Seed Morphobiometry of Wild and Cultivated Taxa of *Phaseolus* L. (Fabaceae)

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A morphobiometric analysis of seeds of *Phaseolus* L. from 50 populations belonging to 31 wild and cultivated taxa was carried out. Based on the outcome of the study an identification key was developed comprising 25 morphotypes of which 23 related to individual taxa [*P. angustissimus* Gray, *P. filifomis* Benth., *P. acutifolius* Gray var. *latifolius* Freeman, *P. lunatus* L. var. *sylvester* Baudet, *P. polyanthus* Greenm., *P. lunatus* L. var. *lunatus* cv.-gr. "Sieva", *P. lunatus* L. var. *lunatus* cv.-gr. "Big Lima", *P. lunatus* L. var. *lunatus* cv.-gr. "Potato", *P. maculatus* Scheele, *P. lunatus* L., *P. microcarpus* Mart., *P. leptostachyus* Benth. var. *leptostachyus*, *P. grayanus* Woot. & Standl., *P. vulgaris* L. var. *aborigeneus* (Burk.) Baudet, *P. marechalii* Delgado, *P. vulgaris* L. var. *vulgaris*, *P. ritensis* Jones, *P. polystachios* (L.) Britt., Sterns & Pogg. var. *polystachios*, *P. hintonii* Delgado, *P. pauciflorus* Don, *P. parvulus* Greene, *P. pluriflorus* Maréchal, Mascherpa & Stainer, *P. micranthus* Hook. & Arn.) and two that refer to several taxa (*P. vulgaris* morphotype with *P. vulgaris* L., *P. coccineus* L., *P. glabellus* Piper, *P. oligospermus* Piper and *P. acutifolius* var. *tenuifolius* morphotype with *P. acutifolius* Gray var. *tenuifolius* (Woot. & Standl.) Gray, *P. acutifolius* Gray var. *acutifolius*, *P. xanthotrychus* Piper, *P. zimapanensis* (Delgado) Jaaska). The different patterns of seminal tegument allowed 31 taxa to cluster into three groups: (1) *Phaseolus angustissimus* Gray group (wrinkled seed coat) with two morphotypes, (2) *Phaseolus lunatus* L. group (smooth tegument with striae) with ten morphotypes and (3) *Phaseolus vulgaris* L. group (smooth tegument without striae) with 13 morphotypes. All the taxa exhibited uniformity in size and variability in tegument colour of seeds irrespective of the source of population and the type of habitat. Characterization of taxa into definite morphotypes and the groups could be useful for biosystematic investigations and the marker-based genetic selection approaches in this important leguminous crop.

Key Words: Biometry, Morphology, *Phaseolus*, Seeds

The bean, cultivated since classical times, is the second most important legume in the world for human consumption after soybean. The change in dietary habits and the globalization of the agrifood sector has led to the spread of bean varieties particularly selected for the canning industry, thus reducing the genetic diversity of *Phaseolus* species grown worldwide. This has had a strong impact on access by people to the large number of its species, varieties and ecotypes, suited to a certain specific environment where they have been selected by generations of farmers. In addition, the loss of this diversity is also accompanied by the loss of agronomic, organoleptic and sensory traits which can no longer be used by people for meeting their livelihood needs. Moreover, the possibility of crosses between different species of *Phaseolus* L., may involve not only an improvement in the production of fruits and seeds but also ensure genetic diversity of all those properties that characterize this genus (Maréchal *et al*., 1978; Mok *et al*., 1978; Leonard *et al*., 1987; Hoc *et al*., 2003; Kulsolwa and Myers, 2005).

There is a great deal of scientific articles on *Phaseolus* that have mainly focused on chemotaxonomy, phytogeography, cultivation practices, *ex situ* conservation, physiological and agronomic characteristics, resistance to abiotic and biotic stresses, etc. (Brücher, 1953; Berglund-Brücher, 1969; Berglund-Brücher *et al*., 1969; Castiñeiras *et al*., 1994a, 1994b; Guerra *et al*., 1994, Hernández *et al*. 1994; Mihálikova and Benková, 1995; Freytag and Debouck, 2002; Gutiérrez *et al*., 2004; Delgado-Salinas *et al*., 2006). Less attention has been paid to investigate the morphological variability in seed and in particular of its shape, size, hilum, chalaza or tegument sculpture. These characters although may appear less significant than others, are in fact very useful for the identification of morphotypes and assist to illustrate evolutionary trends of taxa at the generic level (Benson, 1982; De Leonardis *et al*., 2002). The purpose of this study was to add more light on the morphobiometric variability of *Phaseolus* seeds in order to search for possible morphological markers that can be successfully used

