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Behavior of the pollen tube of *Poincianella pyramidalis* (Tul.) L. P. Queiroz after compatible and incompatible crosses

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Aiming to analyze the pollen tube growth behavior in compatible and incompatible crosses in *Poincianella pyramidalis*, on-field crosses were performed using flowers of 30 randomly selected individuals, which were self- and cross-pollinated. In the cross-pollination, the pollen germinated and the pollen tube grew in the transmitting tissue of the pistil, followed by a succession of callose rings through the growth of the tubes towards the ovary; after 8 h, the ovules were fertilized. On the other hand, in self-pollination, the pollen grains germinated, but the pollen tubes were inhibited during growth at the stylar transmitting tissue, indicating that the self-incompatibility of the species is homomorphic and gametophytic.

Key words: Fabaceae, self-incompatibility, Caatinga, *Poincianella pyramidalis,* incompatible crosses, compatible crosses.

INTRODUCTION

The Caatinga biome is considered one of the largest geographic areas on the planet, distributed in Brazil across the states of Piauí, Ceará, Rio Grande do Norte, Paraíba, Pernambuco, Alagoas, Sergipe, Bahia and part of Minas Gerais (IBGE, 2011). It is a lush and thorny vegetation characterized mostly by its completely deciduous species, which are subjected to water shortage during most part of the year, due to the poor spatial and temporal distribution of rain, elevated evapotranspiration rate and low water-holding capacity of the soils, which are, in general, shallow and stony (Andrade Lima, 1989).

The family, Fabaceae consists of one of the most representative families of the Caatinga, it is the third largest family among the Angiosperms, having 727 genera and approximately 19,325 species (Lewis et al., 2005). The economic importance of the representatives of this family is indisputable, because many genera and

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species are utilized in human feeding (e.g. forages), in the recovery of poor soils, urban greening, and in the logging and chemical industries (Lewis et al., 2005).

Poincianella pyramidalis is an arboreal species (Monteiro et al., 2005) belonging to the family Fabaceae. It is endemic to the Caatinga that inhabits stony areas (Silva and Matos, 1998), and popularly known as *catingueira*, *pau-de-porco* and *catinga-de-porco* (Braga, 1960). It is one of the most widely dispersed species in the Brazilian northeast semiarid, and can be found in several plant associations. It is highly utilized by the communities for firewood and charcoal (Silva et al., 2009).

Regarding the family Fabaceae, its reproductive system has already been studied in at least 451 species, with 152 (33.7%) of them showing self-incompatibility (Kill and Drumond, 2001; Freitas and Oliveira, 2002; Costa et al., 2003; Leite and Machado, 2009). However, the mechanism of incompatibility (gametophytic, sporophytic or late-acting) has not been determined for most of the analyzed species. As to the Caesalpinioideae subfamily, there is still a large knowledge gap with regard to the reproductive system (Lewis et al., 2000; Leite and Machado, 2009).

Self-incompatibility (SI) is the incapacity of a fertile plant to generate seeds when fertilized by its own pollen. It is a gene-based physiological mechanism that promotes allogamy. As a reproductive barrier, prefertilization is one of the least costly mechanisms that impede self-pollination, regarding the allocation of maternal resources (Schifino-Wittmann and Dall'Agno, 2002). Many different forms of self-incompatibility are known, and in some cases, the molecular mechanisms involved have been at least partially elucidated (Franklin-Tong, 2008). However, information on the sites of inhibition or the genetics of the incompatibility, for many plant groups, remains incomplete (Bilinski and Kohn, 2012).

There are two main types of SI: gametophytic (GSI), in which the specificity of the pollen is generated by the S allele of the haploid genome of the pollen grain (gametophyte) and the sporophytic (SSI), in which specificity is determined by the diploid genotype of the adult plant, which originates the pollen grain. The sporophytic self-incompatibility can be homomorphic, when there are no floral modifications in the process, or heteromorphic, when, along with the SI process, there are floral modifications. The self-incompatibility reaction comprises the processes from the prevention of germination of the pollen until the rupture of the pollen tube (De Nettancourt, 2000).

