Two *Vigna* Species Native to Argentina with Ornamental Potential

P.S. Hoc and F.S. Ojeda
Facultad de Ciencias Exactas y Naturales
Universidad de Buenos Aires
PROPLAME-PHRIDEB (CONICET)
Buenos Aires
Argentina

Abstract

***Vigna candida* (Vell.) Maréchal, Mascherpa and Stainier and *V. adenantha* (G.F.W. Meyer) Maréchal, Mascherpa and Stainier have potential as ornamentals and could be used as forage crops in warm areas. They are scandent, stolonate and pluri-annual. In both species the flowering period begins in springtime and last until autumn. The flowers are flag blossoms, disposed in 5-10-flowered racemes of 10 cm long. The standard and the keel are white, the wings are white with violet nectar guides; a variation in the color pattern of these petals occur between the species. During the anthesis, that lasts 1-2 days, an anular nectary that surround the ovary secrete the nectar offered as reward. Legitimate flower visitors are species of *Xylocopa*, *Centris* and *Bombus* (*Hymenoptera*), because of only these individuals can trigger the system of pollen transfer; also, nectar and pollen thieves or robbers were detected but did not affect the reproductive success. In *V. candida*, aroma is also emitted as other secondary attractant. Experiments of controlled pollinations were carried out in order to know the reproductive system of each species. *V. adenantha* is self-compatible but the reproductive success reach the 100% when pollinators visit the flowers, oppositely, in *V. candida*, which is self-incompatible, if pollinators do not work, fruits and seeds cannot be produced. Because of the growing pattern, phenology, the beautiful and conspicuous flowers, and the nitrifying capacity, both species should be appreciated as ornamentals in warm or temperate areas, even in flooded land.**

INTRODUCTION

Eight species of *Vigna* Savi (*Fabaceae*) are represented in Argentina (Palacios and Hoc, 2001). The populations of each species are found along the river edges forests at the east (Paranaense Province, sensu Cabrera, 1976), but only the populations of *V. adenantha* (G.F.W. Meyer) Maréchal, Mascherpa et Stainier reach Buenos Aires. The western populations of *V. adenantha* grow in the slopes of the cloud forests of the Yungas (Cabrera, 1976).

Vigna candida (Vell.) Maréchal, Mascherpa et Stainier and *V. adenantha* are perennial, and develop a hypocotyl, but the stem in the first species is straight while in the later exhibits haptotropic movements (Ojeda et al., 2012). In both species the inflorescences are racemes with 9 or more nodes, in each one of them a short and sphaerical axis bear two flowers and up to seven extrafloral nectaries (Ojeda and Hoc, 2008).

Vigna adenantha has a potential to grow as a forage crop in subtropical or tropical areas (Fernández et al., 1988), and perhaps as an ornamental plant (Hoc and Ojeda, 2011) and *V. candida* as ornamentals (Hoc and Ojeda, 2011). Although studies on the floral biology of paleotropical species of *Vigna* were performed by Hedström and Thulin (1986), Kumar (1976), and Gopinathan and Babu (1987), the knowledge in the neo-tropical species (Di Stilio et al., 1991; Hoc et al., 1993; Agulló et al., 1994) is not readily available. In order to know the floral biology, reproductive system, and reproductive success of *V. adenantha* and *V. candida*, this study was undertaken.

MATERIALS AND METHODS

Plant Materials and Culture

Field work was carried out since the spring of 2009 in Concordia (Entre Ríos Province, Argentina). Germplasm was collected in Concordia and in Salta, Argentina. Experiments were carried out in the field of the University of Buenos Aires. Herbarium vouchers were deposited in the BAFC Herbarium (Holmgren et al., 1990).

