

STUDY OF UNSTABLE PHENOTYPIC BEAN SEEDS

Eliza TEODORESCU, Elena BARCANU, Ion GHERASE, Ovidia AGAPIE

Vegetable Research and Development Station Buzau, 23 Mesteacanolui Street, 120024,
Buzau, Romania

Corresponding authors email: barcanuelena@yahoo.com

Abstract

Since 2010, at the Vegetable Research and Development Station (V.R.D.S.) Buzău there was initiated a program in order to preserve and valorize local bean population (*Phaseolus vulgaris* L.) from Eastern and South – Eastern Romania. In 2020, the germplasm collection contained over 450 accessions. This paper presents the study of 3 climbing bean accessions seeds sown. For each accession sown, there were taken into study 9 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 and qualitative characteristics. The main colors of the control seeds were brown (V_1 - medium, V_2 - light and V_3 - dark). The seeds harvested presented a lot of colors including white (on entire grain - 1.3.; 1.4.; 2.6.; 3.1 or as secondary color 1.5.; 1.7.; 1.8.; 2.7.; 3.3.). The mean weight of 100 seeds varied between 36.52 g (1.4.) and 70.95 g (1 Mt). The results obtained, corroborated by diversity of color and shape of the seeds harvested, show the presence of cross-pollination of climbing bean plants and increase the value of the bean germplasm collection.

Key words: cross-pollination, germplasm collection, new biotypes, *Phaseolus vulgaris* L.

INTRODUCTION

In Eastern and South-East Romania there are favorable conditions for growing common bean production, as well as a great tradition in cultivation (Ruști 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 collecting of local bean population from the main vegetable areas in Romania. Bean germplasm collection of V.R.D.S. Buzău contains over 450 accessions. Similar researches were made in Romania by Rădulescu (1940), Munteanu (1985), Stan et al. (1993 and 1995), Leonte et al. (2004), Riviș and Nedelea (2008), Giurcă and Murariu (2009), Danci et al. (2010) and Madoșă et al. (2010 and 2011).

Normally, in Romania, 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. (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, and only 0.2-0.5% cross-pollinated. In Puerto Rico, Vakili (1976), quoted

by Bliss (1980), reports that carpenter bees (*Xylocopa brasiliatorum* 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).

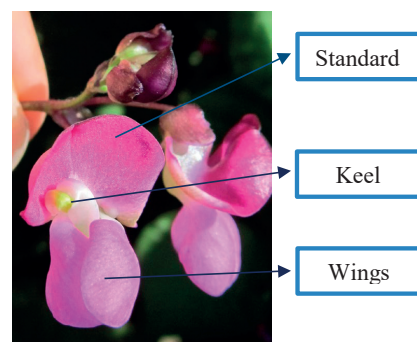


Figure 1. Bean flower morphology
(about Teodorescu, 2016)

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 (Figure 2). 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.

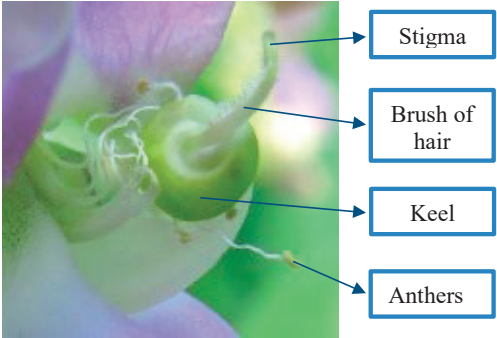


Figure 2. Stigma and anthers were no longer protected by the keel (about Teodorescu, 2016)

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 Eastern and 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; Ruști and Munteanu, 2008). Therefore, the experimental variants were the following:

V ₁		V ₂		V ₃	
1 Mt	35 B	2 Mt	47 BBD	3 Mt	48 B
1.1.	35 BB	2.1.	47 BBDA	3.1.	48 BA
1.2.	35 BD	2.2.	47 BBDC	3.2.	48 BB
1.3.	35 BE	2.3.	47 BBDD	3.3.	48 BC
1.4.	35 BF	2.4.	47 BBDF	3.4.	48 BE
1.5.	35 BG	2.5.	47 BB DG	3.5.	48 BF
1.6.	35 BH	2.6.	47 BB DH	3.6.	48 BG
1.7.	35 BI	2.7.	47 BB DI	3.7.	48 BI
1.8.	35 BJ	2.8.	47 BB DJ	3.8.	48 BJ
1.9.	35 BK	2.9.	47 BB DK	3.9.	48 BK

This germplasm collection was cultivated in green - house covered with polyethylene. The green house’s ventilation space permitted the access of insects.

The determination of plants, pods and seeds characteristics were made according to U.P.O.V. guideline (2015), C.P.V.O. protocol (2009) and color scale (Genchev and Kiryakov, 2005). 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 3).

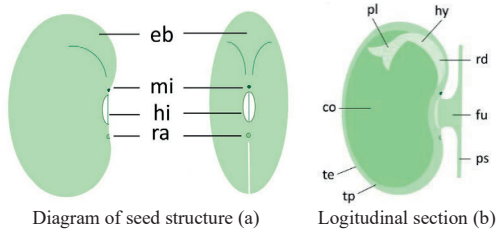


Figure 3. Seed Structure of a Dicotyledon (a): eb - embryo bulge; mi - micropyle; hi - hilum; ra - raphe and (b): pl - plumule; hy - hypocotyl; rd - radicle; co - cotyledon; fu - funicle; ps - pod; te - testa; tp - testa perenchyma (about Chen et al., 2021)

A more detailed overview of the plumule – radicle complex includes 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 4). According to Descriptor for *Phaseolus vulgaris* (IBPGR, 1982), van Schoonhoven A. and Pastor-Corrales (1994) and Debouck D. (2009) 100 seeds 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).



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

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 Fălțiceanu, 2008; Giurcă and Murariu, 2009) was made this way: low variability ($CV < 10\%$), mean ($CV = 10- 20\%$) and high ($CV > 20\%$).

RESULTS AND DISCUSSIONS

In 2020, most of the accessions cultivated presented a high degree of instability. 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 to 9 biotypes in comparison with seeds sown (Figure 5).



Figure 5. Brown - main color of the control seeds

The main colors of the control seeds were brown (V_1 - medium, V_2 - light reddish and V_3 - dark). The secondary color was also present at V_1 (black - in streaks), V_2 (reddish brown - in streaks and in patches) and V_3 (reddish brown - in patches). In what it concerns V_1 , (Table 1) it presents maximum values at 1Mt (weight - 70.96 g and thickness - 7.31 mm), 1.1. (length - 17.01 mm), and 1.8. (width - 9.85 mm) and all minimum values at 1.4. (weight - 37.05 g; length - 12.51 mm; width - 6.82 mm and thickness - 5.68 mm CV had a value of 16.15% (bigger than 10% - mean variability) only regarding weight.

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	70.96 a*	16.04 b	9.23 b	7.31 a	1.74	1.26
1.1.	67.49 a	17.01 a	9.05 b	6.62 bcd	1.88	1.37
1.2.	58.82 bc	13.98 ef	8.88 bc	6.94 abc	1.57	1.28
1.3.	64.48 ab	15.68 bc	8.84 bc	6.68 bc	1.77	1.32
1.4.	37.05 d	12.51 g	6.82 e	5.68 e	1.83	1.20
1.5.	52.73 c	14.16 ef	8.27 d	6.93 abc	1.71	1.19
1.6.	56.39 c	15.07 cd	9.28 b	6.18 d	1.62	1.50
1.7.	64.73 ab	14.73 de	8.93 bc	7.10 ab	1.65	1.26
1.8.	58.82 bc	15.06 cd	9.85 a	6.49 cd	1.53	1.52
1.9.	56.55 c	13.82 f	8.52 cd	6.99 abc	1.62	1.22
Mean	58.80	14.81	8.77	6.69	1.69	1.31
Stand. var.	9.50	1.28	0.81	0.48	0.11	0.12
CV%	16.15	8.61	9.22	7.21	6.71	8.92
Max.	70.96	17.01	9.85	7.31	1.88	1.52
Min.	37.05	12.51	6.82	5.68	1.53	1.19