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by breeders as in other studies of Fabaceae (Gunn, 1970; Ladizinsky and Adler, 1976a, 1976b; Auckland and Singh, 1977; Kupica, 1977; Ahmad and Godward, 1980; Lersten and Gunn, 1981; De Leonardis *et al.*, 1993, 1995, 1996).

Materials and Methods

Seeds originating from 50 populations of 31 taxa of *Phaseolus* were used for this study. The samples were kindly provided by the National Botanic Garden of Belgium from Wild Phaseolinae Collection.

Morphometric data was gathered form a sample of 30 seeds for each taxon and performed under a stereo-microscope (Jeva Citoval) supplied with 16x eye piece and 1x objective lenses (Table 1). Measurements on large-size (≥ 4,0 mm) seeds were performed with a vernier caliper. Each seed was measured for length (Le), width (Wi), thickness (Th), distance between the micropyle and higher end of the seed (D1), distance between micropyle and the center of the chalaza (D2), distance between the center of the chalaza and the lower end of the seed (D3), distance between higher end of the seed and hilar collar near the chalaza (D4), distance between lower end of the seed and hilar collar near the chalaza (D5), hilum length (Le.hi), hilum width (Wi. hi), chalaza shape (Fig. 1). The map of sketch shapes reported in Fig. 2 has been taken from the descriptive terminology edited by Systematics Association (1962). The seed colour, numeric codes were given following a methodology described by Berggren (1969).

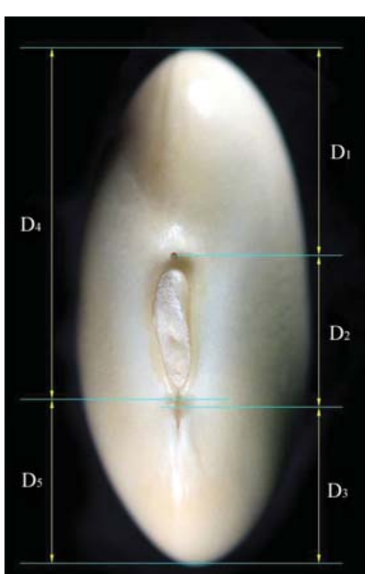

Fig. 1. Measures of some characters made on *Phaseolus* seeds (see abbreviations in Materials and Methods)

Results and Discussion

Table 1 and Fig. 1 show size (in mm) and dimension of seed, respectively. As for length, width and thickness of the seeds of greater size belong to *P. lunatus* (NI 776), while the seeds of smaller size are those belonging to *P. pauciflorus* (Le 1.9). The distance between the micropyle and higher end of the seed (D1) is in the range 0.5 (*P. pauciflorus*) and 6.6 (*P. lunatus* var. *lunatus* NI 426). The distance between micropyle and the center of the chalaza (D2) varies between 0.6 (*P. pauciflorus*) and 5.4 (*P. polyanthus* NI 519). The distance between the center of the chalaza and the lower end of the seed (D3) is in the range 0.9 (*P. acutifolius* var. *tenuifolius* NI 705, *P. micranthus, P. pauciflorus*) and 5.8 (*P. lunatus* var. *lunatus* NI 426). Distance between higher end of the seed and hilar collar near the chalaza (D4) varies between 0.8 (*Ph. pauciflorus*) and 1.30 (*P. polyanthus* NI 519). Distance between lower end of the seed and hilar collar near the chalaza (D5) varies between 1.1 (*P. acutifolius* var. *tenuifolius* NI 705, *P. micranthus*, *P. pauciflorus*) and 6.2 (*P. lunatus* var. *lunatus* NI 426). The hilar length and width vary respectively, between 0.5 (*P. pauciflorus*) and 4.9 (*P. polyanthus* NI 519) and between 0.5 (*P. pauciflorus*) and 0.30 (*P. polyanthus* NI 519). The surface is generally smooth with or without striae, except *P. angustissimus*. and *P. filiformis* having a rugulose surface. The colour varies from white to beige, red to dark brown often with the presence of marbling. In a lateral view the seed range from strictly elliptic to broadly elliptic (rarely oval or rhomboid). The chalaza is heart-shaped, sometimes barely visible

