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Morphology of seeds and seedlings of four species of *Vigna* Savi (Leguminosae, Phaseolinae)

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ABSTRACT

Four neotropical species of *Vigna* Savi (Leguminosae, Phaseolinae) have potential value as forage crops or ornamentals and could be cultivated in tropical or subtropical areas, even on floodplains. In order to obtain useful data for their culture and taxonomy, the seed morphology, germination pattern (hypogeal or epigeal) and seedling development were studied. The studied species belong to different sections of the genus: *V. adenantha* (G.F.W. Meyer) Maréchal, Mascherpa & Stainier (Sect. *Leptospron*); *V. candida* (Vell.) Maréchal, Mascherpa & Stainier (Sect. *Sigmoidotropis*); *V. caracalla* (L.) Verdc. (Sect. *Caracallae*) and *V. luteola* (Jacq.) Benth. (Sect. *Vigna*). The seeds were collected during fieldwork conducted in northwestern and northeastern Argentina. The qualitative and quantitative characters of the seeds were registered, after which they were sown. The development of the emerged seedlings was followed, first in a greenhouse and thereafter in open field. We recorded the type of germination, the thigmotropic movements of the hypocotyl and of the stem, seedling architecture and plant longevity. These traits allowed us to differentiate the species and construct an identification key that could be useful for agronomic or floricultural purposes. The data obtained partially support the current taxonomic treatment of the genus.

Key words: *Vigna*, seed morphology, germination, seedlings, Argentina

Introduction

In Argentina, the genus *Vigna* Savi (Leguminosae) is represented by eight species (Palacios & Hoc 2001). The populations grow throughout the riparian forests from Misiones to Buenos Aires in the east and on the lower slopes of the subtropical cloud forests in the northwest; the former within the Paranaense Province and the latter within the Yungas Province, according to the phytogeographic regions defined by Cabrera (1976). In addition, populations of *V. luteola* have been recorded for disturbed sites from Salta to Córdoba, i.e., from northwestern to central Argentina (Hoc *et al.* 2007). Four species are economically important: *V. adenantha* and *V. luteola* are promising forage crops in subtropical or tropical areas (Fernández *et al.* 1988), even on floodplains, where their nitrifying properties are advantageous; and *V. adenantha*, *V. candida* and *V. caracalla* have potential value as ornamentals (Hoc & Ojeda 2013; Etcheverry, unpublished data).

Considering the economical potential, knowledge of the seed morphology, germination process, seedling development and plant longevity is necessary in order to cultivate these species. Such knowledge could also contribute to clarifying the taxonomy of the group. Anatomical and morphological characters are useful for distinguishing among species at the seed stage (Corner 1976; Alsina 1988; Juan *et al.* 1998). The same is valid for seedlings, for various taxonomic levels, down to the level of subfamily (Silva & Scatena 2011), genera (Rodriguez & Tozzi 2008) or species (Dottori *et al.* 2000; Perícola *et al.* 2004; Mendiondo & Amela García 2005). These characters can be employed to devise keys, such as those designed to categorize seeds or seedlings (Duke 1965, 1969; Csapody 1968) by family (Sánchez Sánchez & Hernández Cepeda 2004), subfamily (Kirkbride *et al.* 2003) or genus (Deginani 2001), as well as those designed to differentiate among plants that grow in a certain region (Marino *et al.* 2008; Zamora-Cornelio *et al.* 2010).

Various authors have emphasized the taxonomic relevance of the morphology and anatomy of the seeds of legumes. De Candolle (1825) distinguished the systematic value of the curvature of the embryo. Capitaine (1912) differentiated the three subfamilies by morphological features, and Corner (1951) underscored those findings with anatomical studies. Recently, Kirkbride *et al.* (2003) and Ubiergo & Lapp (2007) demonstrated the diagnostic value of seed characters at the generic and specific level. The seeds of Papilionoideae species have been described in many reports (Corner 1951, 1976; Gunn 1981; Alsina 1988; Kirkbride *et al*. 2003; Ubiergo & Lapp 2007). Gunn (1981) differentiated

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the type of arils among this group. Despite these numerous contributions, the seeds of only two species of *Vigna* have been analyzed (Kirkbride *et al*. 2003), neither of which was from the neotropics.

