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EVALUATION OF UNSTABLE PHENOTYPIC COMMON BEAN SEEDS: A CASE STUDY

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Abstract

In a normal crop, beans are self-pollinating. The aim of this study was to present the effects of cross-pollination within a bean germplasm collection (over 450 accessions) under climatic conditions from the south-eastern part of Romania. This paper presents the study of L 47 AA climbing bean accession seeds sown, and there were taken into study nine new biotypes harvested. The seeds represented the control variant for the analysis of the harvested biotypes. The seeds were evaluated in what it concerns quantitative characteristics (100 seeds weight, length, width, and thickness) and qualitative characteristics (shape, primary and secondary seed color, shape of the secondary color, intensity and color of the hilum ring). The mean weight of 100 seeds was 42.52 g (with a minimum value of 31.75 g and a maximum value of 55.92 g). At biotype 5 the maximum values of the quantitative characteristics were recorded (weight - 55.92 g, length - 14.93 mm, and thickness - 6.38 mm). A similar coloration to the control was found in biotype 9 (primary color brown, secondary color black in streaks), but white or cream colors were also observed.

Key words*: seeds, Phaseolus vulgaris L., germplasm collection, qualitative characteristics, quantitative characteristics.*

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most widely consumed legumes globally, valued for its high protein content, dietary fiber, and essential micronutrients like iron and zinc. As a staple food in many regions, particularly Africa, Latin America, and Asia, it is crucial for improving food security and addressing malnutrition (Fayaz, 2025).

In 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 for the Vegetable Research and Development Station (V.R.D.S.) Buzău.

Thus, in 2010 started the collection of local bean population from the main vegetable-growing areas of Romania. The bean germplasm collection of V.R.D.S. Buzău contains over 450 accessions.

Similar studies were conducted 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.

Normally, in Romania, the bean (*Phaseolus vulgaris* L.) is considered a self-pollinated plant. Ali et al. 2020 also share the same view. Olaru, 1982 and Ciofu et al., 2003 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, with only 0.2-0.5% cross-pollination. In Puerto Rico, Vakili (1976), quoted by Bliss (1980), reports that carpenter bees (*Xylocopa brasilianorum* L.) were responsible for levels as high as 15 to 20% cross-pollination.

In what it concerns some accessions, over time, the phenotypic instability phenomenon has been more intensive because of some particularities concerning flower (Figure 1) morphology (Debouck and Hidalgo, 1986).

Studying flower characteristics in accessions that presented a high variability, it was observed that the stigma and anthers were no longer protected by the keel.

Under the pressure of different insects (especially bees and thrips) the stigma get out of the keel and makes contact with the full of pollen bees' feet from another flower, or it detaches and leaves the stigma and the anthers free (Figure 2).

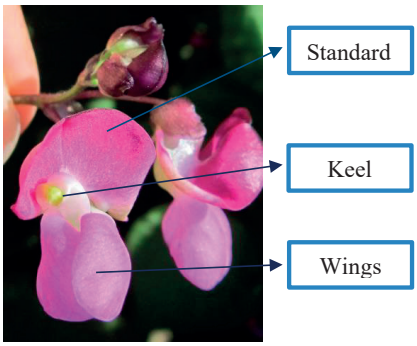


Figure 1. Bean flower morphology (about Teodorescu, 2016)

The turning over inside the keel is prevented by the brush of hairs near the stigma and also because of the style curvature.

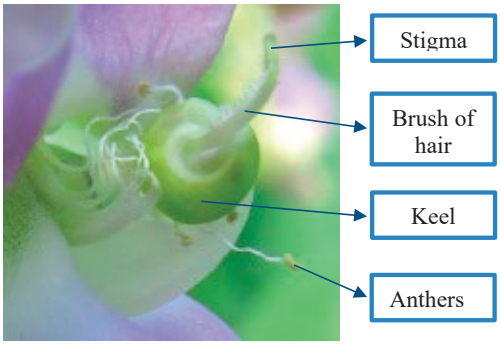


Figure 2. Stigma and anthers were no longer protected by the keel (about Teodorescu, 2016)

At the proveniences that present such flower morphology, 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 under climatic conditions from South - eastern part of Romania.

