Theor Appl Genet (2000) 101:685–689

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Pollen tube behavior in yellow passion fruit following compatible and incompatible crosses

Received: 12 October 1998 / Accepted: 2 December 1999

Abstract Pistil squashes were used to examine the growth of *Passiflora edulis* f. *flavicarpa* Deg pollen tubes in self-compatible and self-incompatible crosses. Compatible tubes typically showed a uniform layer of callose deposition in the walls and in small plugs spaced at regular intervals within the tube. Two sites of rejection were observed in incompatible crosses: the stigma and on some occasions the style, especially in anomalous crosses. In the style the inhibition of pollen-tube growth occurred in both the upper and middle parts of the transmitting tract. These findings are consistent with the hypothesis that suggests the presence of a gametophytic gene which acts in association with the sporophytic S-gene in *P. edulis*.

Key words *Passifloraceae* · Pollen-tube growth · Self-incompatibility · *Passiflora edulis* f. *flavicarpa*

Introduction

Passion fruit is a perennial, vigorous, climbing woody vine that was originally found occurring wild from Brazil and Paraguay to northern Argentina. Being considered as a tropical or subtropical species, it could tolerate a wide range of climatic conditions and is commercially cultivated from sea level in the East Indian region to elevations of 2500 m in Kenya and other parts of Africa. It will tolerate cool periods of 5–13°C and slight frosts of –2.5°C. There are approximately 465 species of *Passiflora*; however, only four species, *Passiflora edulis* Sims., *Passiflora quadrangularis* L., *Passiflora ligularis*, and *Passiflora mollissima*, are cultivated for food. The majority of the cytologically analyzed species of *Passiflora* have shown 2n=18 chromosomes, with regular meiosis and high pollen viability, more than 90%, including the most cultivated species, *P. edulis* f. *flavicarpa* Deg.(Soares-Scott et al. 1995). Its delightful aromatic fruits are eaten fresh or processed into soft drinks, alcoholic drinks, jams, preserves, sweets and sherbets or made into a variety of desserts (Vanderplank 1996). This has led to an ever-increasing demand for passion fruit. Pollination is one of the most important factors affecting fruit set, being effected by pollinating agents such as carpenter bees, *Xilocopa* ssp. (Akamine and Girolame 1959; Ruggiero et al. 1976). The passion fruit flowers are perfect, hermaphrodite but self-incompatible.

The self-incompatibility in *Passiflora* has already been reported by Munro (1868), see de Nettancout (1977). However, references about the incompatibility system and its genetic control are more recent. Akamine and Girolame (1959), Knight and Winters (1962), and Chang (1974) obtained differences of fruit-set rate in reciprocal crosses. Ho and Shii (1986) verified that the incompatible reaction takes place at the papillal cells of the stigma, resembling the sporophytic system of self-incompatibility. Bruckner et al. (1995) studied self-incompatibility in two generations and their results confirmed a homomorphic sporophytic system, probably controlled by a single *S*-gene. Further data indicate that two loci may be involved in the genetic control of the self-incompatibility (Rêgo et al. 1998). The purpose of the present study was to examine the behavior of pollen-tube growth in compatible and incompatible crosses, to determine the site and time of inhibition for incompatible pollen tubes, and finaly to observe the approximate time of fertilization.

Communicated by H.F. Linskens

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Materials and Methods

Plant material

The studies were conducted at the Department of Plant Science, Federal University of Viçosa. The crosses and self-pollinations were done within the progenies BG and BJ from *P. edulis*. These progenies were obtained after self-pollination at the bud-stage (Bruckner et al. 1995). The progeny BG originated from selfing the plant B_3 (of unknown phenotype). The progeny B resulted from the backcross of plant 414.414–10 (S_4) to 414 (S_2). The progeny B had the phenotypes S_2 (B_1, B_2 and B_5) and B_3, an unknown phenotype (the plant died before it was identified), but which was not S_2 nor S_4. Progeny BJ was derived from selfing the plant 808.