Seedlings of legumes have been described by several authors. Compton (1912) related structure, size, form, habit and morphology within each species. Duke & Polhill (1981) reviewed matters of ecology and systematics. Cotyledons of legumes can vary by morphology and function as well as in terms of their exposure at germination (Duke & Polhill 1981). Smith (1981) assigned seedlings to different tribes within the Papilionoideae by cotyledon venation pattern. According to Ye (1983), the seedlings of Papilionoideae can be categorized by morphological type (*Cinnamomum*, *Chimonanthus* or *Sophora*). However, there is as yet little knowledge of the germination and seedling morphology of *Vigna* spp. The characters reported to date are the display of two opposite unifoliate primary leaves (Maréchal *et al.* 1978) and the hypogeal cotyledons (Maréchal *et al.* 1981).

Beyra & Reyes Artiles (2004) pointed out that few characters can be used to distinguish the species within the genus *Vigna*; neither of them involved the seed nor the seedling in detail. The aim of the present work was to analyze the seed morphology, germination type, seedling development and plant longevity of four native species of *Vigna* in order to identify characters with taxonomic value.

Materials and methods

Materials

Seeds of *Vigna adenantha* (G.F.W. Meyer) Maréchal, Mascherpa & Stainier (Sect. Leptospron), *Vigna candida* (Vell.) Maréchal, Mascherpa & Stainier (Sect. Sigmoidotropis), *Vigna caracalla* (L.) Verdc. (Sect. Caracallae) and *Vigna luteola* (Jacq.) Benth. (Sect. Vigna) were collected during fieldwork conducted in northwestern and northeastern Argentina. The material was deposited in the Herbarium of the University of Buenos Aires and the Herbarium of the Instituto de Botánica Darwinion (codes, BAFC and SI, respectively; Thiers 2012) as follows (accession numbers precede herbarium codes):

Vigna adenantha: T. Meyer 755 (SI); E. Pueyrredón 17918 (SI); A. Schinini y C. Quarín 11545 (SI); P. Hoc 429 (BAFC); A. Burkart 7654 (SI).

Vigna candida: P. Hoc 404 (BAFC); A. Burkart 19597 (SI).

Vigna caracalla: A. Krapovickas *et al.* 28312 (SI); P. Hoc 403 (BAFC); A. G. Schulz 1188 (SI).

Vigna luteola: P. Hoc 401 (BAFC); P. Hoc 427, 428 (BAFC).

Seed characters

A quantitative-qualitative analysis of the seeds was performed before a germination experiment.

Quantitative characters. The length and width of thirty seeds of each species were measured under a stereoscopic microscope, with a metric scale; the thickness was measured with a Vernier caliper at the macroscopic scale.

For each quantitative character, ANOVA was performed using the GraphPad Prism 5 software.

Qualitative characters. The shape of the seeds, the hilum and the rim aril were described. The color was determined using the color chart devised by Kelly (1964).

To study the seed surface, the hilum and the rim aril were examined under scanning electron microscopy (SEM), the seeds were dehydrated in a graded ethanol series (50, 60, 70, 80, 90 and 100%), mounted on stubs, coated with gold-palladium alloy and observed with a field emission scanning electron microscope (Supra 40; Carl Zeiss, Oberkochen, Germany). The terminology employed to describe the seed surface follows Barthlott *et al.* (1998).

Seedling characters

Under laboratory conditions, we scarified the seeds by making a cut in the integument, placed them in Petri dishes previously prepared with a base of cotton and Whatman paper wetted with distilled water. After germination, the seedlings were transferred to pots containing 500 mm^3 of soil perlite at 3:1. Seedling stage was set based on the appearance of the leaves. Seedling development was recorded until the first (trifoliate) second leaf expanded and the lateral branches of the main axis began to grow (after approximately one month). We recorded the emergency of the cotyledons, as well as the growth pattern and thigmotropic movements of the hypocotyl and the stem. The length of the epicotyl and the length and width of the primary leaves (pair of opposite unifoliate leaves) were measured with a metric bar made of graph paper. The branching system was described after the development of the first axillary bud. The plants were then transferred to the field and their growth habit was studied for three consecutive years to assess architecture and longevity.