Seeds were scarified after cutting the opposite side of the hilum, and without any sterilization, placed in Petri dishes with cotton and moistened Whatman paper, neither fungal nor bacterial contamination were detected. After germination in seven days, the seedlings were transferred to pots (500 mm^3) filled with a growing medium of soil and perlite (50:50, by volume). In a greenhouse without temperature control the seedlings development was recorded until the first 3-foliolated leaf expanded and the lateral branches of the main stem started to grow. Afterwards, the plants were transferred to the open field and their growing conditions were monitored. Temperatures ranged from 14.3 to 22.6°C and rainfall was 1282.4 mm per year. Thirty plants of each species were maintained under culture until the present. This field is ecologically compatible with the area of origin of both species.

Nectar Secretion and Stigma Receptivity

Stigma receptivity was tested as described by Osborn et al. (1988), taking as a positive result the bubbling in the stigma in presence of hydrogen peroxide. The sequence of the anthesis was followed since the beginning using hand lenses and a stereo-microscope (Hoc and Amela García, 1998) and floral phases and visitors were recorded on ten flowers of ten plants of each species. The spectrum, frequency and behaviour of floral visitors on each plant were recorded during the anthesis.

The amount of the nectar secreted was collected and measured with capillary tubes of 0.5 mm in diameters and then the volume was calculated. To perform this test, flower buds were isolated and the volume of nectar was recorded from anthesis to senescence. The concentration of sugars was determined using a hand refractometer for low volumes (model Eclipse, Bellingham and Stanley, UK).

Reproductive System (Pollination Experiment)

The following 3 treatments were performed, by selecting flower buds approaching anthesis. Some flower buds were exposed to visitors (open pollination). Other flower buds were self-pollinated by the researcher (induced autogamy). Other ones were bagged to check for spontaneous autogamy. Flowers remained until senescence if pollination did not occur or until seed set when pollinated. For each treatment the reproductive success was evaluated using the following formula: (number of seeds / number of ovules) ×100.

Floral Attractants

The colour pattern of the flowers was determined using the color tables of Kelly (1965). An olfactory test was carried out to determine which floral parts emitted fragrance. Each floral piece was placed in sterile glass bottles for 15-20 minutes, and then the bottles were opened to test with the researcher nose.

RESULTS

Vigna adenantha

1. Flowers at Anthesis and Rewards. The flowers are erect flag-blossoms (Faegri and Van der Pijl, 1979). At anthesis, the hooded standard has its margin turned back. Its limb is white (263) in the center, and very pale purple (226) towards the margins; between the limb and the claw there are brilliant yellow green (116) nectar guides. The limb of the wings is white with very deep reddish purple (239) bands. The left wing is the alighting place, both wings are adnated to the keel at their claws (epidermal papilla of both pieces interweave). The keel is white; its apex is coiled 540° to the left. At this moment, the anthers dehisce and the pollen is deposited in the brush of hairs that the style has below the stigma, which is terminal and begins to be receptive. The nectar (reward) is secreted by a nectary disk that surrounds the base of the ovary. The anthesis starts approximately at 8:00 a.m. and finishes at 5:00 p.m. or earlier when the plants are shaded in the afternoon (Fig. 2a).

2. Nectar Secretion. In average, 1.85 µl of nectar is produced during the anthesis, and the sugar percentage recorded is of 42.17% (n = 10).

3. Pollination Process and Behaviour of Pollinators. Pollinators started to visit the flowers since 8:00 am and leave when the patch is shaded. They reach the nectar accumulated at the base of the ovary alighting on the left wing and pressing it downwards with their anterior legs, and introducing its tongue in the staminal tube. In this way, the keel is forced downwards and thus the stigma, stylar brush of hairs and upper free parts of the stamens protrude from the keel touching the left side of the dorsal mesopronote and wings of the visitor. As the visitors leave the flower, the left wing and the keel recover their original position. The insects work quickly, spending no more than two seconds per flower. *Xylocopa ordinaria*, *Centris* sp. and *Bombus atratus* are considered as legitimate visitors (Fig. 2c, d, g). *Xylocopa ordinaria* carry pollen of *V. adenantha* on the dorsal part of the thorax, on the tegula and the wings of the same side. As a result of the process of cleaning that the individuals perform, part of the pollen is transferred to the hind of legs (Fig. 1 a, b, c).

