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Full Length Research Paper

Aspects of the floral biology and pollen properties of *Vigna unguiculata* L. Walp (Fabaceae)

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The present work had the objective of collecting information pertaining to the floral biology and properties related with the viability and germinability of black-eyed bean (*Vigna unguiculata*) pollen grains. The study was conducted in the municipal area of Cruz das Almas, Bahia, Brazil during the months of April to June of the year 2011. In the climate and soil conditions of the study area, *V. unguiculata* anthesis is diurnal, occurring from 5.30 to 9.30 a.m. Over 90% of pollen grains remained viable, but the germinability rate was under 40% *in vitro*. This information is relevant to subsidize bean crop pollination and genetic improvement programs.

Key words: Horticulture, genetic resources, pollination.

INTRODUCTION

Vigna unguiculata L. Walp is a species from the Fabaceae family known technically as cowpea bean, and presents many varieties. It is a rustic plant, tolerant with the water regime in the semiarid region and undemanding when it comes to soil fertility. Reports account that the species came from Africa and was introduced in Brazil in the 16th century (IPAHN, 2011). It expanded primarily throughout the Northeast region and later on throughout the rest of Brazil, and is chiefly produced in Bahia and the State of Pernambuco in areas that add up to 1.2 million hectares, followed by some northern states, which add up to 55.8 thousand hectares (Felippe, 2007; Silva, 2005). Popular local names for *V. unguiculata* varieties are: feijão de corda, feijão macassar, feijão de praia, feijão gurutuba, feijão trepa pau, feijão caupi (cowpea bean), feijão canapu and feijão fradinho (black-eyed peas or beans). As a source of income and vegetable protein, *V. unguiculata* is considered as one of the most important crops in Brazil, representing 15% of the bean production throughout the country (Leite et al., 2009; Sá and Batista, 2009; Teófilo et al., 2008; Rocha et al., 2007; Pio-Ribeiro et al., 2005; FAO, 2005).

The 'black-eyed' variety is generally consumed as green or dry beans (Felippe, 2007; Silva, 2005). This variety is of great economic importance to the State of Bahia because it is used to prepare the *acarajé* dough, a delicacy highly consumed in the region. According to IPAHN (2011), the Institute for National Artistic and Historical Heritage, *acarajé* is an important provider of jobs and income to thousands of people, who stir a consumer market of up to $80,000 in sales considering one single point of sale in Bahia, Brazil (IPAHN, 2011).

Furthermore, there is a great agronomic importance approach associated with the morphological characteristics of bean cultivars, precisely because variations can occur due to genetic differences or dissemination of the world. So, it is important to know the possible genetic differences in selected germplasm for breeding programs (Elias et al., 2007). This is the big reason for studies related to plant phenology and floral biology related to their reproductive mechanisms.

Therefore, this work aimed to carry out experimental studies on aspects of the floral biology *V. unguiculata*,

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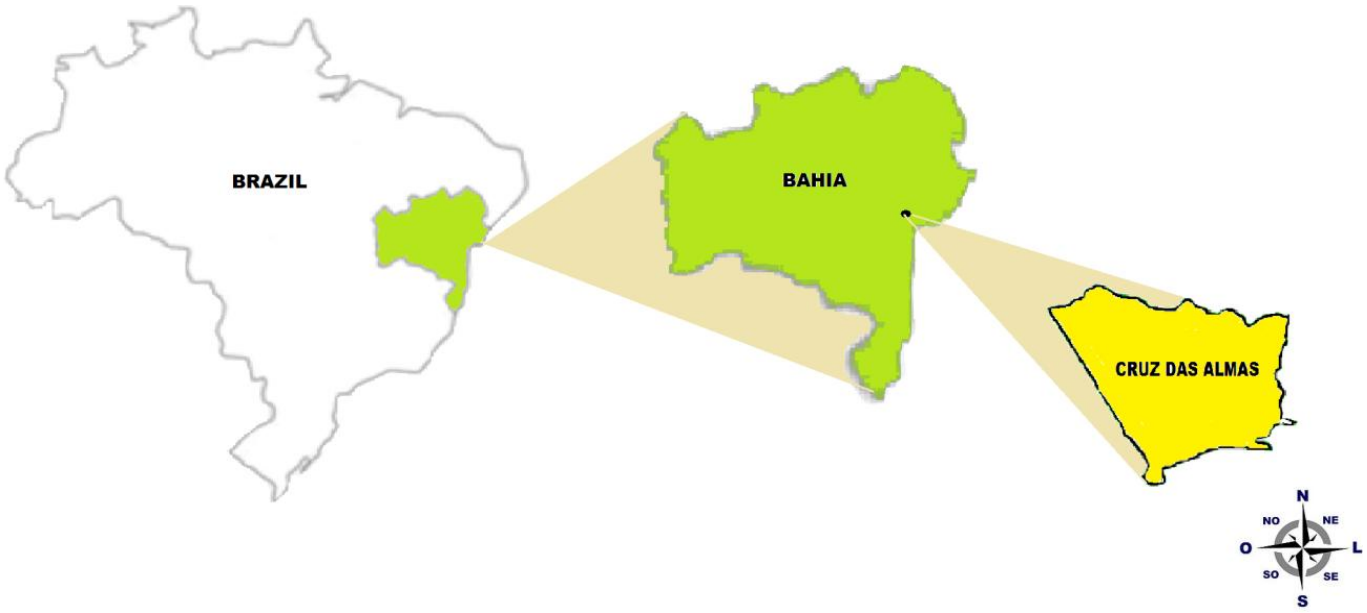