LSD 5% = 6.5887

LSD 1% = 9.0360

LSD 0.1% = 12.2989

LSD 5% = 0.7522

LSD 1% = 1.0316

LSD 0.1% = 1.4042

LSD 5% = 0.4281

LSD 1% = 0.5871

LSD 0.1% = 0.7991

LSD 5% = 0.4617

LSD 1% = 0.6332

LSD 0.1% = 0.8619

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

The CV in what it concerns all quantitative characteristics had a value between 6.71% (length/width) and 9.22 % (width); this shows a low variability for the other parameters analyzed.

The appearance of a large number of biotypes represents the result of the presence of numerous insects in the crop (Figure 6).

According to U.P.O.V. guideline (2015), C.P.V.O. protocol (2009) and also to the color scale (Genchev and Kiryakov, 2005), at accessions with kidney – shaped seeds shape, it is recommended to evaluate also degree of curvature (weak, medium or strong)

Same shape with 1 Mt (kidney) it also found at 1.1., 1.3. and 1.8. The predominant main color at V₁ (Table 2) was brown, except 1.3., 1.4. (white) and 1.9. black.

The predominant secundar color (Figure 7) to this variant was black (1Mt, 1.2., 1.6. and 1.7.) The shape of the secondary striped color was

the most frequently met (1Mt, 1.3., 1.6., 1.7. and 1.8.).



Figure 6. Insects present in the crop and flowers after their visit (Teodorescu, 2024).

Table 2. Main qualitative characteristics of the seeds V₁

V ₁	Seeds shape ¹	Color			Hilum ring	
		Main	Secundar	Shape of secundar	Intensity	Color
1 Mt	kidney ² w	brown	black	in streaks	absent	–
1.1.	kidney s	L brown	–	–	weak	brown
1.2.	elliptic	beige	black	in streaks	medium	reddish brown
1.3.	kidney s	white	–	–	absent	–
1.4.	elliptic	white	–	–	absent	–
1.5.	elliptic	L brown	white	embryo bulge – in patches	strong	brown
1.6.	elliptic	brown	black	in streaks	medium	reddish brown
1.7.	elliptic	reddish brown	black white	in streaks in patches	weak	D reddish brown
1.8.	kidney w	L brown	brown white	in streaks in patches	weak	brown
1.9.	elliptic	black	L brown	in fine in patches	absent	–

¹Shape of median longitudinal section; L=light; D=dark.

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

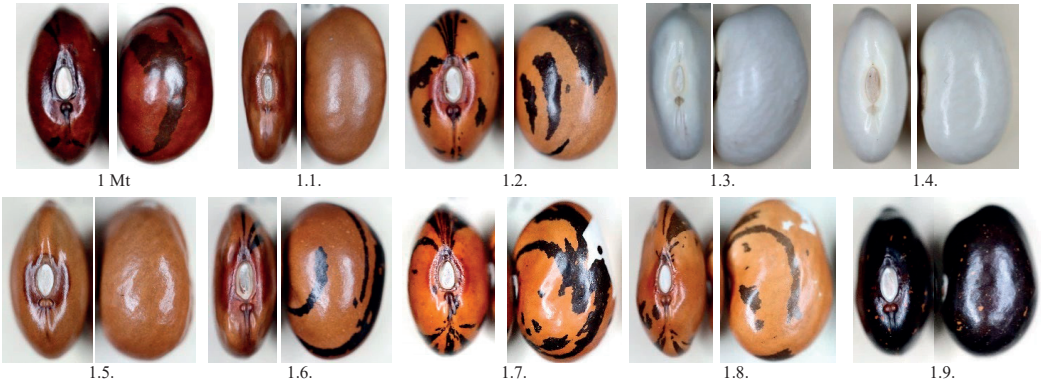


Figure 7. Aspects of qualitative characteristics of the seeds V₁

In what it concerns V_1 , the differences in color of the hypocotyl were evident from the first development stages of plants. (Figure 8 a and b). 1.5. draws attention because the secondary color is localized in the embryo bulge area and also because the color hilar ring was strong (Figure 8