Late-acting self-incompatibility (LSI) is characterized by the uniform abortion of the pistils shortly after self-pollination, even though the pollen tubes have traversed the style and penetrated most of the ovules (Seavey and Bawa, 1986; Sage et al., 1994; Bittencourt, 2008). Although, it is a widely distributed phenomenon that occurs in a clustered manner in some angiosperm families (Gibbs and Bianchi, 1999), the physiological and genetic mechanisms acting on LSI are not well known (Ladoux and Friar, 2006). Late-acting self-incompatibility can basically manifest in three different ways: (1) with inhibition of the pollen tubes, occurring before the ovules are penetrated; (2) with inhibition after the ovules are penetrated, but before their fecundation; or (3) with postzygotic rejection of the ovules (Bittencourt, 2008).

Self-incompatibility in *P. pyramidalis* has already been described by Leite and Machado (2009) as being late-acting, and so, the objective of the present study was to analyze the growth behavior of the pollen tube in compatible and incompatible crosses in *P. pyramidalis* so as to determine the location and time of inhibition for incompatible pollen tubes, in addition to observing the approximate fertilization time.

MATERIALS AND METHODS

Experimental area

The experiments were conducted at the Experimental Station Bacia Escola, at Federal University of Paraíba (UFPB), located in the municipality of João do Cariri/PB, Brazil. The geographic coordinates of the area are 7°23'30" S and 36°31'59"W, at an altitude of 458 m. According to the Köppen classification, the climate of the region is a hot semi-arid BSh-type, with rainy period from January to April, showing average annual temperatures of about 26°C, relative air humidity of approximately 68%, and annual average precipitation of 376.4 mm. The experimental area has 3.20 ha and is composed of a typical vegetation of Caatinga under regeneration. The area was fenced so as to prevent the access of animals.

The laboratory analyses were carried out at the laboratory of Plant Anatomy of the Department of Plant Biology (Department of Plant Biology, DBV) at Federal University of Viçosa (UFV), located in Viçosa/MG, Brazil.

Experimental material

All the crosses were performed from May to June 2010, during the flowering period of *P. pyramidalis*. The individuals utilized in the experiment were randomly selected among those showing leafy crown and good phytosanitary conditions (apparent absence of diseases or parasitic infestations).

For the open pollination (control), five individuals were tagged, and their tagged and uncovered flowers were monitored, under natural conditions of pollination, without manipulation. At cross-pollination (allogamy), five plants were used; their leaves were emasculated and the pollen from flowers of distinct plants which were at 100 m away from the recipient plant was deposited on the stigmas of its flowers. For manual self-pollination (geitonogamy), however, five individuals were tagged, and the same methodology for manual cross-pollination was adopted, but this time with pollen grains from flowers of the same plant.

Evaluation of the growth of the pollen tubes

The self-incompatibility reaction in the stigma and in the style was diagnosed according to the methodology proposed by Martin

Figure 1. *Poincianella pyramidalis*. a. Inflorescence; b. flower exhibiting nectar guide (arrow) in the center of the vexillum.

(1959), with a few alterations. For this purpose, the pistils of the flowers were collected at 10, 20, 30, 40, 50 and 60 min and at 8, 24 and 48 h, respectively, after the pollination, and fixed in ethanol: glacial acetic acid (3:1, v/v). For the observation of the germination of the pollen and growth of the pollen tube, the pistils were removed from the fixative solution, washed in phosphate buffer pH 7.5 and placed in a sodium hydroxide solution (NaOH) 1 M for approximately 8 h, at 60°C. This procedure was performed so as to soften and clarify the plant tissues, and at the end of this period the material was washed again three times in phosphate buffer (K_3PO_4) 7.5, and then placed on a slide. One drop of a blue-aniline solution was added, and it was left to sit for 30 min and then covered with a coverslip, which was slightly pressed upon to allow for the plant tissue to better spread. The observations were made with an Olympus BH2 photomicroscope (epifluorescence illumination using a BP-490 filter) and pollen tubes were identified by the fluorescence of the callose on the walls and on the plugs, and then photographed on a Kodak ISO 400 color negative film.

RESULTS AND DISCUSSION

External structures of the pistil of *P. pyramidalis*

The flowers of *P. pyramidalis* are arranged in a terminal or axillary-terminal inflorescence, in panicle (Figure 1a). Their bracts are ovate, apiculate, concave, slightly pilose, presenting small glandular spots on the back. The flowers are yellow, arranged in racemes (Maia, 2004), and this is the most common type of inflorescence in the family Fabaceae (Tucker, 2003). It presents zygomorphic symmetry, yellow color, a guide and high concentration of nectar, which, according to Faegri and Van der Pijl (1979), are typical attributes of the melittophily syndrome (Figure 1b).