Results

Seed characters

The significant differences among the species, in terms of the quantitative characters of seeds, are shown in Tab. 1.

***Vigna adenantha*.** Seeds are 5.4-7.9 (6.98±0.62) mm long × 4.6-5.8 (5.09±0.31) mm wide × 2.8-4.9 (3.41±0.46) mm thick, truncate-elliptic, rhomboid or broadly rhomboid, with rounded vertex. Smooth, dull, light olive brown (94 l. OlBr) or moderate olive brown (95 m. OlBr) surface. Elliptic hilum. Rim aril with a triangular or broadly triangular expansion. Micropyle in the center of a triangular depression. Triangular or narrowly triangular lens, lobes flat or slightly elevated (Fig. 1, 2).

Table 1. ANOVA and Tukey's multiple comparison test of morphometric data related to the seeds of four *Vigna* species.

Seed character	*Vigna candida*	*Vigna caracalla*	*Vigna luteola*
Length		F=65.91	
Vigna adenantha	8.71*	0.13	16.83*
Vigna candida		8.84*	8.12*
Vigna caracalla			16.96*
Width		F=415.9	
Vigna adenantha	28.82*	18.95*	14.25*
Vigna candida		47.78*	14.57*
Vigna caracalla			33.20*
Thickness		F=63.05	
Vigna adenantha	14.91*	3.337	3.286
Vigna candida		18.25*	11.63*
Vigna caracalla			6.623*

*p<0.05.

SEM: In the seed (Fig. 9) the lax hilum cells disposed in uniseriate filaments (Fig. 10). Spirally arranged tubes developed in the rim aril (Fig. 11). Testa reticulate with quadrangular lumina (Fig. 12).

Vigna candida. Seeds are 4.7-6.3 (5.90±0.36) mm long × 2.4-3.0 (2.68±0.12) mm wide × 2.1-2.7 (2.43±0.14) mm thick, oblong, with slightly rounded vertex. Smooth, dull, light yellowish brown (76 l. yBr), dark orange-yellow (72 d. OY) or deep orange-yellow (69 deep OY), with scattered black (267 Bk) spots on the surface. Narrowly elliptic or narrowly oblong hilum, rim aril with a broadly triangular expansion, cleft at the apex. Micropyle situated at the edge of a spherical protuberance. Mounded, transversely elliptic lens, with a poorly developed groove (Fig. 3, 4).

SEM: In the seed (Fig 13) polyhedric densely disposed cells at the hilum near the rim aril (Fig. 14). The tubules of the seed coat are expanded and fused forming a reticulate pattern (Fig. 15). Testa surface perforate (lumina 1-2 µm in diameter) or fossulate (lumina 2 µm long. × 1 µm wide) (Fig. 16).

Vigna caracalla. Seeds are 6.1-7.7 (6.99±0.40) mm long × 6.1-7.7 (6.68±0.37) mm wide × 3.0-4.4 (3.63±0.35) mm thick, transversely elliptic or broadly oblong. Smooth, dull, deep yellowish brown (75 deep yBr), dark yellowish brown (78 d. yBr), deep brown (59 deep Br) or deep reddish brown (44 deep rBr) surface, with light yellowish brown (76 l. yBr), medium yellowish brown (77 m. yBr), deep reddish brown (44 deep rBr), brownish orange (54 brO), strong reddish brown (40 s. rBr) or black (267 Bk) spots. Sunken hilum with a cushion-shaped structure, rim aril with a trapezoidal expansion with or without a cleft margin. Sunken micropyle in the same depression that contains the hilum. Conspicuous, heart-shaped lens (Fig. 5, 6).

SEM. In the seed (Fig. 17) the cells of the hilum are disposed in uniseriate compact filaments (Fig. 18). Differentiated filaments in the surface fuse forming the rim aril towards the hilum (Fig. 19). Testa reticulate and granulate (Fig. 20).