In *Centris* sp. a well delimited spot of pollen of *V. adenantha* can be observed in the center of the dorsal thorax. Also, illegitimate visitors, as the individuals of *Trigona* sp. are present in the flowers since 12:30 to 4:30 pm They do not trigger the flowering mechanism because alight on the nectar guide of the left wing, walk towards the keel where gather pollen. (Fig. 2h). Also, individuals of *Apis mellifera* visit the flowers but they do not trigger the pollination mechanism.

4. Post Pollination. Following pollination, the ovary start to grow, at this time, the standard folds its limb, which turns brilliant yellow (83), and covers the rest of the floral. The wings parts also turn brilliant yellow, but the bands turns to gray-purplish pink (253). The stigma is not receptive, when the pollen have been removed even if pollinators visited the flower. The legitimate visitors avoid these flowers. A pressure made on the left wing exposes the stigma and the brush of hairs, but the flower pieces do not recover their original position. If pollination did not occur, the flower withers. (Fig. 2k).

5. Reproductive System Success. The seed set improves after the induced autogamy treatment. The results of the spontaneous autogamy indicate that the proximity of pollen grains presented near the stigma do not implies that self-pollination should spontaneously occur without a triggering mechanism (Table 1).

Vigna candida

1. Flowers at Anthesis and Rewards. In the flower recently opened the standard is hooded, with its margin turned back; the limb is white (263), and brilliant violet (206) towards the margins, between the limb and the claw there are brilliant greenish yellow (98) nectar guides. The limb of the wings is white (263) with strong violet (207) bands. Both wings are adnated to the keel at their claw; the right wing is the alighting place. The keel is white (263), its apex is “S” shaped and turned to the right. At this moment, the anthers dehisces and the pollen is presented in the brush of hairs that the style has below the stigma, which is terminal and begins to be receptive, the nectar (reward) is secreted by a nectary disk that surrounds the base of the ovary. A citric odour is emitted during the anthesis which start at 8:00 am and finish at 5:00 pm or when the patch is shaded. (Fig. 2b).

2. Nectar Offered. In average, 2.37 µl (n = 10) was collected during the anthesis, and the sugar percentage recorded is of 45.8%.

3. Pollination Process. Behaviour of the Legitimate Floral Visitors. The pollinators start to visit the flowers since 8:00 am and leave at 5: 00 pm or when the patch is shaded.

They reach the nectar accumulated at the base of the ovary alighting on the right wing, pressing it downwards with their anterior legs and introducing its tongue in the staminal tube. In this way, the keel is forced downwards (epidermal papilla of both parts interweave), and thus the stigma, stylar brush of hairs and upper free parts of the stamens protrude from the keel's apex touching the right side of the dorsal thorax of the insect. As the visitor leave the flower, the right wing and the keel recover their original position. Individuals of *Xylocopa ordinaria, Centris* sp., *Thygater* sp. and *Bombus atratus* are legitimate visitors (Fig. 2e, f, i). *Xylocopa ordinaria* carry pollen of *V. candida* on the dorsal part of the thorax (Fig. 1, d), on the tegula and the wings of the same side and on the third pair of legs (Fig. 1e).

Sometimes, some individuals of *Bombus atratus* alight on the left wing of the flower, sucking nectar but not triggering the pollen transfer mechanism, so, they act as gatherers. Also, in some populations illegitimate visitors, as individuals of *Apis mellifera*, alight on the calyx which previously was perfored by some individuals of Euglossinae and gather the nectar (Fig. 2j).

4. Post Pollination. Following pollination, the ovary starts to grow, at this time, the standard fold its limb, which turns moderate orange yellow (71). The wings turn moderate orange yellow (71), but the bands turns to deep reddish purple (238). The stigma is not receptive and the pollen was almost completely removed. The legitimate visitors avoid these flowers. A pressure made on the right wing exposes the stigma and the brush of hairs, but the flower pieces do not recover their original position. If pollination did not occur, the flower abscises (Fig. 1 l).