c and d). The difference between 1.7. and 1.8. is given by the secondary color in streaks (Figure 8 e). The funicle color varied within this variant from white (Figure 8 f) to brown (Figure 8 g). In the field, sometimes (harvest), the brightness was high.

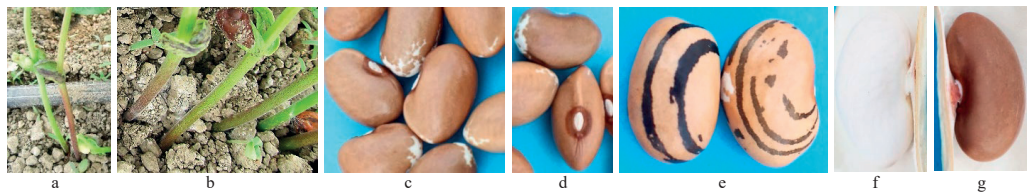


Figure 8. Aspects of crop and seeds belong to V_1 : a and b - differences in anthocyanin coloration of the hypocotyl; c - white secondary color on embryo bulge area at 1.5.; d - strong coloration of hilar ring of 1.5.; e - comparison between the secondary color in streaks to 1.7. and 1.8.; f - white funicle at 1.3. and g - brown funicle at 1.1.

At V_2 (Table 3), the two highest values were registered at the control variant (weight - 54.67g and width - 8.98 mm) and two minimum values at 2.8 (weight - 38.61 g; length - 11.41 mm). The mean weight value was 45.50 g and more than

half of the new biotypes were below this value (2.7. - 44.47 g; 2.5. - 43.56 g; 2.6. - 40.58 g; 2.4. - 39.65 g and 2.8. - 38.61 g). A mean variability is also observed in what it concerns the weight (11.39%).

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	54.67 a*	14.41 bc	8.98 a	6.35 a	1.60	1.42
2.1.	48.49 bc	14.59 ab	8.62 b	6.28 a	1.69	1.37
2.2.	51.29 ab	15.19 a	8.65 b	6.26 ab	1.76	1.38
2.3.	46.17 bc	12.90 d	8.70 ab	6.58 a	1.48	1.32
2.4.	39.65 de	12.65 d	8.47 bc	5.32 d	1.49	1.59
2.5.	43.56 cde	14.39 bc	8.27 cd	5.52 cd	1.74	1.50
2.6.	40.58 de	12.17 d	8.22 cd	5.85 bc	1.48	1.40
2.7.	44.47 cd	13.76 c	7.91 e	6.22 ab	1.74	1.27
2.8.	38.61 e	11.41 e	8.08 de	6.41 a	1.41	1.26
2.9.	47.48 bc	12.90 d	8.70 ab	6.45 a	1.48	1.35
Mean	45.50	13.44	8.46	6.12	1.59	1.39
Stand. var.	5.18	1.21	0.33	0.42	0.13	0.10
CV%	11.39	9.03	3.91	6.84	8.36	7.23
Max	54.67	15.19	8.98	6.58	1.76	1.59
Min	38.61	11.41	7.91	5.32	1.41	1.26

LSD 5% = 5.2321 LSD 5% = 0.7235 LSD 5% = 0.2865 LSD 5% = 0.3930
LSD 1% = 7.1755 LSD 1% = 0.9923 LSD 1% = 0.3929 LSD 1% = 0.5390
LSD 0.1% = 9.7666 LSD 0.1% = 1.3506 LSD 0.1% = 0.5348 LSD 0.1% = 0.7336