With a fluorescence microscope, it was possible to view the external structure of the pistil of *P. pyramidalis* in detail (Figure 2). In the epidermis of this structure, there were numerous simple and secretory trichomes (Figure 2d), homogeneously distributed all over the epidermis, in addition to the presence of stomata (Figure 2e).

The cells from the stigmatic region could be characterized as elongated papillary cells, forming a hollow ring, which bent to the adaxial region (Figures 2a and 2b), and the same characteristic was observed in *Caesalpinia echinata* and *Caesalpinia peltophoroides* (Zaia, 2004). Dulberger et al. (1994) mention that most angiosperms have an exposed stigmatic surface that facilitates the process of capture and deposition of pollen, but in some species of the genera *Cassia*, *Senna* and *Chamaecrista* belonging to family Fabaceae, access to the stigmatic surface is protected by what was named the fringe of trichomes, in which the size and number of trichomes and the degree to which the stigmatic surface is exposed varies according to the species (Dulberger, 1981; Dulberger et al., 1994; Tucker, 1996). The pollen grains germinate in the sub-stigmatic chamber, or under the papillary cells; however, for the vast majority of species, there is still little information on their role in pollination. On *C. calycina*, Lewis and Gibbs (1999) stated that the fringe of trichomes of the stigma plays an important role in removing pollen from the body of the pollinator. In fact, during the visit of the pollinator, the abdomen of the insect comes in contact with these trichomes of the region of the stigma, and they scrape off the pollen present in its body, guiding its entry into the stigmatic region (Arceo-Gomez et al., 2011).

Behavior of the pollen tube in compatible crosses

The compatible crosses originated from cross-pollination, and their behavior occurred as can be seen in Figure 3. It

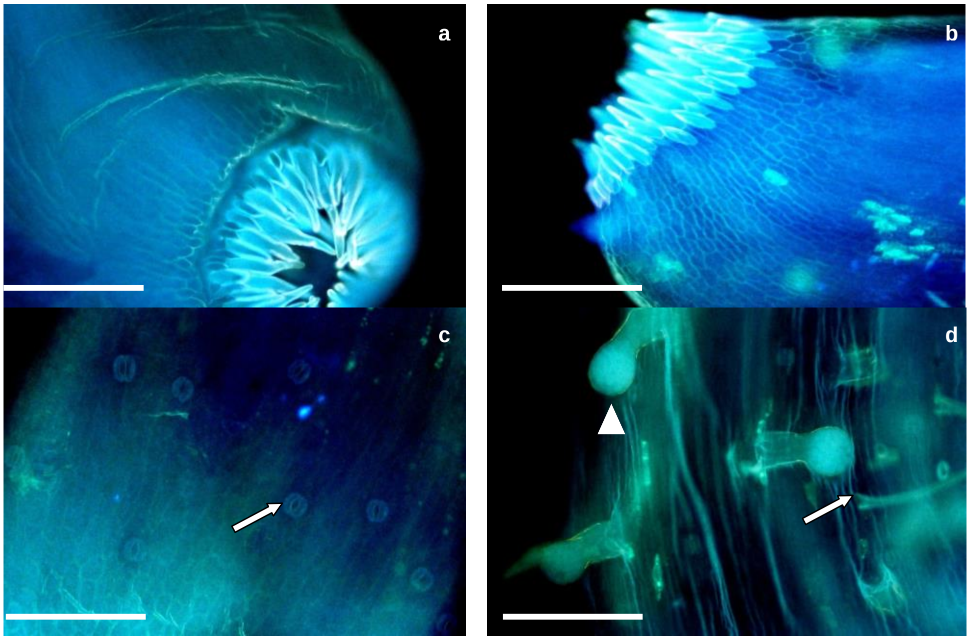

Figure 2. Structures on the pistil of the flower of *P. pyramidalis* Tul. a. Fringe of trichomes (front view); Fringe of trichomes (side view); c. Stomata in the style (arrow); d. Simple trichomes (arrow) and secretory trichomes (arrowhead). Bar = 10 µm.

is noteworthy that it is not possible to observe the germination of the pollen grain on the stigmatic surface, because it is located inside the stigmatic cavity, formed by the fringes of trichome, where the pollen grains fall and germinate, so it is only possible to visualize the several pollen tubes growing in the stylar transmitting tissue, until they reach the ovules.