5. Reproductive System Success. Only the open pollination treatment, produced seeds. The results of the spontaneous and the induced autogamy treatment show that neither the proximity of pollen grains present near the stigma nor the triggering mechanism implies that self-pollination should occur (Table 1).

DISCUSSION

The flowers in both species have the structure, color and reward characters of the flag-blossoms adapted to medium to large sized bees (Faegri and Van der Pijl, 1979). The pollen is presented on stylar subapical trichomes and is transferred to the dorsal side of the pollinators, and during this process the stigma contact the pollen grains carried there. During the anthesis, the triggering mechanism here descript resist many visits, as observed by Hoc et al (1993) in *V. longifolia* (Benth.) Verdc. and Etcheverry (2005) in *V. caracalla* (L.) Verdc. This presentation and transference of the pollen was clarified by Endress (1999) as an economical pollination mechanism evolved in many Papilionoideae. The nectar concentration agrees with the previous report for melitophilous plants; the percentage of sugars is constant throughout the anthesis (Cruden et al., 1983). Rain or drizzle did not affect nectar composition or pollen, because the keel and the staminal tube protect them, according with the observations on *Phaseolus augusti* Harms and *P. vulgaris* L. var. *aborigineus* (Burkart) Baudet (Hoc and Amela, 1998, 1999). Anthesis occurs during the same period of the day in both species and they share the same pollinators (*Xylocopa* sp. and *Centris* sp.), but cross pollinations did not occur. As the keel is coiled to the left in *V. adenantha* and turned to the right in *V. candida*, these species differ in the alighting wing; in consequence, the pollen is transferred as spots to different sides of the dorsal thorax of the pollinators.

It can be inferred that if some genetic compatibility exists between the two species, the hybridization is impossible, according to Endress (1999) who stated that in the asymmetric flowers of the Papilionoideae genetic (specific) isolation exists. The floral morphology to differentiate Subgenera and Sections within the genus (Palacios and Hoc, 2001; Maréchal et al., 1981) indeed points out the incredible evolutionary paths developed during the speciation of the species that in many cases grow in sympatry.

In the species here studied gathering of pollen or nectar occur but do not affect the offer of reward as Hoc et al. (1993) observed. In *V. candida* fragrance is emitted and acts as a secondary attractant for pollinators. In *V. candida* the results of the spontaneous autogamy treatment indicates that the proximity of pollen grains presented near the stigma do not imply that self-pollination should spontaneously occur, so, *V. candida* is self-incompatible, and the action of a visitor is needed to produce seeds. The induced autogamy treatment performed here confirmed this asseveration. *V. adenantha* is self-compatible, because of seeds are produced after free pollination, induced autogamy and spontaneous autogamy treatments. Although, spontaneous autogamy produced the lowest seed set, perhaps the proximity of the hair brush with pollen do not warrant an efficient transfer to the stigma if the triggering mechanism do no act. Present results show a higher production of seeds after de induced autogamy, is possible that the bagging of the flowers after the triggering treatment acted as a protection against frugivores and other predators.

Because of the growing pattern, phenology, the beautiful and conspicuous flowers, and the nitrifying capacity, both species should be appreciated as ornamentals in warm or temperate areas, even in flooded land.

Literature Cited

Agulló, M.A., Hoc, P.S. and Brizuela, M.M. 1994. Síndrome floral en *Vigna candida* (Leguminosae). S.T.Nº 16. VI Congreso Latinoamericano de Botánica. Mar del Plata, Argentina. 2-8 October. p.805.

Cabrera, A.L. 1976. Regiones Fitogeográficas Argentinas. In: L.R. Parodi (ed.), Enciclopedia Argentina de Agricultura y Jardinería. 2(1):1-85, f. 1-3 (2^{nd} ed.). Ampliada y actualizada por W.F. Kluger. Acme. Buenos Aires.

Cruden, R.W., Hermann, S.M. and Peterson, S. 1983. Patterns of nectar production and plant-pollinator coevolution. In: B. Bentley and T. Elias (eds.), The Biology of Nectaries. Columbia University Press, New York.