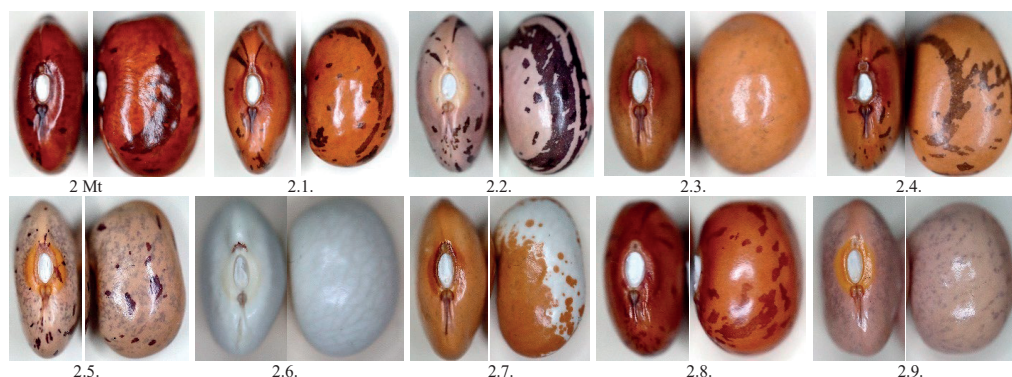
*Different letters between variants denote significant differences (Duncan's test, $p < 0.05$).

The kidney shape (2.2., 2.4., 2.5. and 2.7.) with a weak degree of curvature predominates at V_2 (Table 4). The other biotypes had an elliptic (2Mt, 2.1. and 2.3.) or circular to elliptic (2.3., 2.6. and 2.9.) shape. The main color of control variant (light reddish brown) was found at 2.1. 2.8. The other three biotypes (2.2., 2.5. and 2.9.) had various shades of beige as their main color.

The white color (Figure 9) was present at 2.6. as main color and at 2.7. as secondary color (on half of grain). A strong veining and difference in color of caruncula (raphe) can be observed at 2.6. Weight at V_3 (Table 5) had the lowest values at 3.7. (47.65 g) and the highest values at control variant – 1 Mt (60.45 g).

Table 4. Main qualitative characteristics of the seeds V₂

V ₂	Seeds shape ¹	Color			Hilum ring	
		Main	Secondar	Shape of secundar	Intensity	Color
2 Mt	elliptic	L reddish brown	reddish brown	in streaks and in patches	very weak	brown
2.1.	elliptic	L reddish brown	D brown	in streaks and in patches	weak	brown
2.2.	kidney ² w	beige	mauve	in streaks and in patches	weak	yellowish
2.3.	circular to elliptic	L brown	grey	in very fine in patches	medium	brown
2.4.	kidney w	L brown	brown	in streaks and in patches	strong	brown
2.5.	kidney w	beige	reddish brown grey	in patches in fine patches	strong	orange
2.6.	circular to elliptic	white	brown	above the micropyle	absent	–
2.7.	kidney w	café au lait	white	half of grain	medium	brown
2.8.	elliptic	L reddish brown	reddish brown	in streaks and in patches	absent	–
2.9.	circular to elliptic	beige	grey	in very fine patches	strong	orange

¹Shape of median longitudinal section; L = light; D = dark.²Degree of curvature for kidney - shaped only: weak (w), medium (m) and strong (s).Figure 9. Aspects of qualitative characteristics of the seeds V₂

According to van Schoonhoven A. (1994), seeds belonging to this variant had a large size (more than 40 g). Seeds length registered the maximum value at 3.2 (16.76 mm) and the minimum value

at 3.4. (13.36 mm). The coefficient of variation values for all parameters studied at V₃ were less than 10% (weight - 9.45% was the highest value), indicating low variability.