MATERIALS AND METHODS

The study was carried out in V. R. D. S. 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).

Therefore, the experimental variants were the following:

Mt - 47 AA	5 - 47 AAI
1 - 47 AAA	6 - 47 AAJ
2 - 47 AAB	7 - 47 AAK
3 - 47 AAE	8 - 47 AAL
4 - 47 AAF	9 - 47 AAM

This germplasm collection was cultivated in green – house covered with polyethylene. The greenhouse's ventilation space permitted access of insects.

The determination of plants, pods, and seeds characteristics was made according to the U.P.O.V. guideline (2015), the C.P.V.O. protocol (2009) and the color scale (Genchev and Kiryakov, 2005). For characterization, there were used 10 seeds from each accession, according to the *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 to width in cross section (Figure 3).

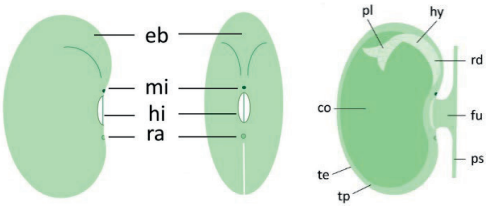


Diagram of seed structure (a) Logitudinal section (b)

Figure 3. Seed Structure of a Dicotyledon **(a)**: eb – embryo bulge; mi – micropyle; hi – hilum; ra – raphe; and **(b)**: pl – plumule; hy – hypocotyl; rd – radicule; co – cotyledon; fu – funicle; ps – pod; te – testa; tp – testa perenchyma (about Chen et al. 2021)

A more detailed overview of the plumule–radicle complex includes the epicotyl and primary leaves (Debouck and Hidalgo, 1986). Rădulescu (1940) quoted by Olaru (1982) pays particular attention to the coloring area around the hilum, otherwise, the hilum is always white. The edge surrounding the hilum may have a different coloration, with various intensities (absent, weak, medium, and strong) than the rest of the testa, and it is also interrupted at the micropyle and caruncle.

Caruncle (raphe) represents a heart – shaped lift with the tip facing the end of the seed. The stripe of the caruncle represents a narrow band that

leaves from the top of the caruncle, it extends to the end of the seed – were it ends like a swallow`s tail, and it has a variable length. (Figure 4).

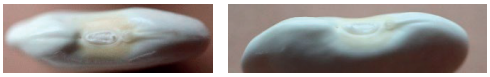


Figure 4. Aspects of the embryo bulge, micropyle, hilum, caruncle, and the stripe of the caruncle (about Teodorescu, 2024)

According to the Descriptor for *Phaseolus vulgaris* (IBPGR, 1982), van Schoonhoven A. and Pastor - Corrales (1994) and Debouck D. (2009) 100 seed mass were randomly chosen for measuring seed mass (small - less than 25 g, medium - 25 g to 40 g, and large - more than 40 g). In order to determine seed weight there was used an electronic balance reading accuracy of 0.0001 g (Partner WAS220/x).

The experimental design was a randomized block design with 3 replicates for each variant. The seeds represented the control variant for the analysis of the harvested biotypes. In order to analyze the results, analysis of variance and multiple comparison methods, using 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 set variation (Ireland, 2010). The coefficient of variation in a single sample with observations is defined as CV= *s/m,* where *m* is the mean and *s* is the standard deviation (Forkman, 2009). Variability assessment according to the CV values (Munteanu and Fălticeanu, 2008; Giurcă and Murariu, 2009) was made as follows: low variability (CV < 10%), mean (CV= 10-20%) and high (CV > 20%).

RESULTS AND DISCUSSIONS

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 5).

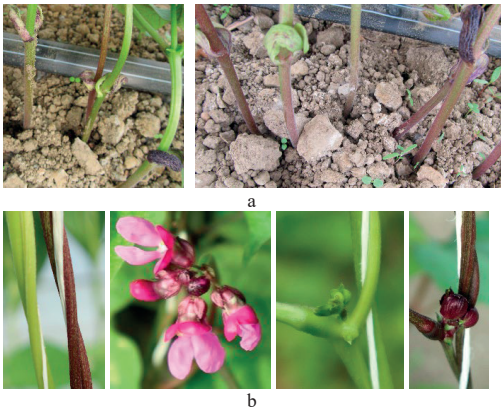
From the first phases of vegetation, it was observed differences in the color of the hypocotyl (Figure 6, a).