Di Stilio, V., Agulló, M.A., Brizuela, M.M., Hoc, P.S. and Palacios, R.A. 1991. Relación unidad de polinización-visitantes florales en *Vigna adenantha*. Resúmenes XXIII Jornadas Argentinas de Botánica. San Carlos de Bariloche, Argentina. 14-18 October. p.21.

Endress, P. 1999. Symmetry in flowers: Diversity and evolution. Int. J. Plant Sci. S3-S23.

Etcheverry, A.V. 2005. Biología reproductiva de *Vigna caracalla* (*Fabaceae*). PhD Thesis. FCEN, Universidad de Buenos Aires, Buenos Aires.

Faegri, K. and Van der Pijl, L. 1979. The Principles Pollinations Ecology. Pergamon Press, Oxford.

Fernández, J., Benítez, C.A., Pizzio, R.M. and Royo Pallares, O. 1988. Leguminosas forrajeras nativas del este de la provincia de Corrientes. Serie Técnica No. 26. Estación Experimental Agropecuaria Mercedes (Corrientes), INTA, Argentina.

Gopinathan, M.Ch. and Babu, R. 1987. Breeding systems and pollination in *Vigna minima* (Leguminosae, Papilionoideae). Pl. Syst. Evol. 156:117-126.

Hedström, I. and Thulin, M. 1986. Pollination by a hugging mechanism in *Vigna vexillata* (Leguminosae, Papilionoideae). Pl. Syst. Evol 154:275-283.

Hoc, P.S., Di Stilio, V., Agulló, M.A., Brizuela, M.M., Palacios, R.A., Hazeldine, P. and Genise, J.F. 1993 Biología floral de *Vigna longifolia* (*Leguminosae*, *Phaseoleae*). Darwiniana 32:27-39.

Hoc, P.S. and Amela García, M.T. 1998. Floral biology and reproductive system of *Phaseolus augusti* (Fabaceae). Beitr. Biologie d. Pflanzen/ Duncker & Humblot. 70: 121-140.

Hoc, P.S. and Amela García, M.T. 1999. Biología floral y sistema reproductivo de *Phaseolus vulgaris* var. *aborigineus* (*Fabaceae*). Rev. de Biol. Trop. 47:53-61.

Holmgren, P.K., Holmgren, N.H. and Barnett, L.C. 1990. Index Herbariorum. Part I: The herbaria of the world: 1-693. Regnum vegetabile 120. New York Botanical Garden, New York.

Kelly, K.L. 1965. ISCC-NBS. Color-name Charts Illustrated with Centroid Colors. Standard Sample No. 2106 Suppl. to Nat. Bur. Standards Circ. 53. U.S. Government Printing Office, Washington, D.C.

Kumar, P., Prakash, R. and Haque, M.D.F. 1976. Floral biology of cowpea (*Vigna sinensis* L.). Tropical Grain Legume Bull. 6:9-11.
Maréchal, R., Masherpa, J.M. and Stainier, F. 1981. Taxonometric study of the *Phaseolus-Vigna* complex and related genera. p.329-335. In: R.M. Pollhill and P.H. Raven (eds.), Adv. in Legume Syst. 1.
Ojeda, F.S. and Hoc, P.S. 2008. Morfología de los nectarios extraflorales y sus posibles implicancias taxonómicas en especies nativas de *Vigna* (*Leguminosae*). Inter. J. Morph. 26:769.
Ojeda, F.S., Hoc, P.S. and Amela García, M.T. 2012. Species of *Vigna* (*Leguminosae*, *Phaseoleae*) in Argentina. Morphology of seeds and seedlings. Acta Bot. Bras. (in press).
Osborn, M.M., Kevan, P.G. and Lane, M.A. 1988. Pollination biology of *Opuntia polyacantha* and *Opuntia phaeacantha* (*Cactaceae*) in Southern Colorado. Plant Syst. and Evol. 159:85-94.
Palacios, R.A. and Hoc, P.S. 2001. *Phaseoleae*. In: Proflora. Flora Fanerogámica Argentina. Fasc. 75, 128. *Fabaceae*, parte 12. CONICET, Buenos Aires.

