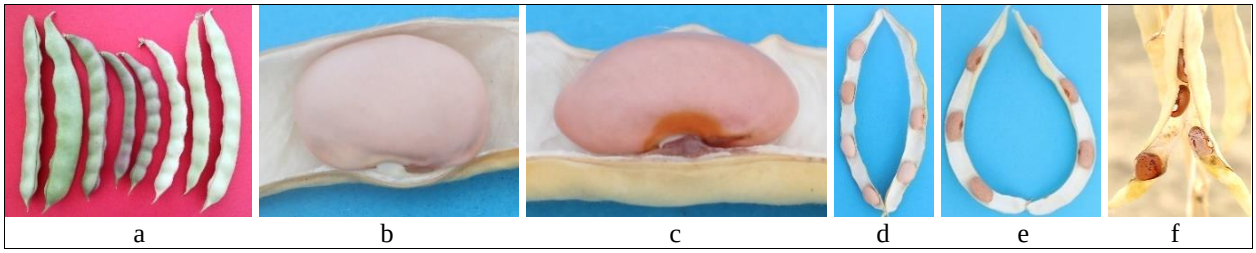

Figure 4. Different aspects from V_1: a - immature pods; b - white funicle at 1.1.; c - brown funicle at 1.2.; d - weak to medium degree of curvature at 1.1.; e - strong to very strong degree of curvature at 1.4. and f - a pod with two types of seeds

Most of biotypes harvested from V_1 (Figure 5) had the same shape with 1Mt (circular to elliptic), except 1.4. (kidney-shaped with a small degree of curvature) and 1.8. (rectangular).

Figure 5. Aspects of qualitative characteristics of the seeds V_1

According to U.P.O.V. guideline (2015), C.P.V.O. protocol (2009) and color scale (Genchev and Kiryakov, 2005), at accessions with kidney - shaped seeds shape (Table 2), it is recommended to evaluate also degree of curvature (weak, medium or strong). A similar color to that of 1Mt (brown) it is found in four variants (light - 1.3., 1.8. and dark - 1.6., 1.9.), another four variants have various shades of beige as main color (1.1., 1.2., 1.4. and 1.7.). Among the shades of brown and beige, 1.5. has white as its main color. Only three biotypes show a single secondary color (1.4. - grey, 1.5. - brown and 1.6. - garnet) and 1.7. shows two secondary colors (grey and garnet) and a hilum ring strong colored in orange.

Table 2. Main qualitative characteristics of the seeds V_1

V_1	Seeds shape[1]	Color			Hilum ring	
		Main	Secondar	Shape of secondar color	Intensity	Color
1 Mt	circular to elliptic	brown	–	–	medium	D brown
1.1.	circular to elliptic	L beige	–	–	absent	–
1.2.	circular to elliptic	beige	–	–	medium	L red
1.3.	circular to elliptic	L brown	–	–	absent	–
1.4.	kidney-shaped w[2]	L beige	grey	patches	medium	L red
1.5.	circular to elliptic	white	brown	above the micropyle	absent	–
1.6.	circular to elliptic	D brown	garnet	streaks and patches	weak	brown
1.7.	circular to elliptic	L beige	grey garnet	patches patches and small streaks	strong	orange
1.8.	rectangular	L brown	–	–	strong	brown
1.9.	circular to elliptic	D brown	–	–	weak	brown

[1] Shape of median longitudinal section L = light D = dark
[2] Degree of curvature for kidney - shaped only: weak (w), medium (m) and strong (s).

All maximum values at V_2 (Table 3) were recorded at 2.1. (weight - 57.67 g, length - 16.30 mm, width - 9.25 mm and thickness - 6.41 mm) and the minimum at 2.5. (weight - 31.15 g, length - 12.39 mm, width - 7.41 mm and thickness - 5.30 mm). Variability was mean for weight (16.72%) and low for the other parameters studied (length - 8.14%, width - 6.89% and thickness - 6.34%).