Vigna luteola. Seeds are 3.3-5.0 (4.42±0.50) mm long × 3.0-4.9 (3.57±0.44) mm wide × 2.3-3.5 (3.02±0.22) mm thick, elliptic, transversely oblong or ovate. Smooth, dull, black (267 Bk) or deep grayish yellow brown (81 deep Gyish yBr) surface. Narrowly elliptic, elliptic, narrowly ovate or narrowly oblong hilum. Rim aril without expansion. Small micropyle, on a dome. Conspicuous, heart-shaped lens (Fig. 7, 8).

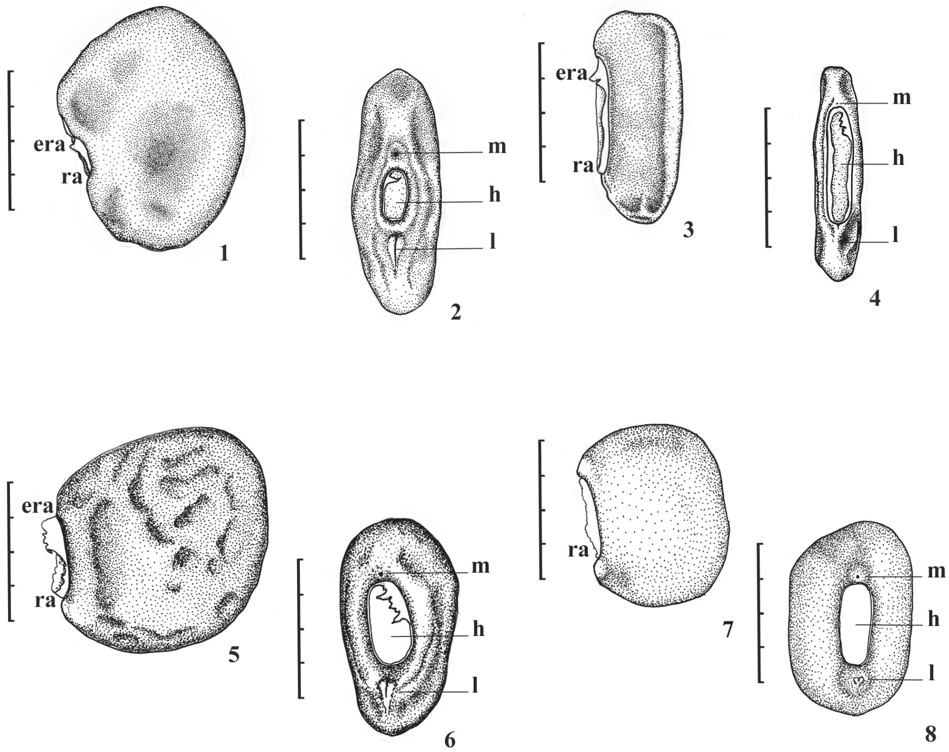
SEM: The cells of the hilum are disposed in moniliform uniseriate to triseriate interwoven filaments (Fig. 22). In the seed (Fig. 21) the elements of the reticulum are fused in a strictly striate pattern towards the rim aril (Fig. 23). Reticulate-striate testa (Fig. 24).