Pollen tubes have a fluorescent green color when stained with clarified aniline blue solution and viewed under UV light on an epifluorescence microscope. The first evidence of the pollen germination was observed in general 20 min after pollination (Figure 3a). After 50 min the pollen tube growth was limited to the beginning of the first third of the pistil. By 60 min, the tube was found, on average, at the central third of the pistil (Figures 3b and c). Its growth was strictly guided in the transmitting tissue of the pistil and followed by a succession of callose rings along the growth of the tubes towards the ovary, resembling a flight of stairs (Figure 3c). After 4 h, the tubes began to approach the ovarian cavity (Figure 3d). The fertilization of the ovules (Figure 3e) started 8 h after pollination, and by 24 h, most of the ovules had already been fertilized (Figure 3f). In this sense, Leite and Machado (2009) observed, for the same species, ovules penetrated 24 and 48 h after cross-pollination.

Behavior of the pollen tube in incompatible crosses

In *P. pyramidalis*, pollen grains germinated on the surface of the stigma, within the stigmatic cavity, but this did not occur due to the presence of the trichomes fringes, which cover the surface, as previously mentioned (Figure 2). Characteristics of an incompatible gametophytic pollination were observed in these crosses. The pollen grains germinated approximately 20 min after pollination. In approximately 30 min, the pollen tube reached the beginning of the first third of the pistil, and at 50 min, the tube was found near the central region of the pistil (Figure 4). It is worth stressing that each tube was able to germinate and grow in the transmitting tissue of the stylus, and was inhibited within it, when the pollen tube expanded and burst. The callose synthesis is induced by the incompatible pollen, probably through proteins expressed by the S alleles, which were broadcast from the anther tapetum, a nutritive tissue (Sood et al., 1982) to the pores of the exine during the development of pollen; these are features found in species of gametophytic self-incompatibility. This is the most common system among plants, and it is also thought to be the most primitive (De Nettancourt, 2000; Schifino-Wittmann and Dall'Agnol, 2002).

The manual self-pollination showed self-incompatibility. In this case, the pollen grains germinated, penetrated the stigmatic tissue and were inhibited, in most cases, in the central region of the stylar transmitting tissue (Figure 4a). Two types of abnormalities were observed in the style: (a) narrowing of the pollen tube wall (Figure 4b) and (b) formation of bulb at the apex of the pollen tube (Figure 4c). The occurrence of these abnormalities and