Figure 1. Location of the Cruz das Almas municipal area, Bahia, Brazil.

black-eyed variety, and on the viability and germinability properties of the pollen, using techniques of tissue culture plants, and information about what increases their pollination biology, that can subsidize with future genetic improvement programs, because of its economic importance to Africa, Brazil and other countries. Research on agricultural practices related to bean pollination is very important in order to increase productivity and grain production.

MATERIALS AND METHODS

The study was conducted between April and June 2011 in a greenhouse located at the Insect Study Nucleus of Universidade Federal do Recôncavo da Bahia, municipal area of Cuz das Almas, Bahia, Brazil (Figure 1). The average temperature in the period was 25°C (77°F) at 80% relative humidity. The local climate is of type Am, according to the Koppen climate typology (Baptista and Oliveira, 1998).

The grains used were selected from smallholder plantations in the region where this study was conducted. The bean seeds were planted in 4010 L pots containing a mixture of soil, washed sand and sheep manure in the proportion 7:2:1 and hand watered. 45 days after the seeds were planted, the first flowering occurred, lasting 30 days.

The study was broken down into the following phases:

(a) Anthesis: This was carried out in the first week of flowering, when two flowers buds in their initial phase were marked per pot having 80 buds. Observations were carried out in the pre-anthesis, anthesis and post-anthesis periods, according to the methodology described by Almeida et al. (2004). The assessments began at 4 o' clock in the morning, and consisted of focal observations every hour until senescence of the petals, for a period of 30 days.

(b) Receptiveness of the stigma: We used the technique described by Dafni et al. (2005), consisting of marking 20 stigmas at each hour in the pre-anthesis, anthesis and post-anthesis periods, placing two drops of hydrogen peroxide (3%) on the stigmatic papillae in order to verify peroxidase activity, which indicates reception of the stigma to pollen grains.

(c) Floral diagnosis: It carried out in the anthesis phase on 20 flowers, which had their parts dissected, measured with a digital pachymeter and analyzed for their anatomy.

(d) Pollen viability: This was carried out with a staining technique using acetic carmine at 1% (Lyra et al., 2011), and consisted of collecting pollen grains from 20 flowers at 10 different times, that is, at periods contemplating the pre-anthesis, anthesis and post-anthesis phases. The pollen was collected with a sterile brush and submerged in test tubes containing acetic carmine. Soon after each collection period, 10 slides per sample were prepared, and 100 grains were counted at random per slide using an optical microscope (40 x) and a slide scanning technique. Grains colored red were considered viable and non-colored grains were considered non-viable.

(e) Pollen germinability: In order to assess the pollen germinability rate the tissue culture, *in vitro* method was used in standard Murashige and Skoog (MS) 1962 culture medium, with added saccharose at 10%, incubated in biological oxygen demand (BOD) for 24 h and stained with acetic carmine at 1% to obtain the contrast for pollen tube growth. This methodology consisted of collecting the pollen grains of 20 flowers at 10 different times at periods that contemplated pre-anthesis, anthesis and post-anthesis. The pollen was collected with sterile brushes and sowed on Petri dishes containing culture medium. 24 h after each sowing activity, 10 slides of each sample were prepared and 100 grains were counted at random per slide. Pollen grains considered germinated were those whose pollen tube size were of the same size as or bigger than them, according to the methodology used by Ribeiro et al. (2007) and Almeida et al. (2004).

The viability and germinability test results were submitted to Regression Analysis with statistical analysis program SISVAR 4.2 (Ferreira, 2003).