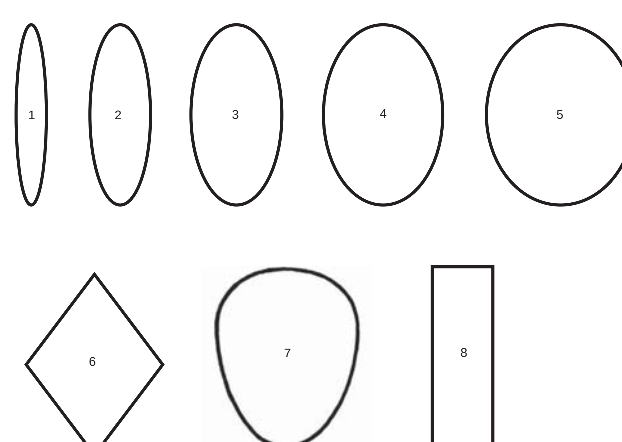

Fig. 2. The map of sketch shapes: 1-2) narrowly elliptic, 3-4) elliptic, 5) broadly elliptic, 6) rhombic, 7) broadly obovate, 8) narrowly oblong

Table 1. Mean values (expressed in mm) of the seed parameters and morphological characters of 50 populations of *Phaseolus*

Codes	Taxa	Le	Wi	Th	D1	D2	D3	D4	D5	Lateral shape	Transversal	Le.hi	Wi.hi	Chalaza shape	Seed coat	Colour	Countries
1	*P. acutifolius* Gray var. *acutifolius* NI 576	4.3	3.1	2.1	1.5	1.3	1.5	2.4	1.7	4,5	10	1.1	0.9	H-s	S	19,39 M 33	Mexico
2	*P. acutifolius* Gray var. *acutifolius* NI 851	4.1	3.4	2.2	1.8	1.0	1.3	2.5	1.6	4,5	7,8,9,10,11	0.9	0.7	H-s C	S	18 M 37; 20 M 28; 29 M 33	Mexico
3	*P. acutifolius* Gray var. *latifolius* Freeman NI 901	7.5	5.2	3.8	2.7	1.8	3.1	3.5	3.2	2,3	8	1.8	1.3	H-s C	Ss	10	El Salvador
4	*P. acutifolius* Gray var. *latifolius* Freeman NI 588	8.1	5.4	3.5	3.1	1.6	3.4	3.9	3.6	4	2,10	1.4	1.1	H-s C	S	4	Mexico
5	*P. acutifolius* Gray var. *tenuifolius* (Woot. & Standl.) Gray NI 705	3.3	2.7	1.5	1.3	1.1	0.9	2.2	1.1	5	9,10,11	0.9	0.7	H-s C	S	20 M 36; 33 M 20	Mexico
6	*P. acutifolius* Gray var. *tenuifolius* (Woot. & Standl.) Gray NI 1272	3.3	2.7	1.8	1.1	1.0	1.2	1.8	1.4	4	9	0.8	0.7	H-s C	S	20 M 14,36; 30 m 36	Mexico
7	*P. angustissimus* Gray NI 788	4.0	3.2	2.4	1.3	0.9	1.8	1.8	2.2	4	9	0.8	0.7	H-s	R	28,32,33 M 37	Arizona
8	*P. angustissimus* Gray NI 1303	5.1	3.9	2.7	2.0	1.2	2.1	2.7	2.4	5	9	1.1	0.8	H-sfv C	R	19 M 37	Mexico
9	*P. coccineus* L. NI 722	7.0	5.7	3.8	2.5	2.8	2.3	4.2	2.8	4,5	9	1.9	1.2	H-sfv C	S	33 M 29; 33 M 20	Mexico
10	*P. coccineus* L. NI 819	5.5	4.8	2.6	1.6	2.3	1.7	3.4	2.0	5	10	1.9	1.4	H-s	S	33 M 20	Mexico
11	*P. coccineus* L. NI 1430	9.2	8.3	6.6	2.5	3.6	2.0	6.2	3.1	28	8	3.1	2.0	H-s	S	36	Mexico
12	*P. filiformis* Benth. NI 689	3.2	2.6	1.6	1.3	0.7	1.1	1.8	1.3	4	9	0.6	0.6	H-s	R	19 M 37	Arizona
13	*P. filiformis* Benth. NI 859	3.1	2.9	1.6	1.3	0.9	1.1	1.8	1.3	5,28	9,10	0.7	0.7	H-s	R	19 M 33	Mexico