Table 3. Main quantitative characteristics of the seeds V_2

V_2	100 seeds mass (g)	Seeds dimensions (mm)			Length/ Width	Width/ Thickness
		Length	Width	Thickness		
2 Mt	46.95 bc *	14.23 c	8.13 cd	5.88 bc	1.75	1.38
2.1.	57.67 a	16.13 a	9.25 a	6.41 a	1.74	1.44
2.2.	42.97 d	14.16 c	8.53 b	5.35 e	1.66	1.60
2.3.	45.63 bcd	14.28 c	8.23 bc	6.00 bc	1.74	1.37
2.4.	44.81 cd	13.73 cd	7.81 de	6.11 ab	1.76	1.28
2.5.	31.15 f	12.39 f	7.41 f	5.30 e	1.67	1.40
2.6.	38.17 e	13.40 de	8.54 b	5.48 de	1.57	1.56
2.7.	46.11 bcd	14.97 b	8.39 bc	5.72 cd	1.79	1.47
2.8.	48.40 b	15.43 b	9.03 a	5.91 bc	1.71	1.53
2.9.	36.22 e	12.79 ef	7.74 ef	5.43 de	1.65	1.43
Mean	**43.81**	**14.15**	**8.31**	**5.76**	**1.70**	**1.45**
Stand. var.	7.33	1.15	0.57	0.37	0.07	0.10
CV%	**16.72**	**8.14**	**6.89**	**6.34**	**3.82**	**6.61**
Max	57.67	16.13	9.25	6.41	1.79	1.60
Min	31.15	12.39	7.41	5.30	1.57	1.28
	LSD 5% = 3.2305 LSD 1% = 4.4303 LSD 0.1% = 6.0302	LSD 5% = 0.6510 LSD 1% = 0.8928 LSD 0.1% = 1.2151	LSD 5% = 0.3431 LSD 1% = 0.4705 LSD 0.1% = 0.6404	LSD 5% = 0.3371 LSD 1% = 0.4623 LSD 0.1% = 0.6292		

* Within a column, the means followed by the same letter are not significantly different (Duncan test, α=0.05)

Differences between control variant (2 Mt) and two biotypes (2.3. and 2.4.) were statistically insignificant.

All seeds from V_2 had a kidney - shaped shape, most showed a weak degree of curvature, except 2.1. (strong), 2.6. and 2.9. (medium). Brown (2 Mt and 2.2.), light brown (2.3., 2.5. and 2.6.), beige (2.4. and 2.8.) and light beige (2.1.) were the predominant colors in V_2. The other two variants (Table 4) had black (2.7.) and khaki (2.9.) as the main colors.

Table 4. Main qualitative characteristics of the seeds V_2

V_2	Seeds shape[1]	Color			Hilum ring	
		Main	Secondar	Shape of secondar color	Intensity	Color
2 Mt	kidney-shaped w[2]	brown	L brown black	fine patches patches and streaks	absent	–
2.1.	kidney-shaped s	L beige	grey black	fine patches patches and streaks	weak	beige
2.2.	kidney-shaped w	brown	L brown black	fine patches patches and streaks	medium	D brown
2.3.	kidney-shaped w	L brown	beige brown	fine patches patches	absent	–
2.4.	kidney-shaped w	beige	–	–	medium	D brown
2.5.	kidney-shaped w	L brown	–	–	medium	D brown
2.6.	kidney-shaped m	L brown	brown	streaks and patches	weak	D brown
2.7.	kidney-shaped w	black	beige	patches	absent	–
2.8.	kidney-shaped w	beige	black	streaks and patches	medium	D brown
2.9.	kidney-shaped m	kaki	–	–	absent	–

[1] Shape of median longitudinal section L = light D = dark

[2] Degree of curvature for kidney - shaped only: weak (w), medium (m) and strong (s).

Three variants (Figure 6) had no secondary color (2.4., 2.5. and 2.9.) and three other variants had only one secondary color (2.6. - brown, 2.7. - beige and 2.8. - black). Two secondary colors were observed at 2 Mt and 2.2. (light brown and black); 2.1. (grey and black) and 2.3. (beige and brown). A similarity was observed between 2 Mt and 2.2.

Figure 6. Aspects of qualitative characteristics of the seeds V_2

Quantitative, there is one significant difference (length - 0.74 mm) between 2 Mt and 2.7., but qualitatively they only have one thing in common: shape.

Differences in the anthocyanin coloration of the hypocotyl were observed from the early stage of plant development at V_2, then different types of pods appeared and, finally, different types of seeds were harvested (Figure 7).

Figure 7. Different aspects from V_2

CONCLUSIONS

Various intensities of anthocyanin coloration of the hypocotyl show that the sown seeds were uniform only from a phenotypic point of view.

Under these conditions, the percentage of cross-pollination determined by insects cannot be specified.

A complete phenotypic characterization of bean seeds must include their quantitative and qualitative evaluation.

All biotypes will be cultivated to determine the characteristics of the plant. Only those that meet the breeding objectives will be kept and introduced into germplasm collection.

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