Figure 2. Different phases of *V. unguiculata* flowers. A, pre-anthesis; B, anthesis and C, post- anthesis.

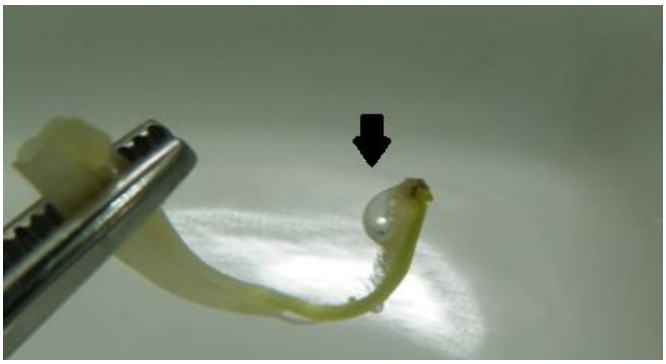

Figure 3. Peroxidase activity on *V. unguiculata* stigma, demonstrating receptiveness.

RESULTS AND DISCUSSION

Within 10 days of observation, anthesis occurred between 5:00 to 5:30 a.m. on all flowers previously marked. In pre-anthesis, at 4:00 a.m, the greenish flowers remained. However, during anthesis, when the petals closed, became yellow color (Figure 2).

In the stigma receptivity test, peroxidase action was observed at all times. As noted by Rocha et al. (2007), the floral opening of cowpea bean flowers begins around 5.30 a.m and continues until 9.30 a.m when the stigma is still receptive (Figure 3). These authors also observed that the stigma is receptive for a period of one day before anthesis until midday on the day of anthesis.

Floral diagnosis was carried out according to the morphological classification of Vidal and Vidal (1999), and Gonçalves and Lorenzi (2007). The flowers are hermaphrodite, deciduous, cyclic, dichlamydeous and heterochlamydeous with zygomorphic symmetry. The five sepals and petals present are in free condition, denominated polysepalous and polypetalous, respectively. Their corolla is papilionaceous, that is, it has an upper petal called standard, two side petals called wings and two lower, inner petals jointly called keel. One of the stamens is longer than the other nine (heterostemonous); the stamens are free; they are diadelphous; with simple ramification; the anthers are free and basifixed, enclosed in relation to the corolla, longitudinally dehiscent and introrsed. They are multicarpellary, syncarpous, with insertion in the terminal style. The hylum, chalaza and micropyle are in the same straight line, that is, their ovule is classified as orthotropous (Figure 4). This study of the cowpea bean flower morphology is fundamental to subsidize assisted pollination programs involving the Fabaceae family, because knowing how the flower offers food to insects makes it easier to understand the insect-plant relationship (Terra and Ferreira, 2009; Pizzamiglio-Gutierrez, 2009; Lorenzon et al., 2003), and to help phytoimprovement programs (Ribeiro et al., 2007; Almeida et al., 2004).

It was established that pollen grains are available during the entire anthesis period (5.00 am to 9.30 am). The pollen viability test (Figure 5) in relation to the collection time demonstrated a linear effect when submitted to regression analysis (Figure 6). This demonstrates that pollination is possible even in the post-anthesis period. This result is in accordance with Rocha et al. (2007). The cowpea bean pollen can remain viable for up to 42 h depending on air temperature and relative humidity. In germinability tests, a downward linear effect was established in the regression analysis (Figure 7). This means that even when genetically apt, as demonstrated in the viability tests, *V. unguiculata* pollen grains had a small possibility of germination.

Conclusion

In the Cruz das Almas, Bahia, Brazil, the anthesis of flower *V. unguiculata* occurs between 5:00 to 5:30 a.m. The pollen *V. unguiculata* are genetically viable in over 90% of cases as confirmed by colorimetric assay, but fertilization of carpels is less than 40%, which is demonstrated by the low rate of development of pollen tubes in *in vitro* culture medium.