14	*P. glabellus* Piper NI 1304	4.9	4.7	3.2	1.5	2.2	1.3	3.5	1.4	5	8	1.9	1.3	H-sfv C	S	20 M 33	Mexico
15	*P. grayanus* Woot. & Standl. NI 724	5.4	4.1	3.1	1.4	1.5	2.6	2.5	2.9	4,40	8,9	1.5	1.5	H-s	S	19, 30 M 36	Mexico
16	*P. hintonii* Delgado NI 716	3.8	4.0	1.9	1.2	0.9	1.6	1.9	1.8	28	9,10	0.7	0.7	H-s	S	36	Mexico
17	*P. leptostachyus* Benth. var. *leptostachyus* NI 714	3.6	3.5	2.3	1.1	0.9	1.5	1.8	1.8	5	9	0.7	0.7	H-s	S	20 M 33,39; 36	Mexico
18	*P. leptostachyus* Benth. var. *leptostachyus* NI 1036	3.6	3.2	2.2	1.2	1.1	1.4	2.0	1.6	5	9	0.9	0.7	H-sfv C	S	40 M 37; 21 M 31; 23 M 37	Mexico
19	*P. lunatus* L. NI 776	18.1	8.6	4.1	4.3	3.7	3.8	7.3	4.7	5	9,10	3.4	1.8	H-s C	Ss	10; 36 M 21,22	Mexico
20	*P. lunatus* L. NI 1414 A	8.5	6.6	3.6	4.0	3.1	2.2	5.6	3.0	4	10	1.8	1.5	H-s	S	36	Mexico
21	*P. lunatus* L. var. *lunatus* cv.-gr. “Lima”NI 426	15.9	13.2	6.7	6.6	4.6	5.8	9.0	6.2	4	9,10	4.2	2.2	H-s C	Ss	17	Florida
22	*P. lunatus* L. var. *lunatus* cv-gr. “Potato” NI 783	9.9	8.4	5.8	3.6	3.0	3.4	4.9	3.6	4,5	8,9	2.7	2.0	H-s C	Ss	23,29 M 30; 30 M 32; 31 M 30	Brazil
23	*P. lunatus* L. var. *lunatus* cv-gr. “Sieva” NI 549	12.2	8.9	4.1	4.2	3.6	4.5	7.3	4.9	4	9,10	3.3	1.6	H-s	Ss	31	California
24	*P. lunatus* L. var. *silvester* Baudet NI 745	9.0	7.2	4.1	2.8	2.8	3.8	5.1	4.4	4	9,10	2.4	1.6	H-s C	S	19,20,29,31 M 37	Mexico
25	*P. lunatus* L. var. *silvester* Baudet NI 1605	7.8	6.0	3.1	2.4	3.0	4.5	4.5	3.4	5	10,11	2.2	1.7	H-s	S	19,33 M 36	Costa Rica
26	*P. maculatus* Scheele NI 1237	7.9	6.5	3.8	1.9	2.0	3.7	3.8	4.1	4,5	37,38,39	2.1	1.5	H-sfv C	R	18,19,22,23,40	Mexico
27	*P. marechalii* Delgado NI 402	5.6	4.5	3.4	1.7	2.2	1.7	3.4	2.2	5	8	1.9	1.5	H-s	S	21,29,30 M 36	Mexico
28	*P. micranthus* Hook. & Arn. NI 1590	2.6	2.3	1.4	1.1	0.8	0.9	1.5	1.1	5	9,10	0.7	0.7	H-sfv C	S	20,29 M 36; 36	Mexico
29	*P. microcarpus* Mart. NI 809	4.1	2.8	1.9	1.6	0.9	1.6	2.3	1.8	28	32,44	0.7	0.8	H-s	S	19,22 M 36	Mexico
30	*P. oligospermus* Piper NI 1116	5.6	5.2	3.1	1.8	1.9	1.4	3.4	1.7	4,5	9	2.1	1.8	H-s	S	20,22,23 M 36	Guatemala

