A PUTATIVE MUTANT OF A SELF-COMPATIBLE YELLOW PASSION FRUIT
WITH THE CORONA COLOR AS A PHENOTYPIC MARKER (1)

MARGARETE MAGALHÃES SOUZA (2); ALEXANDRE PIO VIANA (3);
TELMA NAIR SANTANA PEREIRA (3)

ABSTRACT

Yellow passion fruit (Passiflora edulis f. flavicarpa) is a self-incompatible crop with purple corona flowers (PCF). Plants exhibiting white corona flowers (WCF) were observed in an experimental field located in Campos dos Goytacazes - RJ, Brazil. This study was performed in order to test the fertility of these genotypes through studies of in vivo pollination, meiotic behavior and pollen viability. Using in vivo pollination, the mean fruit set percentage, obtained from self-pollinations at the moment that flowers open, was 70.83% in WCF and 0% in PCF. In terms of cytological analysis, for both kinds of plants, WCF and PCF, the meiotic behavior was considered regular, considering the high meiotic index estimated for both of them, 96.3% and 97.7%, and pollen viability, 98% and 99.5%, respectively. Such results suggest that the genotype WCF is self-compatible and interesting for use in genetic improvement of yellow passion fruit.

Key words: Passion flower, self-compatibility, in vivo pollination, meiotic behavior, pollen viability.

RESUMO

UM PROVÁVEL MUTANTE AUTOCOMPATÍVEL DE MARACUJAZEIRO AMARELO
COM A COR DA CORONA COMO MARCADOR FENOTÍPICO

O maracujazeiro amarelo (Passiflora edulis f. flavicarpa) é autoincompatível e com flores de corona púrpura (PCF). Plantas com flores de corona branca (WCF) foram observadas em um campo experimental localizado em Campos dos Goytacazes (RJ), Brasil. Este estudo foi realizado para testar a fertilidade dos genótipos WCF por meio de estudos de polinização in vivo, comportamento meiótico e viabilidade polínica. Utilizando polinização in vivo, o percentual médio de frutos obtidos das autopolinizações realizadas no momento de abertura da flor foi de 70,83% em WCF and 0% em PCF. Quanto às análises citológicas, para ambos os tipos de plantas, WCF e PCF, o comportamento meiótico foi considerado regular, com alto índice meiótico para ambas, 96,3% e 97,7%, e viabilidade polínica de 98% e 99,5% respectivamente. Tais resultados indicam que o genótipo WCF é autocompatível e de interesse para o uso em programas de melhoramento genético do maracujazeiro amarelo.

Palavras-chave: Maracujazeiro, autocompatibilidade, polinização in vivo, comportamento meiótico, viabilidade polínica.

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(2) Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Rod. Ilhéus-Itabuna, Km 16, 45662-900 Ilhéus (BA). E-mail: souzamagg@yahoo.com.br (*) Corresponding author.

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1. INTRODUCTION

Yellow passion fruit (Passiflora edulis Sims f. flavicarpa O. Deg.) is an allogamous and diploid (2n = 18; Storey, 1950) species. Its origin is unknown, being involved in an apparent case of edaphically induced speciation in the Amazon Forest (Gentry, 1981). It is an agronomically important species, which grows in rainy areas of the tropics and warm subtropics, whose fruits are considered delicious (Turner et al., 1996). Its flowers are very distinct: light smell, perfect and hermaphroditic, 4-5-cm wide. It has an androgyrnephore which supports its reproductive parts, five white petals and sepals, five anthers and two lines of purple coronas in the white base of the apex (Vanderplank, 2000). One of the main characteristics of the Passifloraceae is the size of the flowers and the coloration of the corona (Vanderplank, 2000; Ulmer and MacDougall, 2004). In plants, the main pigments are carotenoids (reddish, and from orange to yellow), anthocyanins (red, blue, and purple or violet) and chlorophylls (green). Anthocyanins belong to a group of phenolic compounds known as flavonoids, which are related to plant protection against damages caused by ultra-violet irradiation (Strack and Wray, 1989; Holton and Cornish, 1995) and against diseases caused by microorganisms (Gray, 1989). Probably, one of the important functions of flavonoids is related to pollination and sexual reproduction of plants (Peter and Verma, 1990).