Seedling morphology

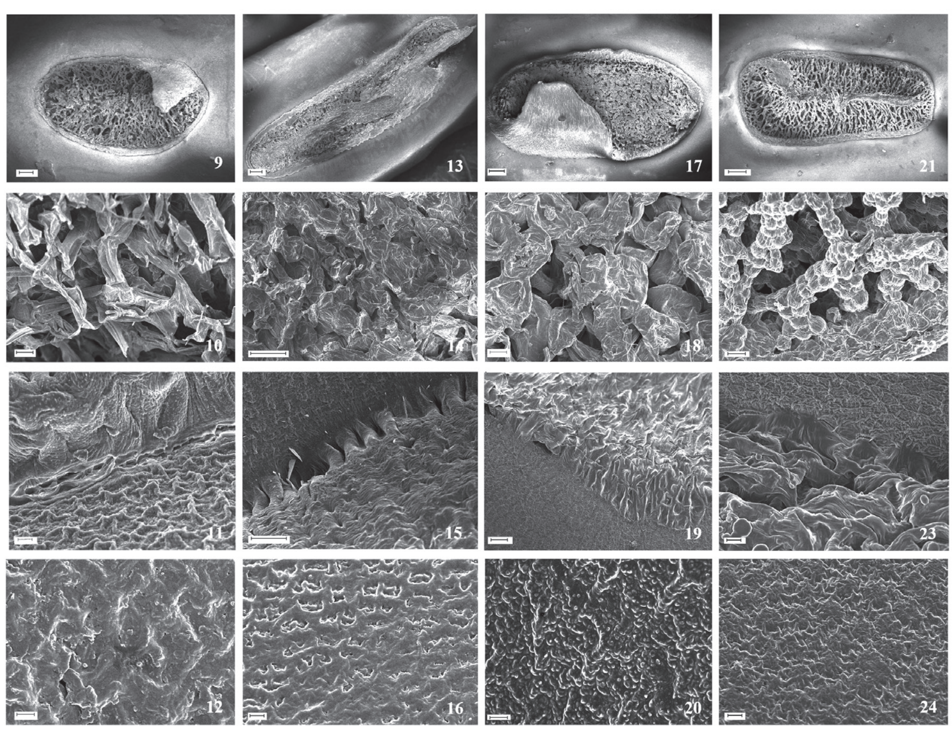
Vigna adenantha. Phanero-epigeal germination, fleshy cotyledons (prioritizing nutrient storage over photosynthesis), epicotyl 21-116 (66.17±6.78) mm long, primary leaves 18-35 (25.61±4.45) mm long × 18-36 (25.80±4.59) mm wide, simple, broadly ovate with acute apex and cordate base. Straight epicotyl, stem with thigmotropic movements from the node of the primary leaves. Branches develop after the 15th second leaf is produced (Fig. 25).

Growth pattern: with thickened hypocotyl, pluriannual.

Vigna candida. Phanero-epigeal germination, fleshy cotyledons, epicotyl 21-116 (45.81±4.87) mm long, primary leaves 24-38 (30.52±3.54) mm long × 16-29 (22.73±3.39) mm wide, simple, triangular to broadly triangular, with acute apex and cordate base. Straight epicotyl, stem with



Figures 1-8. Morphology of the seeds of *Vigna* species. Lateral and hilar view, observed with stereoscopic microscope: 1-2. *Vigna adenantha* (G.F.W. Meyer) Maréchal, Mascherpa & Stainier; 3-4. *Vigna candida* (Vell.) Maréchal, Mascherpa & Stainier; 5-6. *Vigna caracalla* (L.) Verdc.; 7-8. *Vigna luteola* (Jacq.) Benth. Scale bars = 4 mm.
era = expansion of the rim aril, **h** = hilum, **l** = lens, **m** = micropyle, **ra** = rim aril.



Figures 9-24. Surface topography of the seed with scanning electronic microscopy (SEM) of *Vigna* species: 9-12. *Vigna adenantha* (G.F.W. Meyer) Maréchal, Mascherpa & Stainier; 13-16. *Vigna candida* (Vell.) Maréchal, Mascherpa & Stainier; 17-20. *Vigna caracalla* (L.) Verdc.; 21-24. *Vigna luteola* (Jacq.) Benth.; 9,13,17,21. Hilum and rim aril; 10,14,18,22. Hilum cells (detail); 11,15,19,23. Transition from testa to rim aril; 12,16,20,24. Testa. Scale bars: 9,13,17,21 = 100 μm; 10,18,22,15,19 = 10 μm; 14 = 30 μm; 11,23,16,24 = 2 μm; 12,20 = 1 μm.

briefly developed internodes between the primary leaves and the 5th-6th second leaves, with thigmotropic movements from the 6th node. Branches develop since the primary leaves are produced (Fig. 26).

Growth pattern: with thickened hypocotyl, pluriannual.

Vigna caracalla. Crypto-hypogeal germination, nutrient-rich cotyledons, epicotyl 30-83 (37.56±8.84) mm long, primary leaves 21-80 (49.37±17.82) mm long × 20-55 (37.21±9.78) mm wide, simple, ovate, with emarginate apex and shortly attenuate or broadly triangular base. Straight epicotyl, stem with short internodes between the primary leaves and the 3rd second leaf, with thigmotropic movements from this node, not branched during the first year of development (Fig. 27). Primary leaves occasionally bifoliate of trifoliate rather than unifoliate.