Codes	Taxa	Le	Wi	Th	D1	D2	D3	D4	D5	Lateral shape	Transversal	Le.hi	Wi.hi	Chalaza shape	Seed coat	Colour	Countries
31	*P. parvulus* Greene NI 800	3.3	2.2	2.1	1.1	1.1	1.1	2.0	1.3	2	8	0.9	0.7	H-sfv C	S	19,40 M 36	Arizona
32	*P. pauciflorus* Don NI 711	1.9	1.8	1.4	0.5	0.6	0.9	0.8	1.1	4,5	8	0.5	0.5	H-sfv C	S	22,29 M 36	Mexico
33	*P. pluriflorus* Maréchal, Mascherpa & Stainier NI 869	3.0	2.3	2.1	0.9	1.0	1.1	1.5	1.5	2,4,6	9	0.9	0.6	H-sfv C	S	30 M 36	Mexico
34	*P. polyanthus* Greenm.NI 519	15.4	11.7	6.0	4.8	5.4	5.2	9.7	5.8	5	9	4.9	2.8	H-s	Ss	29	Mexico
35	*P. polyanthus* Greenm.NI 1011	12.4	10.1	5.5	4.0	4.5	3.8	8.3	4.2	4	9	4.2	2.1	H-s	Ss	10	Colombia
36	*P. polystachyos* (L.) Britt., Sterns & Pogg. var. *polystachous* NI 563	6.1	4.5	3.2	1.9	2.7	1.5	4.2	1.9	4,5	8,9,10	2.6	1.5	H-s C	S	33	Florida
37	*P. ritensis* Jones NI 795	6.8	5.4	2.8	2.4	1.6	2.7	3.6	3.0	5,40	9	1.4	1.1	H-s	S	14 M 20; 18 M 36; 30 M 23	Arizona
38	*P. ritensis* Jones NI 797A	6.0	5.1	3.2	2.4	1.7	2.3	3.4	2.7	4,28	9	1.6	1.1	H-sfv C	S	29 M 33	Arizona
39	*P. vulgaris* L. NI 575	6.4	4.4	2.7	2.4	1.7	2.4	3.7	2.7	4	9	1.5	1.2	H-s	S	20 M 33; 30 M 36	Mexico
40	*P. vulgaris* L. NI 613	6.9	4.9	3.5	2.4	2.4	2.1	4.3	2.7	4	9	2.3	1.4	H-s	S	22,23,40	Mexico
41	*P. vulgaris* L. NI 695	7.6	4.5	3.1	2.6	2.2	2.1	4.5	2.6	4	9	2.1	1.3	H-s	S	22,23,33,40	Mexico
42	*P. vulgaris* L. NI 928	6.1	4.2	2.6	2.0	2.0	2.1	3.7	2.4	4	9	2.0	1.6	H-s C	S	20,30 M 36	Colombia
43	*P. vulgaris* L. var. *aborigeneus* (Burk.) Baudet NI 573	7.9	5.8	2.8	2.9	2.1	2.9	4.7	3.3	4	9	2.0	1.5	H-s	S	28,30 M 36	Brazil
44	*P. vulgaris* L. var. *aborigeneus* (Burk.) Baudet NI 621	7.0	4.9	2.8	2.4	1.9	2.7	4.2	2.9	4	9	1.6	1.2	H-s	S	19,20 M 33	Argentina
45	*P. vulgaris* L. var. *vulgaris* NI 637	8.0	5.8	4.4	3.6	2.5	1.9	5.7	2.3	4	9	2.5	1.7	H-s	S	29	Brazil
46	*P. vulgaris* L. var. *vulgaris* NI 659	9.9	5.9	4.0	3.8	3.1	3.0	6.5	3.4	4	9	2.9	1.6	H-s	S	36	Colombia
47	*P. xanthotrichus* Piper NI 1332	3.4	3.5	1.7	1.0	1.1	1.4	1.8	1.6	5	9,10	0.8	0.7	H-s C	S	19,20,29 M 36	Guatemala
48	*P. xanthotrichus* Piper NI 1558	2.7	2.3	1.4	0.9	0.7	1.3	1.4	1.5	4,14	9,10,31	0.8	0.6	H-s	S	21,23 M 36	Mexico
49	*P. zimapanensis* (Delgado) Jaaska NI 1239	2.5	2.4	1.6	0.8	0.8	1.0	1.4	1.2	5	8,9	0.8	0.7	H-s C	S	20 M 36	Mexico
50	*P. zimapanensis* (Delgado) Jaaska NI 1600	3.4	3.2	1.6	1.1	1.1	1.3	1.9	1.6	5	9,10	0.9	0.7	H-s C	S	20,23 M 36	Mexico