Tables

Table 1. Relative Reproductive Success (RRS).

Species	No.	No. of seeds		RSS (%)
		Seeds	Ovules	
		Open pollination		
V. adenantha	54	2.320	4.294	54
V. candida	190	2.473	3.122	79
		Induced autogamy		
V. adenantha	5	48	54	89
V. candida	10	0	0	0
		Spontaneous autogamy		
V. adenantha	10	20	194	10
V. candida	10	0	0	0

Figures

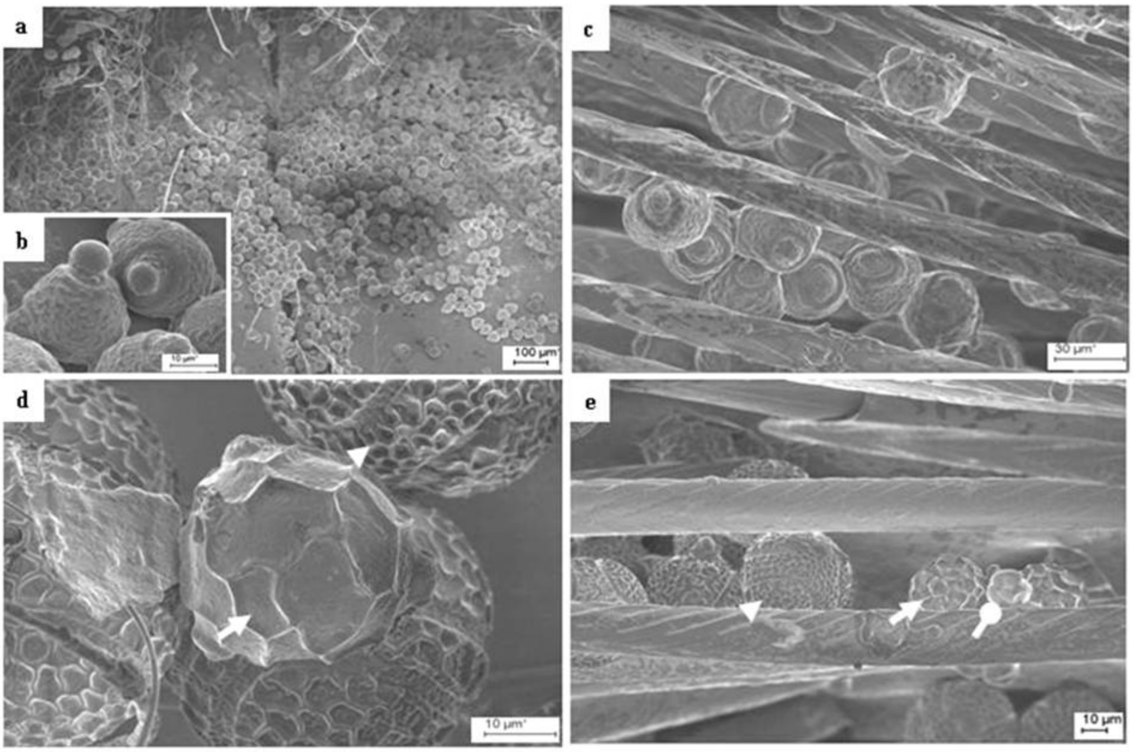

Fig. 1. Pollen transported by *Xylocopa ordinaria*: a, pollen of *V. adenantha* deposited in dorsal prothorax and metathorax; b, detail of a; c, pollen of *V. adenantha* in the third pair of legs; d, pollen of *V. candida* and *Passiflora caerulea* deposited in dorsal thorax; e, pollen of *V. candida* (arrow), *P. caerulea* (head of arrow) and *Solanum bonariensis* (circular arrow) in third pair of legs.