Self-pollination and reciprocal crosses

All plants from the progenies were selfed to confirm the self-incompatibility. Five or more flowers per plant were protected in the morning, with a paper bag, self-pollinated after 1-h pm and covered again. After 7 days, the rate of fruit set was evaluated. The compatibility among the plants within progenies was determined in reciprocal crosses. Each progeny was reciprocally crossed to the next one in the row, forming a chain of reciprocal crosses (Wallace 1979). The plants in which the crosses were incompatible were grouped as self-incompatible groups. Additional crosses were made when the crosses between adjacent plants were compatible. The crosses were classified as compatible when fruit-set occurred or incompatible in the absence of it. With the aim of identifying their phenotypes, some plants of self-incompatible groups within each progeny were crossed with known phenotypes. Incompatible crosses indicated that the plants belonged to the same phenotype.

Pollen-tubes growth evaluation

This experiment was performed to evaluate the self-incompatibility reaction in stigma and style utilizing the techniques described by Martin (1959). Flowers were pollinated after opening and pistils were harvested at intervals of 10, 20, 30, 40, 50 and 60 min and 12, 24 and 48 h, respectively. The pistils were removed from the flowers and fixed in ethanol/acetic acid (v/v,3:1) for 24 h, softened, and stained for callose with a fluorochrome in aniline blue. Pollen tubes within the pistil (stigma, style and ovary) were displayed on slides by dissection followed by light-squashing under a cover slip. Pistil squashes were viewed on an Olympus BH2 photomicroscope (epifluorescence illumination using a BP-490 filter set) and pollen tubes were identified by the fluorescence of the callose on the walls and plugs, and then photographed on negative polychromatic film Kodak ISO 100.

Results

Self-pollination and reciprocal crosses

Self-pollinations made in progenies BG and BJ did not set fruit, indicating that all plants were self-incompatible.

The results of the reciprocal crosses in the BG progeny led to the occurrence of three self-incompatible groups, IX (S_1), X(S_4) and XI (S_3). In group IX one anomalous result was observed, with 37.5% fruit set after crossing the plants 24×26. Reciprocal differences were observed between groups IX and X. The crosses 24×30, 24×34 and 31×32 were incompatible, while their reciprocals were compatible. The cross 35×24 was incompatible, but the reciprocal was not made (see Rêgo et al. 1998).

In progeny BJ, 18 plants were classified as three self-incompatible groups, XII (S_6) XIII (S_3) and XIV (needs additional investigation, since it has the same pattern as group XIII when used as a male parent). Two anomalous result were observed within group XIII by crossing the plants 2×1 and 7×8. The crosses among the plants of groups XIII and XIV were compatible when plants of group XIII were used as the male parent, and incompatible in the reciprocal crosses.

Pollen tubes behavior following compatible crosses

All compatible crosses resulted from pollination between plants of different phenotypes. The first evidence of pollen-grain germination was obtained 30 min after pollination, each papillar cell being occupied by one pollen tube, indicating that the reaction is cell-to-cell (Figs. 1–6). Approximately 60 min after crossing it was possible to observe the pollen tubes in stylar transmission tissue (Fig. 1). They had walls that fluoresced faintly and evenly with aniline blue, except at the tip, which was rarely visible. Callose plugs were spaced at regular intervals. Plugs located above the entrance to the ovary were about 12-µm long (Fig. 3). At 12 h after the pollination the pollen tubes were observed penetrating and fertilizing the ovules (Fig. 2).