Le: length seed; Wi: width seed; Th: thickness seed; Le.hi: length hilum; Wi.hi: width hilum; D1, D2, D3, D4, D5: see in Materials and Methods; H-s: heart-shaped; H-s C: heart-shaped cracked; H-sfv C: heart-shaped few visible and cracked; S: smooth; Ss: smooth with striae; R: rugulose; M: marbling.

with stereo-microscope, with or without fissure. Fig. 3 shows the direct proportionality between length, width and thicknes of the seeds belonging to fifty *Phaseolus* populations.

An analysis of the morphobiometric data allowed to develop the following identification key for 25 morphotypes (Fig. 4) selected in the study.

1	Rugulose seed coat	**2**
1	Smooth seed coat with striae	**3**
1	Smooth seed coat without striae	**11**
2	Seed Thickness 2.2-2.6	*P. angustissimus* type
2	Seed Thickness < = 2.1	*P. filiformis* type
3	Seed Length > 6.0	**4**
3	Seed Length < 6.0	**10**
4	Bright hilum edge	*P. acutifolius* var. *latifolius* type
4	Dark hilum edge	5
5	Narrowly elliptic hilum shape	**6**
5	Elliptic hilum shape	**7**
5	Obovate hilum shape	*P. lunatus* var. *silvester* type
6	Seed Thickness > 4.1	*P. polyanthus* type
6	Seed Thickness < 4.1	*P. lunatus* var. *lunatus* cv.-gr. “Sieva” type
7	Seed Thickness > 4.9	**9**
7	Seed Thickness < 4.9	**8**
8	Distance between the micropyle and higher end of the seed > 3.0	*P. lunatus* type
8	Distance between the micropyle and higher end of the seed < 3.0	*P. maculatus*
9	Seed Width > 10	*P. lunatus* var. *lunatus* cv.gr. “Big Lima”type
9	Seed Width < 10	*P. lunatus* var. *lunatus* cv.gr.“Potato” type
10	Obovate hilum	*P. microcarpus* type
10	Subcircular hilum	*P. leptostachyus* var. *leptostachyus* type
11	Hilum Length > 1.1	**12**
11	Hilum Length < 1.1	**17**
12	Elliptic hilum	*P. vulgaris** type
12	Obovate hilum	**13**
12	Narrowly elliptic hilum	**15**
13	Distance between higher end of the seed and hilar collar near the chalaza ≤ 2.9	*P. grayanus* type
13	Distance between higher end of the seed and hilar collar near the chalaza > 2.9	**14**
14	Seed shape elliptic in lateral view	*P. vulgaris* var. *aborigineus* type
14	Seed shape broadly in lateral view	*P. marechalii* type
15	Distance between higher end of the seed and hilar collar near the chalaza > 5.3	*P. vulgaris* var. *vulgaris* type
15	Distance between higher end of the seed and hilar collar near the chalaza < 5.3	**16**
16	Hilum Length < 2.0	*P. ritensis* type
16	Hilum Length > 2.0	*P. polystachios* var. *polystachios* type
17	Circular hilum	*P. hintonii* type
17	Subcircular hilum	*P. acutifolius* var. *tenuifolius** type
17	Obovate hilum	**18**
17	Elliptic hilum	**19**
18	Seed Length< 2.1	*P. pauciflorus* type
18	Seed Length > 2.1	*P. parvulus* type
19	Seed Thickness > 1.6	*P. pluriflorus* type
19	Seed Thickness < 1.6	*P. micranthus* type

Only two morphotypes include several taxa:

*P. vulgaris** morphotype: *P. vulgaris* NI 575, NI613, NI 695, NI 928; *P. coccineus* NI 722, NI 819, NI 1430; *P. glabellus*, *P. oligospermus.*

P. acutifolius var. *tenuifolius** morphotype: *P. acutifolius* var. *tenuifolius* NI 705, NI 1272; *P. acutifolius* var. *acutifolius*, NI 576, NI 851; *P. xanthotrychus* NI 1332, NI 1558; *P. zimapanensis* NI 1239, NI 1600.