Growth pattern: with thickened hypocotyl, pluriannual.

Vigna luteola. Crypto-hypogeal germination, nutrient-rich cotyledons, epicotyl 10.1-140 (110.08±5.80) mm long, primary leaves 17-32 (26.20±3.69) mm long × 9-16 (12.17±1.80) mm wide, ovate, with acute apex and shortly attenuate base. Epicotyl with thigmotropic movements, stem with branches from the 10th second leaf (Fig. 28).

Growth pattern: without thickened hypocotyl, annual.

We based the key below on the seed and seedlings characters, as well as on the plant longevity of the species.

Key to the species

1. Annual growth pattern, without thickened hypocotyl, epicotyl with thigmotropic movements. Seed without an expansion of the rim aril . Testa reticulate-striate.......... ***V. luteola*** **(Sect. *Vigna*)**

1'. Pluriannual growth pattern, with thickened hypocotyl, straight epicotyl, without thigmotropic movements. Seed with an expansion of the rim aril. Testa not reticulate-striate.......... **2**

2. Seeds 2.4-3.0 (2.68±0.12) mm wide × 2.1-2.7 (2.43±0.14) mm thick, oblong. Straight stem, with briefly developed internodes between the primary leaves and the 5th-6th second leaves. Thigmotropic movements from the 6th node. Branches develop after the primary leaves are produced. Testa perforate-fossulate.......... ***V. candida*** **(Sect. *Sigmoidotropis*)**

2'. Seeds wider than 2.4-3.0 mm and thicker than 2.1-2.7 mm, truncate-elliptic, rhomboid or broadly rhomboid with rounded vertex, transversely elliptic or broadly oblong. Stem with thigmotropic movements from the node corresponding to the primary leaves or the 3rd second leaf. Branches develop from the 15th node, or branching does not occur. Testa not perforate-fossulate.......... **3**

3. Seeds 4.6-5.8 (5.09±0.31) mm wide, surface without colored spots. Elliptic hilum, rim aril with a triangular or broadly triangular expansion. Phanero-epigeal germination. Stem with thigmotropic movements from the node corresponding to the primary leaves. Branches develop from the 15th second leaf. Primary leaves cordate at the base. Testa reticulate, lumina without granules.......... ***V. adenantha*** **(Sect. *Leptospron*)**

3'. Seeds 6.1-7.7 (6.68±0.37) mm wide, surface with colored spots. Sunken and cushioned hilum, rim aril with a trapezoidal expansion with its margin cleft or not. Crypto-hypogeal germination. Straight stem, with short internodes between the primary leaves and the 3rd second leaf, with thigmotropic movements from this node, not branched during the first year of development. Primary leaves shortly attenuate or broadly triangular at the base. Testa reticulate, lumina with granules. .... ***V. caracalla*** **(Sect. *Caracallae*)**

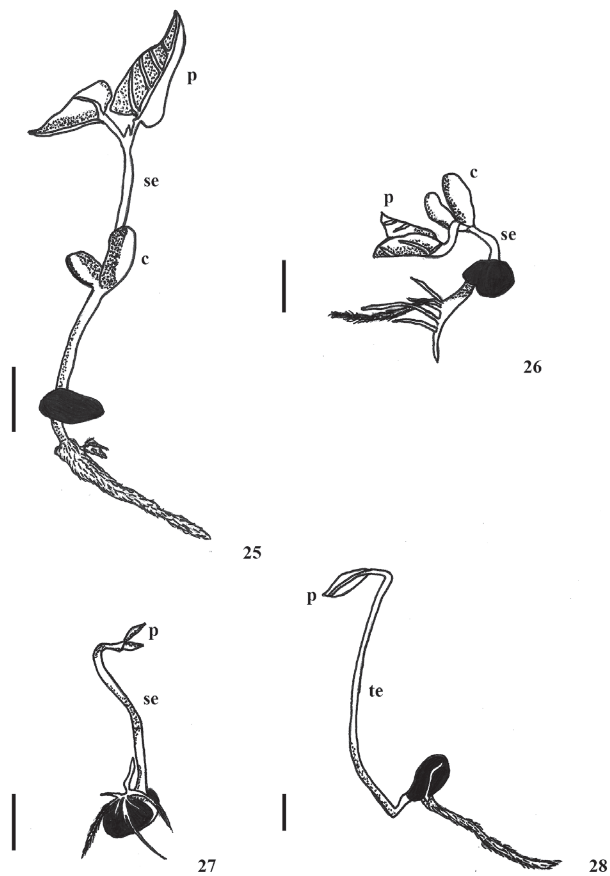
Discussion

The seeds of all four species studied, except those of *V. luteola*, have an expansion of the rim aril, which corresponds to the dry tongue aril typified by Gunn (1981) for some Papilionoideae taxa.