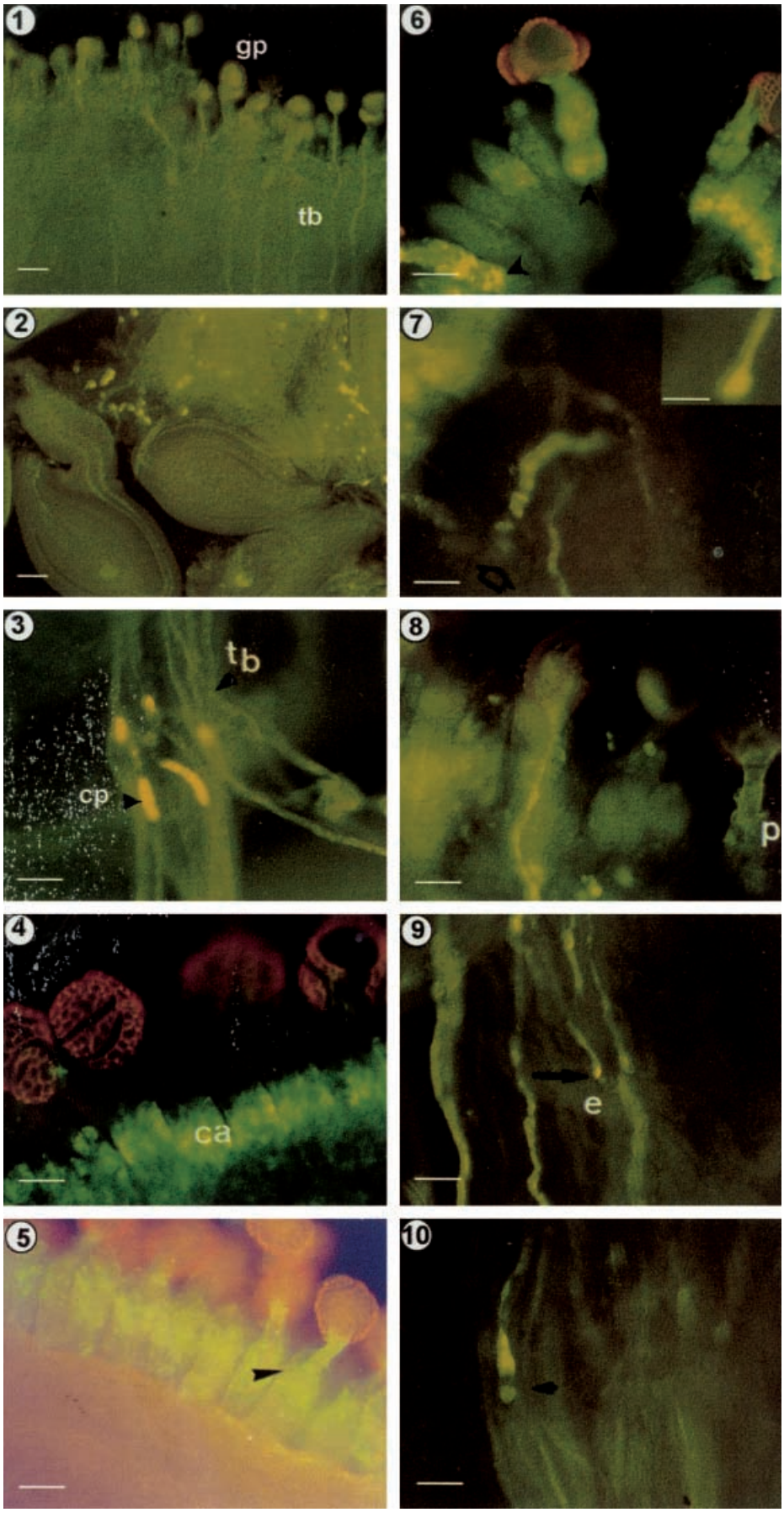
Pollen-tube behavior following incompatible crosses

The incompatible crosses were studied after self- and cross-pollination. Two inhibition sites of pollen-tube growth were identified: stigma and style.

It was observed that the flower presents a dry stigma and elongated papillae, which is composed of multi-seried cells with intercellular spaces. The arrest of pollen-tube development in incompatible crosses occurred in the surface of the stigma. However, in the case of the germinated pollen grains, each tube penetrated the cuticle and the cellular walls, grew approximately two-fold their diameter within the papillae and was inhibited by callose deposition. This took place 30 min after pollination (Fig. 4), and could be seen as a bright yellowish-green fluorescence under the UV fluorescence microscope. The synthesis of callose is dependent on direct contact between both papillae and the adjacent incompatible pollen grain, indicating the need of an elicitor by either synthesis. Therefore, the papillae perform an important function in the recognition and rejection of incompatible pollen, in a relatively short time.