The International Biotechnology and Plant Genetic Resources Institute had carried out an international collaboration in 1982 and in 2001 to develop a list of

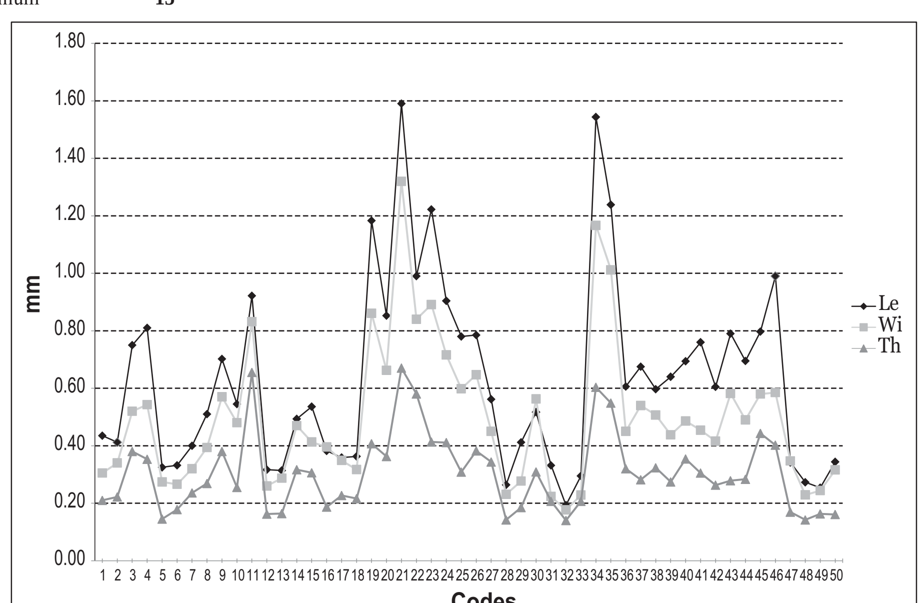

Fig. 3. Proportional dimensions between length, width and thickness of the seeds belonging to 50 *Phaseolus* populations

Fig. 4. Stereomicroscope micrographs of *Phaseolus* taxa: a) *P. angustissimus,* b) *P. filiformis,* c) *P. maculatus,* d) *P. acutifolius* var. *latifolius,* e) *P. lunatus* var. *silvester,* f) *P. polyanthus,* g) *P. lunatus* v. *lunatus* cv.gr. "Sieva", h) *P. lunatus,* i) *P. lunatus* var. *lunatus* cv.gr. "Big Lima", j) *P. lunatus* var. *lunatus* cv.gr. "Potato", k) *P. microcarpus,* l) *P. leptostachyus* var. *leptostachyus,* m) *P. vulgaris,* n) *P. grayanus,* o) *P. vulgaris* var. *aborigineus,* p) *P. marechalii,* q) *P. vulgaris* var. *vulgaris,* r) *P. ritensis,* s) *P. polystachios* var. *polystachios,* t) *P. hintonii,* u) *P. acutifolius* var. *tenuifolius,* v) *P. pauciflorus,* w) *P. parvulus,* x) *P. pluriflorus,* y) *P. micranthus*

descriptors for *P. vulgaris* (IBPGR, 1982) and *P. lunatus* (IBPGR, 2001) in order to (i) better understand the genetic variability of germplasm accessions maintained in gene banks, (ii) facilitate the sharing of information among experts, and (iii) promote a more effective use of the genetic diversity of *Phaseolus* around the world.

In a research on the phenotypical characters of *P. vulgaris* ecotypes, McClean *et al*. (2002) have shown that the variability in the colour of the seeds belonging to same or different populations, seems to be regulated by epistatic genes. Casini (2003) reported variable sizes of seeds within *P. vulgaris* from different geographical areas and opined that ecological conditions such as extreme humidity, high temperatures, low sunlight, unbalanced supply of nutrients and poor soil fertility play an important role in causing infra-specific variability. On the basis of form, colour and size of the seeds of *P. vulgaris* and *P. lunatus* Gutiérrez *et al*. (2004) have distinguished six groups in populations present in Andean region of Venezuela.