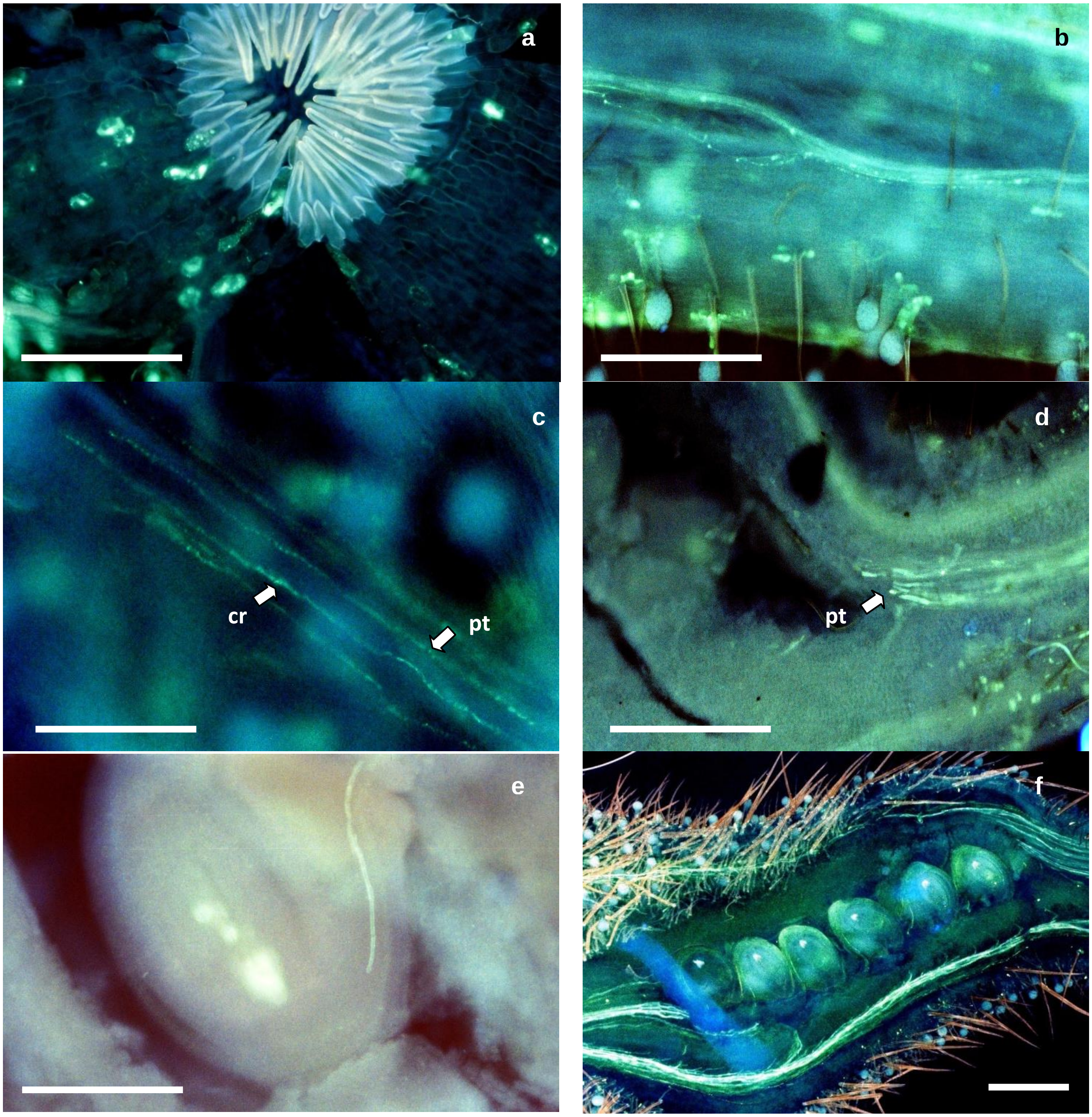

Figure 3. Behavior of the pollen tube (pt) in compatible crosses. a. Start of the elongation of the compatible pollen tubes after the passage through the chamber of trichomes, in the transmitting tissue, stained with clarified aniline blue and observed on a fluorescence microscope; b and c. Several pollen tubes developing in the style, with successive callose rings (cr); Lower part of the style and ovary, displaying several tubes reaching the ovary. e. Ovule being fertilized, displaying the entry of the pollen tube 8 h after pollination. f. Newly-fertilized ovules. Bar = 10 µm.

frequencies were constant among the slides of incompatible crosses analyzed. After formation of the bulb in the style, in some cases, the pollen tube wall is disrupted. These characteristics are usually found in species that express gametophytic self-incompatibility (De Nettancourt, 1977).

Based on the observations, *P. pyramidalis* appeared to be a species that has gametophytic self-incompatibility, and inhibition of the growth of the pollen tube occurred due to the callose deposition in the style, as verified in studies with many woody species (Oliveira and Gibbs, 2000). As opposed to the present study, Leite and