Some anomalous results were observed in some reciprocal crosses within and between groups of the families BG and BJ. In these cases, pollen-tube growth began as in the compatible crosses, but was inhibited generally in the upper third of the style. Here, three categories of ab-

Figs. 1–10 Fluorescence micrographs of *P. edulis* f. *flavicarpa* Deg. pistils pollinated with compatible and incompatible pollen. Pistils were stained with decolorized aniline blue. **Fig. 1** Compatible pollen tubes in the stylar region. pollen grain (*gp*) and pollen-tube elongation (*tb*). **Fig. 2** Ovules at fertilization 12 h after pollination. Bar= 100 µm. **Fig. 3** Growth of pollen tubes (*tb*) with successive callose plugs (*cp*) arriving at the ovary. Bar=50µm. **Fig. 4** Incompatible pollen tubes in the stigmatic region. Beginning of callose synthesis (*ca*) 30 min after pollination. **Fig. 5** Inhibition in stigmatic papillae (*arrow head*) by callose deposition. **Fig. 6** Rejection reaction is cell-to-cell and callose synthesis is restricted to papillae with incompatible pollen (*arrow heads*). **Fig. 7** Incompatible pollen tubes in the stylar region. Irregular growth of the pollen tubes with consequent arrest and breakage in the style. Swelling and breakage of the pollen tube, in detail. **Fig. 8** Evidence of two sites where the incompatibility reaction occurs in the papillar cell (p) and at the upper third of the style (e). **Fig. 9** Sequence of Fig. 8, showing the pollen-tube abnormalities with consequent arrest and callose accumulation at the tip. **Fig. 10** Detail of pollen-tube breakage



normalities were observed in the style: (1) no directional growth of the pollen-tubes (see Fig. 7); (2) thinning of the wall tubes (see Fig. 9); and (3) formation of swellings at the tip of the pollen-tube (see Fig. 7). After the formation of the swelling, the bursting of the tip of the pollen-tube wall was sometimes observed (see Fig. 10). These abnormalities were seen in 50 studied slides of these anomalous crosses.

Discussion

Reciprocal crosses

All the compatible crosses resulted from cross pollinations. Reciprocal differences were observed in progenies BG and BJ (see Rêgo et al. 1998). Differences in reciprocal crosses were reported by Akamine and Girolami (1959), Knight and Winters (1962), and Chang (1974) in yellow passion fruit. Such differences are characteristic of the sporophytic system and can be influenced by variable ambient conditions, such as rainfall, temperature and photoperiod at the time of crossing. Bruckner et al. (1995) noted little difference in reciprocal crosses, and attributed the observed variability to randomness. It is important to note that the phenotype S_3 reappeared after self-fertilization of the plant B_3 (unknown phenotype), which originated from the backcross of the S_4 plant (414.414–10) to its parent 414 (S_2). These finding would not be expected by the monofactorial hypothesis, as earlier proposed by Bruckner et al. (1995); however, they confirm the recent evidence of a two-locus genetic control of self-incompatibility in this species (Rêgo et al. 1998). A possible hypothesis to be investigated is the expression of a second gene which depends on an allele of the *S* series, as reported by Zuberi and Lewis (1998) and Lewis et al. (1988) when studied differences in reciprocal crosses in *Brassica*.

Compatible pollination

In the compatible crosses, several pollen grains germinated and the pollen tube had penetrated the stigmatic surface, growing intercellularly in the transmitting tissues of stigma, style, and ovary. In the ovary, the tube penetrated the funiculus and entered the unfertilized ovule through the micropyle to effect fertilization (Fig. 2), approximately 12 h after pollination. By contrast, Ishihata (1991) and Ho and Shii (1986) reported that the majority of ovules were fertilized between 18 and 24 h after pollination, respectively. According to Heslop-Harrison (1975) similar differences are common and can be attributed to environmental factors, such as temperature, photoperiod and the relative humidity of the air. According to Dumas et al. (1983), the normal development of pollen tubes is the result of the mechanics and nutritive conditions provided by the transmitting tissues of the style. Since the pollen tubes grow for several hours in the gynoecium, ample opportunities exist for interactions between these tubes and the tissues along the length of pistil. The most important interactions are with the callose plugs, induced by physical stress, and which are related to the decreased turgor pressure during tube elongation. Turgor pressure in the tube may be maintained by the formation of a series of these plugs. Pressure is needed for tube penetration into the pistil, which provides a virtual mechanical facilitation pathway for the tubes. Physical-stress changes are known as inductors of callose formation and a new plug would restore the turgor pressure (Aist 1976). The result is the building of a succession of plugs, resembling a ladder, along the elongating pollen tubes (Fig. 3).