The morphological characters of the seedlings of the studied species correspond to the usual ones in Phaseoleae enumerated by Duke & Polhill (1981). The seedlings of *V. caracalla* and *V. luteola* can be assigned to the *Cinnamomum* type; and those of *V. adenantha* and *V. candida* can be assigned to the *Sophora* type (Ye 1983). *Vigna luteola* is assigned to a different subgenus than are the rest of the species, so these characters do not fit the actual infrageneric categories.

Occasional variation in the blade division of the primary leaves has also been observed for several species of *Sesbania* (Veasey *et al.* 1999). This could be a teratological case. Future studies will allow to corroborate or disallow this assumption.

Maréchal *et al.* (1981) mentioned that the germination type in the *Phaseolus*-*Vigna* complex allows the differentiation of the genera included in the group (epigeal in *Phaseolus* and hypogeal in *Vigna*). In contrast, in the present study, we identified both phanero-epigeal and crypto-hypogeal germination within *Vigna*. Duke (1969) stated that many genera have species with both germination types. Two of the species studied here, included in the subgenus *Sigmoidotropis*, exhibited phanero-epigeal germination, whereas the other two, included in the subgenera *Sigmoidotropis* and *Vigna*, exhibited crypto-hypogeal germination. Therefore,



Figures 25-28. Morphology of the seedlings and type of germination of *Vigna* species: 25. *Vigna adenantha* (G.F.W. Meyer) Maréchal, Mascherpa & Stainier; 26. *Vigna candida* (Vell.) Maréchal, Mascherpa & Stainier; 27. *Vigna caracalla* (L.) Verdc.; 28. *Vigna luteola* (Jacq.) Benth.; 25,26. Epigeal germination; 27,28. Hypogeal germination. Scale bars: 25 = 6.98 mm; 26 = 5.90 mm; 27 = 6.99 mm; 28 = 4.42 mm.
c = cotyledons (nutrient storage turning photosynthetic), **te** = epicotyl with thigmotropic movements, **p** = primary leaves, **se** = straight epicotyl.

germination type is not useful for distinguishing between *Vigna* and *Phaseolus*, not even for distinguishing between subgenera within *Vigna*.

Seed size and shape, as well as hilum shape, had diagnostic value at the species level, in accordance with the findings of Alsina (1988) and Paulino *et al.* (2010) for other genera of legumes, underscoring the importance of these characters for taxonomy in the family Fabaceae. Although the testa of all the species consists of crusts corresponding to the classification of Barthlott *et al.* (1998), its varied ornamentation was useful for differentiating among species, as in other Papilionoideae (Alsina 1988).

The seed characters that contributed to the differentiation of the studied *Vigna* species were the width, thickness, shape, as well as the presence and shape of the rim aril expansion and of the coat sculpturing. Although the coloration pattern also differed among species, this feature should be confirmed through studies of seeds from additional populations, as variation in this trait can occur within a single species, exemplified in various genera of legumes (Werker, 1997). Our SEM studies allowed us to describe the morphology of the testa in detail, as well as the transition from the seed coat to the rim aril. The seed characters described may be useful for agronomic purposes, such as seed recognition and quality analysis. Plant longevity also differed among species belonging to different subgenera, *V. luteola* (subgen. *Vigna*) being annual, whereas the other three species (subgen. *Sigmoidotropis*) were pluriannual. Further studies of this trait in more *Vigna* spp. are warranted in order to determine whether the genus merits subdivision. The germination type, the growth pattern of the seedlings during the first year of development and the (previously unknown) plant longevity are also relevant for future taxonomic, systematic and agronomic treatments.

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