Table 5. Main quantitative characteristics of the seeds V₃

V ₃	100 seeds mass (g)	Seeds dimensions (mm)			Length/ Width	Width/ Thickness
		Length	Width	Thickness		
3 Mt	60.45 a*	16.23 ab	8.45 b	6.13 c	1.92	1.38
3.1.	48.78 d	14.55 c	7.64 cd	6.07 cd	1.90	1.26
3.2.	60.35 a	16.76 a	8.93 a	6.29 c	1.88	1.42
3.3.	56.59 abc	13.64 d	8.67 ab	6.72 b	1.57	1.29
3.4.	53.56 bcd	13.36 d	8.38 b	7.37 a	1.59	1.14
3.5.	51.58 cd	16.44 ab	7.83 c	5.67 d	2.10	1.38
3.6.	58.92 ab	16.12 ab	8.45 b	5.68 d	1.91	1.49
3.7.	47.65 d	14.75 c	7.46 d	5.66 d	1.98	1.32
3.8.	49.11 d	14.08 cd	7.56 cd	6.16 c	1.86	1.23
3.9.	49.32 d	15.84 b	7.91 c	5.63 d	2.00	1.40
Mean	53.63	15.18	8.13	6.14	1.87	1.33
Stand. var.	5.07	1.25	0.51	0.56	0.17	0.10
CV%	9.45	8.21	6.29	9.09	8.92	7.84
Max	60.45	16.76	8.93	7.37	2.10	1.49
Min	47.65	13.36	7.46	5.63	1.57	1.14

LSD 5% = 5.4448

LSD 1% = 7.4671

LSD 0.1% = 10.1636

LSD 5% = 0.7474

LSD 1% = 1.0250

LSD 0.1% = 1.3952

LSD 5% = 0.3346

LSD 1% = 0.4589

LSD 0.1% = 0.6246

LSD 5% = 0.4141

LSD 1% = 0.5679

LSD 0.1% = 0.7730

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

The most common shape to this variant (Table 6) was kidney (3 Mt, 3.1., 3.2., 3.5., 3.6., 3.9.), but it was met elliptical (3.3., 3.4.) and rectangular shape (3.7., 3.8.).

The combinations: main color beige, secondary color gray in patches (3.2.) or main color white, secondary colors brown and black on half the seed (3.3.) are much more suggestive in the field (Figure 10) that in the laboratory (Figure 11).

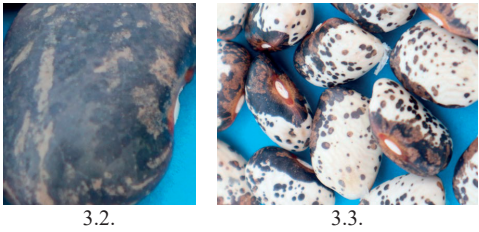


Figure 10. Aspects of seeds in the field

Table 6. Main qualitative characteristics of the seeds V₃

V ₃	Seeds shape ¹	Color			Hilum ring	
		Main	Secondar	Shape of secondar	Intensity	Color
3 Mt	kidney ² m	D brown	reddish brown	in patches	very weak	D brown
3.1.	kidney m	white	–	–	absent	–
3.2.	kidney m	beige	grey	in patches	medium	brown
3.3.	elliptic	white	brown and black	on half of grain	medium	brown
3.4.	elliptic	brown	black	in patches	absent	–
3.5.	kidney m	D beige	grey	in very fine patches	medium	reddish brown
3.6.	kidney s	brown	grey	in fine in patches	weak	brown
3.7.	rectangular	D mauve	–	–	absent	–
3.8.	rectangular	D reddish brown	L brown	in patches	absent	–
3.9.	kidney s	grayish brown	–	–	medium	L reddish brown

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



Figure 11. Aspects of qualitative characteristics of the seeds V₃

CONCLUSIONS

In what it concerns weight of the seeds the maximum values were registered at 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 germplasm collection.

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