Inhibition sites

In the majority of the examined incompatible crosses, the inhibition site was found at the stigmatic surface. However, in some incompatible crosses pollen-tube-growth inhibition was found in the stylar tissue.

Stigmatic inhibition

P. edulis presents a flower with a dry stigma and solid transmitting tissue. The papilla is composed of elongated cells with a thick intercellular space (Fig. 5), rather than being uniseriated as classified by Heslop-Harrison and Shivanna (1977). In addition, this is also the principal site of inhibition of incompatible pollen tubes. These two characteristics are often associated with sporophytic self-incompatability. In incompatible crosses, the pollen germinated and the tube penetrated the stigmatic surface and grew until approximately double the pollen diameter. The arrest of pollen-tube development occurred at the papillar cells of the stigma (Figs. 5 and 6). In contrast, in *Raphanus sativus* (Dickinson and Lewis 1973) and *Brassica oleracea* (Elleman et al. 1988) where pollen germination took place only in certain genotypes, the pollen tubes often had an abnormal morphology and, more important, they failed to invade the wall of the papillae. In this experiment, most of the incompatible crosses exhibited the characteristic pollen-stigma interaction of a homomorphic sporophytic system. Such inhibition occurs when the same allele of the *S* locus is active in both the pistil and the developing pollen tube, whether pistil and pollen are derived from same plant or from different plants that carry the same allele. The rejection response is manifested in papillar cells by the deposition of lenticules of callose in the periplasm of the stigma papillae adjacent to incompatible pollen and within the germinating pollen tubes, especially at the tips of these rejected tubes. This is a cell-to-cell reaction and occurs 30 min after the initial contact between the incompatible pollen and the papillar cells (Fig. 4). The elicitor of this incompatible reaction is a protein pro-

duced by the *S* locus, which we presume to be synthesized in the tapetum and then incorporated into the pollen exine during pollen development, as suggested by Heslop-Harrison (1975). These proteins of the *S* locus, in both pistil and pollen, interact in an unknown manner to elicit a series of biochemical and physiological responses. These changes lead to inhibition of the growth of the incompatible pollen-tube. Rêgo et al. (1998) identified proteins associated with four self-incompatibility *S* alleles in yellow passion fruit, which co-segregated with their respective *S* alleles. These peptides were present mainly in the stigma and at lower amount in the style, where their maximum expression was reached 1 day before anthesis.

Stylar inhibition

Another site where the inhibition of pollen-tube growth occurred was the style. Some reciprocal intra-group crosses within the families BG and BJ were incompatible. Especially in progeny BJ, all crosses of BJ20 (Group III) with the plants BJ17, BJ16 and BJ21 (Group XIV) were compatible when BJ20 was used as the male parent, and incompatible in the reciprocal crosses (Figs. 7, 8, 9). In this case, the pollen-tube growth was inhibited in the upper-third of the stylar transmitting tissue, when flowers of BJ20 were pollinated with pollen of any one of the three other plants (Group XIV). The arrest of pollen tubes within the style was characterized by an irregular deposition of callose and abnormalities in pollen-tube growth, which are both characteristic for the gametophytic system (Heslop-Harrison 1975; de Nettancourt 1977; Dumas and Knox 1983). Rêgo et al (1998) found evidence of two loci controlling the self-incompatibility in yellow passion fruit. The results of the present work also suggest an additional gene that works together with *S* but follows the rules detemined by the gametophytic system, as described by Zuberi and Lewis (1988) in *Brassica campestris*.

Acknowledgements This research was supported by CAPES, CNPq and FAPEMIG. The autors are grateful for the valuable collaboration of Drs. Carvalho, C.R. and Vieira M.F. for technical assistence. The experiments comply with the current laws of Brazil.

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