The self-incompatibility of yellow passion fruit is an important factor to be considered, when planting this species aiming fruit production. Studies on the inheritance of this characteristics have been performed in this species, but the results are not conclusive yet (Bruckner et al., 1995; ReGo et al., 1999, 2000; Falleiro et al., 2000). The self-incompatibility in yellow passion fruit has been reported since the 19th century (Nettancourt, 1977) and many years after (Akamine and Girolami, 1957). ReGo et al. (1999, 2000) identified six alleles (from S1 to S6) and found evidence that the self-incompatibility in yellow passion fruit is controlled by two gene loci, instead of one, probably due to the presence of gametophyte gene which acts in association with sporophytic gene. Falleiro et al. (2002) concluded that the heredity of self-incompatibility in passion fruit is not a result of alleles of S series, or of other loci, but should be explained by a gene complex. This research aimed to study the fertility of a putative self-compatible mutant of yellow passion fruit which has flowers with white corona, through the analysis of the meiotic behavior, pollen viability, in vivo pollination and fruit set.

2. MATERIAL AND METHODS

Germplasm and experimental design

Twenty yellow passion fruit plants with white corona flowers from the experimental station, located in Campos dos Goytacazes – RJ, were analyzed in the randomized complete block design, with three replications and four plants per plot. The plants were obtained by cuttings derived from commercial populations, cultivated in two different places, Campos dos Goytacazes and Macaé, in Rio de Janeiro State, Brazil. When the plants were identified in the field, they were marked, and the genotype was named White Corona Flower - WCF. These plants called attention, not only due to the color of their filaments, but also due to the higher number of fruits, which suggests that the genotype could be self-compatible. The normal genotypes were called Purple Corona Flower – PCF.

In vivo pollination

In order to observe the fruit set in the putative mutant genotypes, three WCF and PCF plants were marked and eight flowers per plant were self-pollinated at the blossom moment (around 12 a.m.) and protected with paper bags until the following day, according to recommendations by Bruckner and Otoni (1999), totaling 72 flowers analyzed for each type of plant (WCF and PCF). The fertility percentage was checked 20 days after pollination, in the beginning of fruit development.

Meiotic behavior and pollen viability

Flower buds of the putative mutant genotype (WCF) and of the normal genotype (PCF) (Figure 1) were fixed in ethanol-glacial acetic acid (3:1) for 2 to 3 hours in room temperature and, after three changes of fixative during this period, the samples were stored at -20°C. Temporary slides were prepared using the squash technique and the cells were stained with 1% acetic carmine. At least 50 cells were analyzed in each meiotic phase per genotype. The analysis included the calculation of the meiotic index according to Love (1951). For pollen viability analysis, pollen grains (PG) of flowers at the anthesis were stained with Alexander solution (Alexander, 1969), which contracts cell wall and cytoplasm, considering the stained and whole PGs viable. The anthers were squashed in a drop of stain and after 2 to 5 minutes in room temperature the PGs were observed for morphological and staining aspects and counted. The values for meiotic index and for pollen viability considered in this study were the mean of four

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randomly sampled slides (repetitions), and each slide these was from two anthers of different flowers, totalizing four plants analyzed. ANOVA was performed using the computer program GENES (Cruz, 2006).

**Figure 1.** Flower with purple corona in the base (PCF; arrow) and flower with putative mutant genotype with white corona (WCF) in yellow passion fruit (*Passiflora edulis f. flavicarpa*).

### 3. RESULTS AND DISCUSSION

The mean percentage values of fertilization in WCF plants, originating from self-pollination of just opened flowers, in plant 1, 2 and 3, respectively, was: a) block 1 – 75%, 62.5%, 75%; b) block 2 – 50%, 87.5%, 62.5%; c) block 3 – 87.5%, 62.5%, 75%. The fertilization index for the WCF genotype was 70.83%. In PCF genotypes, the fertilization index was 0%. The ANOVA for the mean number of fertilized flowers within WCF plants was not significant (p<0.05). No abnormality was observed in fruits from self-pollination.

In both genotypes, low percentages of meiotic irregularity were observed (Table 1). All plants analyzed presented bivalent associations. However, quadrivalent associations were observed in some cells of the WCF genotype (Figure 2 a,b). In the overall, at meiosis I, chromosomes laggard (Figure 2 c,d) and bridges (Figure 2 e) were found in WCF and PCF. Chromosome bridges were maintained until the end of prophase II (Figure 2 f). Meiocytes of both genotypes show irregularities in the spindle fiber orientation during meiosis II, producing two chromosome groups with different positions in the same cell, characterizing the formation of tripolar or transverse spindle fiber (Figure 2 g,i). Irregularities in the spindle were the main abnormality found during the segregation of chromosomes, due to the high percentage of affected cells. Still in meiosis II, asynchrony was verified within the same cell during cell division, and WCF plants were the most affected ones by such irregularity. Some of those cells presented a group of chromosomes in the metaphase while the other nucleus were still disorganized (Figure 2 j). Other cells presented only one chromosome group starting anaphase (Figure 2 k). In telophase II, some cells did not present a symmetric position, but a disorganized position, compatible with the irregular organization of spindle fibers in anaphase II (Figure 2 l) or the cells presented three groups of chromosomes, instead of four. Consequently, post-meiotic products with reduced and non-reduced nucleus were detected (Figure 2 m,n), and sterile PG were observed (Figure 2 o). The mean percentages of meiotic index and pollen viability for WCF and PCF plants (Table 2) were not statistically different (p<0.05).