Prior to flowering, three biotypes were observed in the culture (Figure 6, b): a green shoot that later produced white flowers, a shoot with strong anthocyanin coloration that resulted in violet flowers, and a shoot with intermediate coloration.



Figure 5. Insects present in the crop and flowers after their visit (Teodorescu, 2024)

This paper presents quantitative (100 seed weight, length, thickness, and width) and qualitative (shape, seed color, veining, and hilum ring – intensity and color) characteristics of seeds harvested from 9 biotypes in comparison with seeds sown.



a

b

Figure 6. Aspects from culture: a. Different hypocotyl colorations and b. Differences between shoots and flower buds

A medium mass (between 25 and 40 g) had four variants (V_8 - 31.75 g, V_1 - 34.05 g, V_2 - 34.47g and V_7 - 36.32 g), and the other six variants, including the control variant (V_5 - 55.92 g, Mt - 53.93 g, V_3 - 46.68 g, V_9 - 44.59 g, V_4 - 44.27 g and V_6 - 43.18 g) had a large mass - greater than 40 g (Table 1).

Table 1. Main quantitative characteristics of the seeds

Variants	100 seeds mass (g)	Seeds dimensions (mm)			Length/ Width	Width/ Thickness
		Length	Width	Thickness		
Mt	53.93 a*	14.15 ab	8.46 a	5.98 ab	1.67	1.42
V_1	34.05 c	14.20 ab	7.43 b	4.06 d	1.91	1.83
V_2	34.47 c	13.92 bc	6.88 c	4.99 c	2.02	1.38
V_3	46.68 b	13.38 c	8.28 a	5.91 ab	1.62	1.40
V_4	44.27 b	12.21 d	8.18 a	6.20 a	1.49	1.32
V_5	55.92 a	14.93 a	8.12 a	6.38 a	1.84	1.27
V_6	43.18 b	12.25 d	8.42 a	5.91 ab	1.46	1.42
V_7	36.32 c	14.27 ab	7.37 b	5.05 c	1.94	1.46
V_8	31.75 c	13.19 c	6.47 c	5.62 b	2.04	1.15
V_9	44.59 b	12.45 d	8.26 a	6.38 a	1.51	1.29
Mean	**42.52**	**13.50**	**7.79**	**5.65**	**1.75**	**1.39**
Stand. var.	8.34	0.95	0.70	0.74	0.23	0.18
CV %	19.61	7.06	9.03	13.15	12.95	12.79
Max.	55.92	14.93	8.46	6.38	2.04	1.83
Min.	31.75	12.21	6.47	4.06	1.46	1.15
	LSD 5% = 5.2191 LSD 1% = 7.1576 LSD 0.1% = 9.7423	LSD 5% =0.7293 LSD 1% =1.0002 LSD 0.1% =1.3614	LSD 5% =0.4243 LSD 1% =0.5818 LSD 0.1% =0.7920	LSD 5% =0.4742 LSD 1% =0.6503 LSD 0.1% =0.8851		

*Different letters between variants denote significant differences (Duncan's test, $p<0.05$).

The coefficient of variability was medium for two parameters (weight - 19.61% and thickness - 13.15%) and low for the other two parameters studied (length - 7.06% and width - 9.03%).

Seed length ranged between 12.21 mm (V_4) and 14.93 mm (V_5), with no significant differences recorded among the first four values (V_8 - 14.27 mm, V_1 - 14.20 mm, Mt - 14.15 mm and V_2 - 13.92 mm).

The highest width value was observed in the control variant (8.46 mm), while significant differences were noted in four of the variants (V_1 - 7.43 mm, V_7 - 7.37 mm, V_2 - 6.88 mm and V_8 - 6.47 mm).