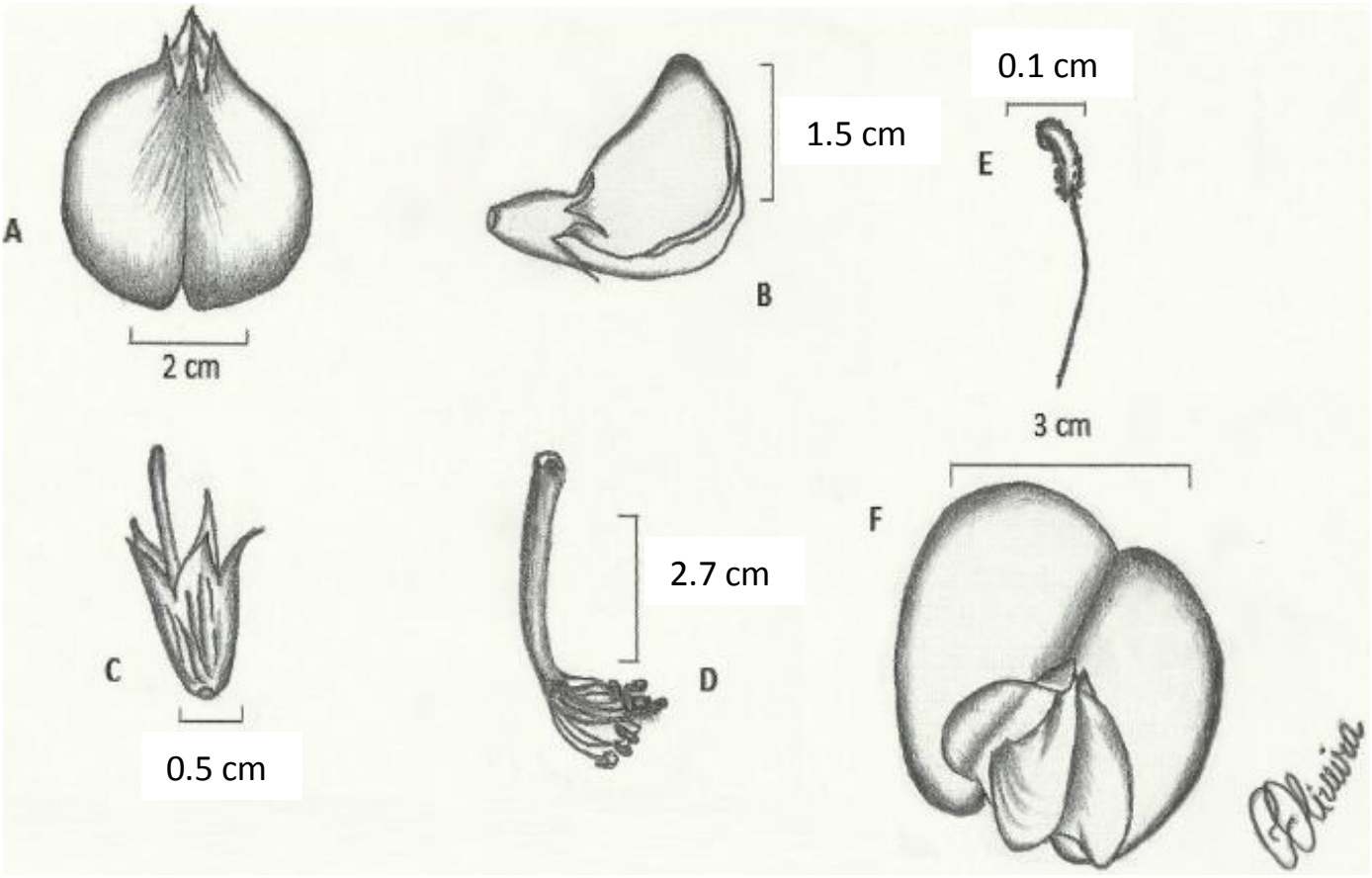

Figure 4. *V. unguiculata* floral pieces. A, standard; B, post-anthesis flower structure; C, sepals; D, stamen and pistil; E longer stamen; F, keel formation.

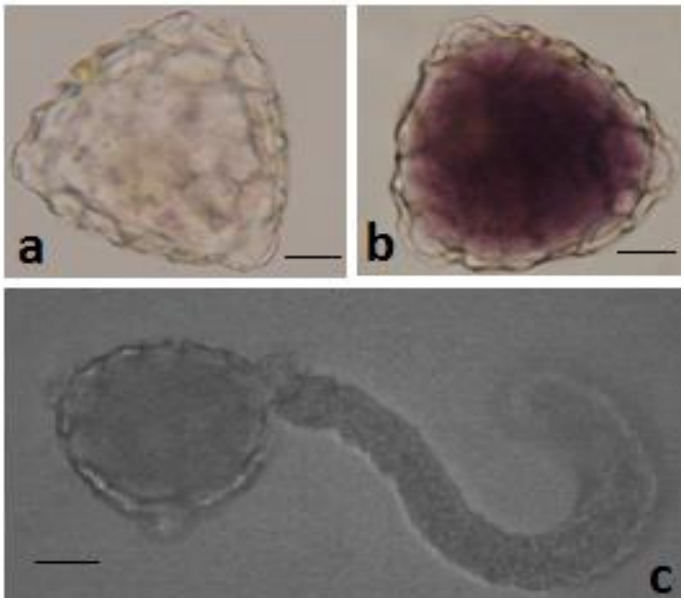

Figure 5. (a) Non-viable pollen grain. (**b)** Viable pollen grain stained with acetic carmine at 1%. **(c)** Pollen tube growth. Scale: 10 µm.