In cytological analysis for both plants, WCF and PCF, the meiotic behavior was considered regular, once the meiotic index and the pollen viability were higher than 70%. Some nuclear restitution mechanisms probably occurred (Shaw and Moore, 1998), leading to frequency of lagging chromosomes to up to 3% in anaphase II, but disappearing in telophase II. Some mutations may affect the pollen development and plant fertility, as it has been reported for species which have agricultural importance such as soybean and maize (Horner and Palmer, 1995).

<table>
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<tr>
<th>Irregularities</th>
<th>Plants with WCF</th>
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M: metaphase; A: anaphase; T: telophase; I: meiosis I; II, meiosis II. WCF: flowers with white corona; PCF: flowers with deeper-purple corona.
Figure 2. Meiotic behavior in yellow passion fruit (*Passiflora edulis* f. *flavicarpa*). a) Nine bivalents at the end of diakinesis in PCF plant. b) Seven bivalents and one quadrivalent at the end of diakinesis in WCF plant. c) Lagging chromosomes at metaphase I in PCF plant. d) Lagging chromosome at telophase I in WCF plant. e) Chromosome bridge at anaphase I in PCF plant. f) Chromosome bridge at prophase II in WCF plant. g) Tripolar spindle at metaphase II in WCF plant. h) Transverse spindle at metaphase II in PCF plant. i) Transverse spindle and lagging chromosomes at anaphase II, and irregular alignment of nuclei at telophase II with micronucleus (arrowhead) resulting from tripolar spindle in WCF plant. j-k) Asynchrony during the cellular division at meiosis II: j) cell presenting one of the groups of chromosomes in metaphase while the other was disorganized in WCF plant, k) cells showing only one of the groups having initiated the anaphase in PCF plant. l-n) Irregular post-meiotic products: l) normal tetrads and monad; m) normal tetrads and dyad; n) Polyad; o) Well-stained, viable pollen grain (dark color) and non-stained, sterile pollen grain. Bar = 20 μm.
Although other meiotic irregularities such as asynchrony of meiosis II within the same cell and abnormalities in the orientation of spindle have been observed in the genotypes WCF and PCF of yellow passion fruit. Such irregularities, apparently, have not affected the pollen viability, which was higher than 98% in such plants. However, the production of non-reduced or unbalanced gametes may not be excluded, for which the cytoplasm has been stained and remains integral. Stains like carmine overestimate pollen viability, and chromosome fragments, fragmented extra nuclei or disintegrated nucleus are better observed when DAPI or vital stains are used (RODRIGUES et al., 2005).

In this study, an average fertilization percentage of 70% was observed in genotypes with white coloration of the corona - WCF plants. If, probably, one of the important functions of anthocyanin (red, blue, and purple or violet pigments) is related to pollination and sexual reproduction of plants (PETER and VERMA, 1990), in WCF plants the pollination by insect may not be necessary, justifying the absence of corona color in these genotypes.

The index obtained through self-pollination in WCF plants indicated that they are self-compatible. The self-incompatibility may be basically gametophytic and sporophytic. HO and SHII (1986) suggested that the self-incompatibility in yellow passion fruit was of the sporophytic type, since the pollen rejection occurs in the stigma. Studies made by BRUCKNER et al. (1995) indicated that at least five S alleles are responsible for self-incompatibility in yellow passion fruit. FALLEIRO et al. (2000) concluded that self-incompatibility in yellow passion fruit is a result not only of S alleles action or of other loci, but also of a gene complex. Literature reports that the percentage of fruits obtained from self-pollination in yellow passion fruit varied from 0 to 20.4% (AKAMINE and GIROLAMI, 1959; KNIGHT and WINTERS, 1962; RUGGIERO et al., 1978). CHANG (1974) obtained 2% to 4% of frutification by self-pollination in yellow passion fruit and around 80% in purple passion fruit. BRUCKNER et al. (1995) pollinated flower buds before anthesis, and self-pollinated buds resulted in 14.8% fruit set, which indicates that self-fertilization is possible. The self-compatibility may be explained by the presence of compatible S alleles or by other locus which control the self-compatibility (ZUBERI et al., 1981; LEWIS et al., 1988).