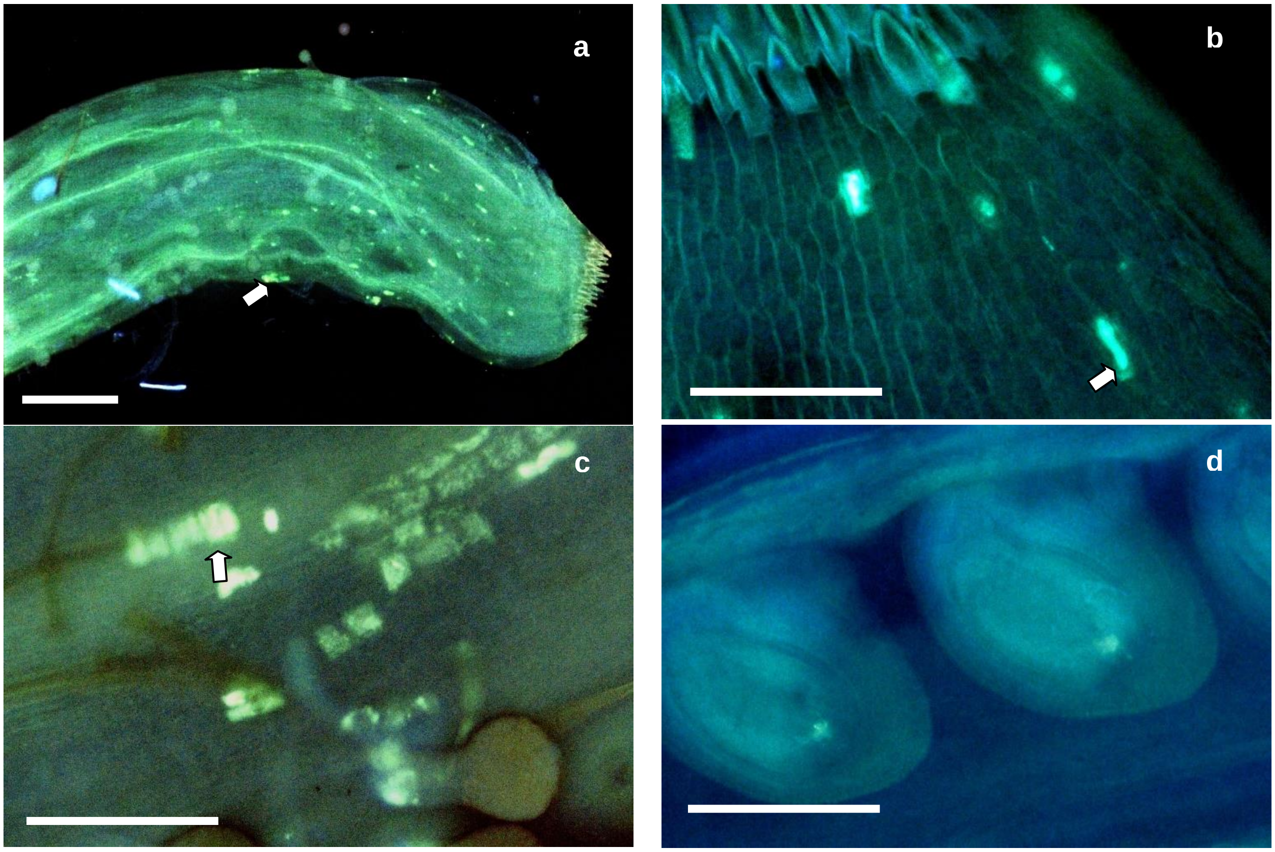

Figure 4. Development of the pollen tube in incompatible crosses. a. Inhibition of growth of pollen tubes in the style; b. Formation of bulb at the apex of the pollen tube; c. Narrowing of the pollen tube wall; d. Non-fertilized ovules. Bar = µm.

Machado (2009), working with *P. pyramidalis* in an area of the Caatinga, with observations on cross- and self-pollinations, verified growth of pollen tubes of manual self-pollination until the ovule and flowers fell off, from this treatment, between 24 and 72 h; these events were classified as late-acting self-compatibility (LSI). The gametophytic self-incompatibility is determined by the S locus, which codes two genes that determine the phenotype in the alleles of the pistil and of the pollen (Kao and Tsukamoto, 2004; Guerra et al., 2012).

Another point for discussion, to justify the fact that the studied species is shown to be gametophytic self-incompatible, contradicting the observations of Leite and Machado (2009), is the variation of the expression of self-incompatibility among populations of a species (Pailler and Thompson 1997; Sage et al 2001) expected particularly for some circumstances, e.g. in populations with different sizes and in the geographical limits of distribution of the species (Fausto et al., 2001; Stone et al., 2006). Thus, the fact that the results of Leite and Machado (2009) suggest that there is a mechanism of late-acting incompatibility (though inconclusive) does not exclude the possibility of existence of a mechanism of gametophytic incompatibility, provided it is partial, in which its expression varies according to both differences between the composition of the pollen load carried by the pollinators and differences between populations of the species (Wolowski and Freitas, 2010).

Conclusions

The site of inhibition of the pollen tube growth is the style. The inhibition of the pollen tube in the style takes place approximately 60 min after pollination. Fertilization occurred 8 h after pollination, in the crosses between compatible plants.

Conflict of Interests

The authors have not declared any conflict of interests.

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