In this study, we did not detect variability in seed size within the same populations. However, varietal differences were conspicuous in some of the taxa (*e.g. P. acutifolius* var. *acutifolius*, var. *latifolius*, var. *tenuifolius*). With regard to colour, we confirmed the variability within the same population or in distinct populations of the same taxon; whereas the form of seeds was found to be generally elliptical both in the same population and in distinct populations of the same taxon (three types of elliptic conditions refer to hilum: see number 12 in the key). More rarely, we encountered rhombic shapes (*P. coccineus* NI 1430, NI 859; *P. filiformis*, *P. hintonii* NI 716, NI 809; *P microcarpus*, *P. ritensis* NI 797A), broadly obovate shapes (*P. grayanus* NI 724, *P. ritensis* NI 795) and narrowly oblong shapes (*P. xanthotrichus* NI 1528). These findings are consistent with the work on *P. vulgaris* by Anderberg (1994) who described this species as being characterized by "reniform-ellipsoid shape, spotted, mottled and marbled colour, lustrous, glossy and smooth surface".

Unlike in *Origanum syriacum* L., a non leguminous species (De Leonardis *et al*., 2007), the traits related to the size of the seed (length, width, thickness) were directly proportional to the dimensions of all the other features investigated such as hilum and chalaza (Fig. 3). Seed size in cultivated crops is known to be usually greater than their wild taxa (De Leonardis *et al*., 1993; 1996). Our data also confirm the expected increased seed size of cultivated taxa (*P. acutifloius*, *P. coccineus*, *P. lunatus*, *P. polyanthus*, *P. vulgaris*) compared with wild taxa (Table 1).

The study allowed the development of an identification key composed of 23 morphotypes which are ascribable to specific taxa along with other two morphotypes that were ascribable to more taxa. If we consider the surface tegument of the seeds (result of genetic expression) the study confirms the uniformity in the expression of this trait in populations of the same taxon even when present in different habitats. These findings led us to cluster the taxa examined in three groups: 1) *P. angustissimus* group (rugulose seed coat) that includes two morphotypes, 2) *P. lunatus* group (tegument smooth with striae) which brings together 10 morphotypes; 3) *P. vulgaris* group (smooth seed coat without striae) that includes 13 morphotypes.

Based on our seed coat data, *P. lunatus* (smooth with striae) and *P. polystachios* (smooth without striae) are found in distinct taxonomic groups, in accordance with the work of Delgado-Salinas *et al*. (2006), but in disagreement with that of Freytag and Debouck (2002). Our observations demonstrate that *P. maculatus* and *P. ritensis* are morphologically distinguishable entities According to Freytag and Debouck (2002) only *P. maculatus* can be separated from the group containing *P. polystachios* species because of its seminal tegument.

P. vulgaris and *P. lunatus* appear to be taxonomically distant entities, findings which are not in agreement with what reported earlier in literature (Degreef & Baudoin, 1996; Duran *et al*., 2005; Delgado-Salinas *et al*., 2006; Sammour *et al*., 2007). The taxonomic affinities among these species reported by Marechal *et al*. (1978), based on a vast morphological study of the *Phaseolus*-*Vigna* complex, are partially validated in this study. Their study has shown that (i) the similarity between *P. vulgaris*, *P. coccineus* and *P. glabellus*, (ii) the taxonomic distance of the *Phaseolus*-*Vigna* complex from *P. lunatus*, (iii) the affinities between *P. angustissimus* and *P. filiformis* (both with rugulose seed coat) and (iv) the intermediate position of *P. acutifolius* with the complex *P. vulgaris*-*P. coccineus* and *P. lunatus*. However, our study demonstrates a greater affinity between *P. vulgaris*, *P. polystachios* var. *polystachios* and *P. ritensis* and between *P. lunatus* and *P. polyanthus*. The elaboration of the identification key based on morphobiometric characters and/or grouping into three affinity groups based on type of sculpture of tegument have allowed the identification of morphotypes that could be useful in biosystematic studies and for assisting breeders in marker-guided genetic selection of *Phaseolus*.

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