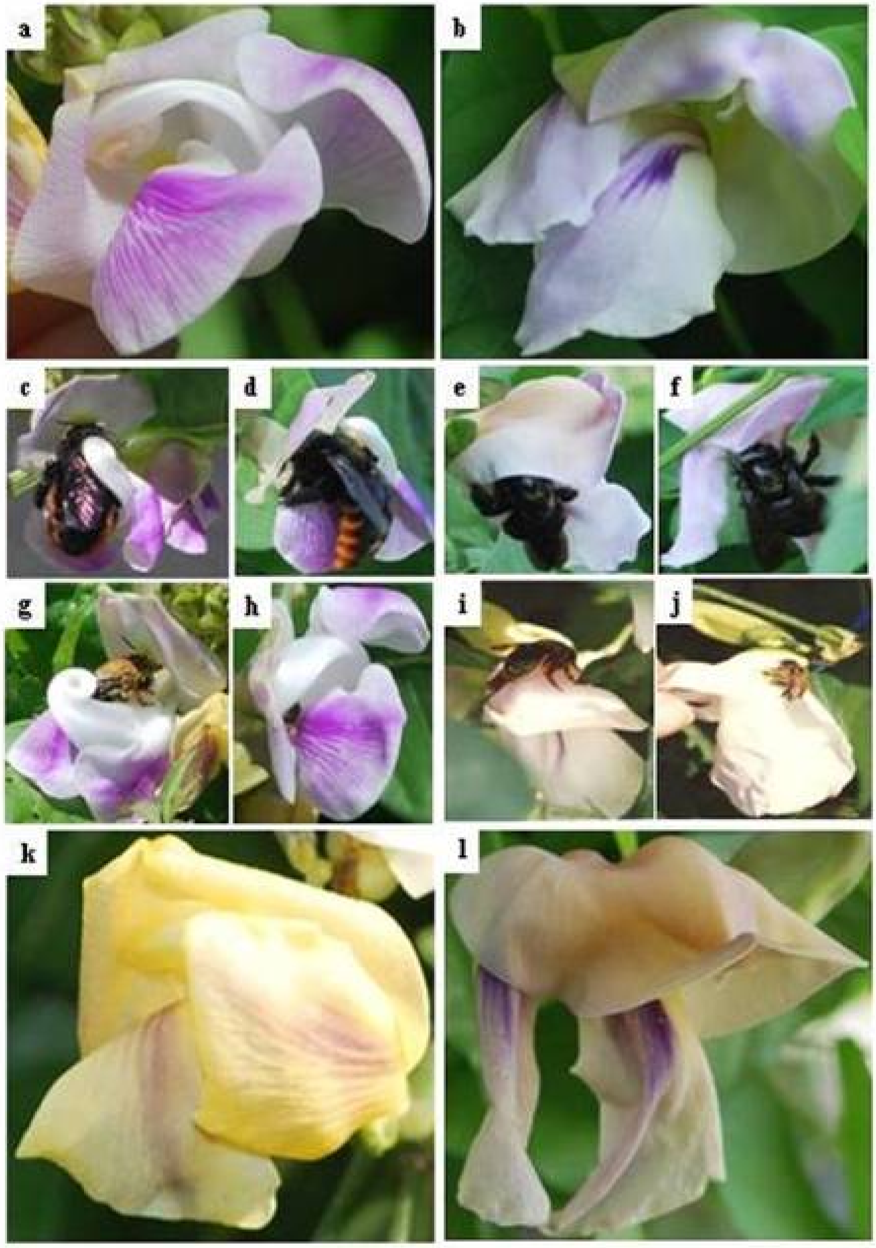

Fig. 2. Flower morphology and floral visitors. *Vigna adenantha*: a, flower in anthesis; c, d, *Xylocopa ordinaria*; g, *Centris* sp.; h, *Trigona* sp.; k, post-pollination syndrome. Pollinators: *Xylocopa ordinaria* and *Centris* sp. Pollen robbers *Trigona* sp. *Vigna candida*: b, flower in anthesis; e, f, *Xylocopa augusti*; i, *Ptyloglossa* sp.; j, *Apis mellifera*; l, post-pollination syndrome. Pollinators: *Xylocopa augusti*, *Centris* sp. and *Ptyloglossa* sp. Nectar robbers: *Apis mellifera*.