The average thickness was 5.65 mm, with a maximum value of 6.38 mm (V_5) and a minimum of 4.06 mm (V_1); significant differences were recorded compared to the last two variants (V_2 - 4.99 mm and V_7 - 5.05 mm).

The same elliptic shape as the control variant (Table 2) was found in V_5 and three biotypes had a circular to elliptic shape (V_4, V_6 and V_9).

Table 2. Main qualitative characteristics of the seeds

Variants	Seeds shape[1]	Color			Hilum ring	
		Main	Secondar	Shape of secondary	Intensity	Color
Mt	elliptic	brown	black	in streaks very fine patches	strong	black
V_1	kidney s[2]	white	–	–	absent	–
V_2	kidney m	red	–	–	absent	–
V_3	kidney w	beige	gray brown	very fine patches in fine patches	medium	beige
V_4	circular to elliptic	reddish brown	brown	very fine patches	weak	reddish brown
V_5	elliptic	reddish brown	brown	in fine patches	absent	–
V_6	circular to elliptic	L brown	black	in streaks in patches	weak	reddish brown
V_7	kidney w	L brown	black	in streaks in patches	medium	black
V_8	kidney s	brown	black	in streaks very fine patches	strong	black
V_9	circular to elliptic	brown	black	in streaks very fine patches	strong	black

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

According to U.P.O.V. guideline (2015), the C.P.V.O. protocol (2009) and also to the color scale (Genchev and Kiryakov, 2005), for accessions with kidney – shaped seed shape, it

is recommended to evaluate also the degree of curvature (weak - V_3, V_7, medium - V_2 and strong - V_1, V_9).
The main color brown was found in the control variant, V_8 and V_9, light brown in V_6 and V_7, reddish brown in V_4 and V_5, red in V_2, beige in V_3 and white in V_1.
The secondary (Figure 7) color black was found in five variants (Mt, V_6 V_7, V_8 and V_9) and brown in two variants (V_4 and V_5).

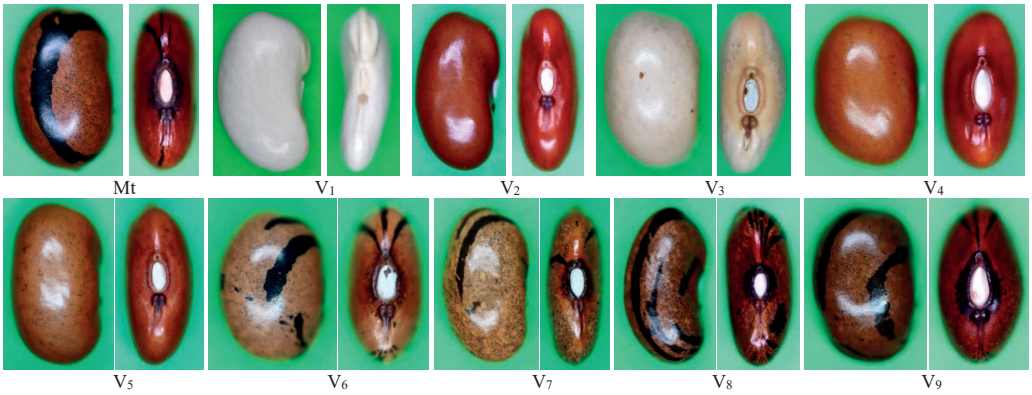


Figure 7. Aspects of qualitative characteristics of the seeds

A similar coloration to the control variant was found in V_9 (main color brown; secondary color black).
Only one color was found in V_1 and V_2. No secondary color was observed in V_1 and V_2, while V_3 exhibited two secondary colors (gray and brown).
The hilar ring is absent in three variants (V_1, V_2, and V_5). The caruncle of V_1 is off-white, and the vascularization is moderate.
At V_4 and V_6, the hilar ring is weak and reddish-brown in color.
A medium intensity of the hilar ring is found in V_3 and V_7. V_3 stands out due to the size and color of the caruncle (darker than the rest of the seed). The control variant, V_8 and V_9, are characterized by high intensity and a black color.

CONCLUSIONS

In what it concerns weight, the maximum values were registered at V_5 following the control variants.
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 the germplasm collection.

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