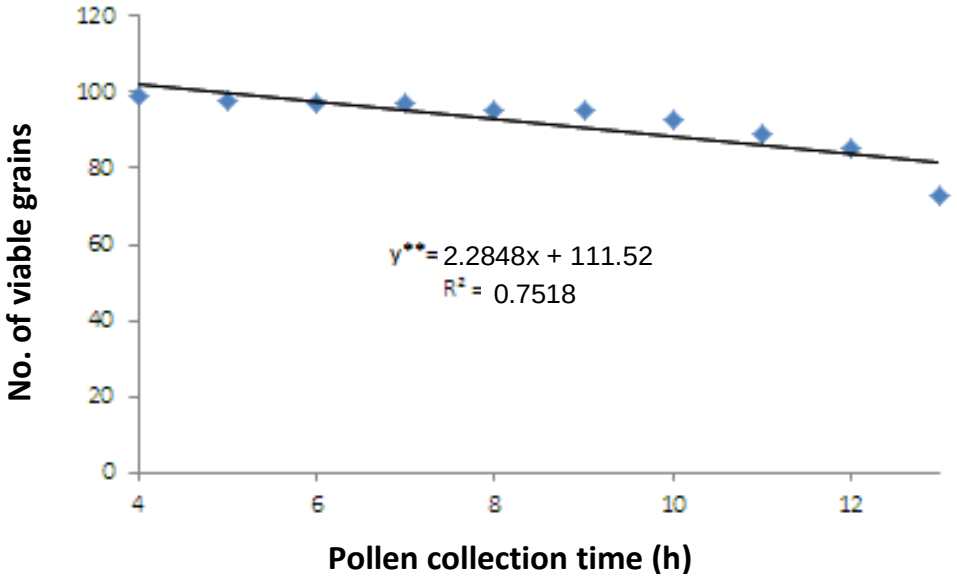

Figure 6. Linear effect (R^2 = 0.7518) of the number of viable pollen grains in relation to the pollen availability time of *V. unguiculata*.

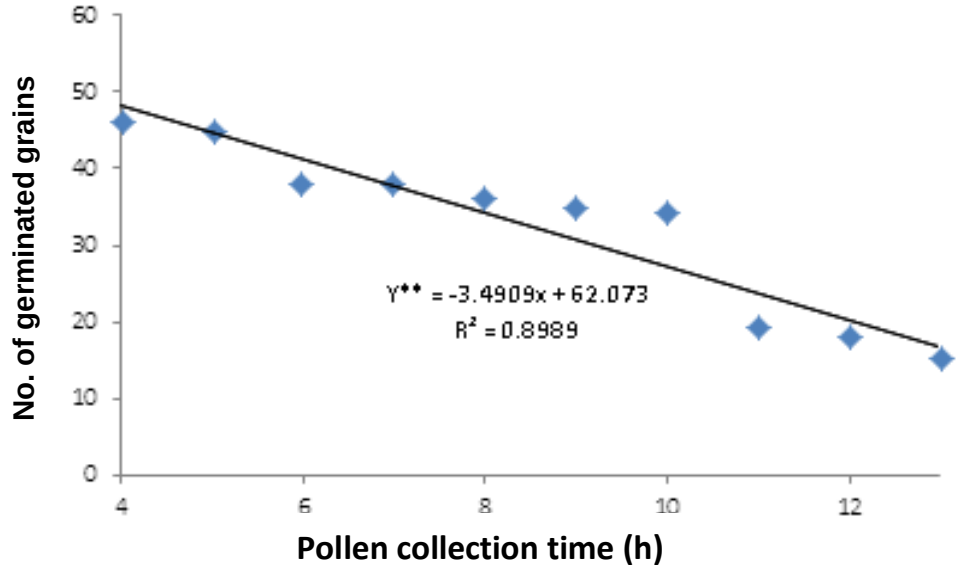

Figure 7. Linear effect (R^2 = 0.8989) of the number of viable pollen grains in relation to the pollen availability time of *V. unguiculata*.

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