In Australia, the occurrence of self-compatible genotypes in purple passion fruit or in hybrids between the Golden variety and forms of purple passion fruit was mentioned by MENZEL et al. (1989). Through reciprocal crossings, BRUCKNER et al. (1995) obtained plants of yellow passion fruit which were partially compatible (5 - 59% fruit set). The partial or pseudo-compatibility may occur through the presence of S alleles which have low dominance level (OCKENDON, 1974) or through the presence of other gene, or of gene complex, which modifies the incompatibility expression (LEWIS et al., 1988; ZUBERI and LEWIS, 1988). VIANA et al. (2003), while dealing with the same population in which WCF genotypes were found, observed that this genotype presented good agronomic performance, thus being included among the plants which bore higher quantity of fruits per plant and heavier fruits, and such characteristics generally are related to self-compatibility.

Some factors may explain the 30% of absence of frutification in WCF genotypes. One of them is the peculiarity of different kinds of curvature of the style in passion fruit. The style can be positioned in the flowers as follow: a) completely curved (CC), when stigmas are near the anthers; b) partially curved (PC), when the stigmas are over the anthers, making a 45° angle; c) without curve (WC), when the style does not curve and the stigmas make 90° angle. In this last type, the flowers are sterile (RUGGIERO et al., 1978) and their frequency may vary between 0 to 50% on the same plant (SOUZA et al., 2002a).
The flowers did not exhibit any marking which would make it possible to soon distinguish among the ones with different kinds of style curvature, and genetic information is not available for such characteristic (Souza et al., 2004). In yellow passion fruit plants, style curvature occurs before one or two hours only, and at 12 a.m. it is still not possible to know which would be the style curvature. However, it is known that the time of flower opening influences negatively the pollen viability (Souza et al., 2002b), and late pollinations could harm the self-pollination experiment results.

Factors such as pesticides (Silva et al., 1999) and improper temperature (Halterlein et al., 1980; Iapichino and Loy, 1987) have a strong negative effect on pollen germination. Among the biotic factors which influence the development of yellow passion fruit, high air humidity during pollination may affect the frutification and fertilization percentage (Rojas and Medina, 1996). When anther opening coincides with high humidity, the high osmotic pressure of the cell content of pollen grains, together with low resistance of their walls, reduce the pollen viability (Souza, 1994). Studies in other species have showed that flower buds are particularly sensitive to stress caused by warming, principally in the development stages which occur five to three days before anthesis and which correspond to microporogenesis, bringing reduction in the pollen production and viability (Gross and Kigel, 1994; Vara Prasad et al., 1999).

Biotic factors such as the physiological stages of the plant interfere with the frutification and seed production (Lederman, 1987). According to Dumas and Knox (1983), the normal development of the pollen tube is the result of good nutritional conditions provided by the transmission tissue of the pistil. Different interactions may exist hours after the growing pollen tube reaches the style tissue (Rego et al., 2000).

The WCF plants, identified as self-compatible, exhibited their corona filaments without attractive color. According to Mol et al. (1998), the main function of pigmentation is to attract pollinators, in that the coloring standard of flowers make them easily recognizable, contrasting with the other parts of the plant. Generally, differences in flower pigments are related to attraction of specific pollinators (Proctor et al., 1996), such that the behavior of pollinators is influenced by the floral morphological characteristics (Dusenbery, 1992). However, not all pollinators see flower colors in the same way (Mol et al., 1998). In Passiflora, the anthocyanins are the most frequently found pigments in flowers. Other factors are responsible for differentiation in floral parts, including co-pigmentation, vacuolar pH and cell format (Mol et al., 1998). The ultraviolet-visible spectral absorption of Passiflora flowers seems to be adjusted to the pollinators’ visual spectral sensitivity (Varassin et al., 2001).

Flower colors are so important for plant biology that mutations which inactivate few structural genes involved in the form and coloring of flowers are enough to change the species of pollinators, which may bring genetic isolation and, possibly, speciation (Vickery, 1995). The pigmentation patterns are established by the cell-specific accumulation of anthocyanins biosynthetic genes (Holton and Cornish, 1995). In Datura stramonium, the presence of anthocyanins has been used as phenotypical marker. Plants of this specie which produce anthocyanins have purple flowers and dark-green stems (‘purple’ plants), while plants which lack anthocyanins have white flowers and light-green stems (‘white’ plants): the anthocyanin marker is dominant and expressed by only one locus, in which polymorphism is kept by selection, and selective agents may include both biotic and abiotic interactions (Stone, 2000). Mutations in which the synthesis of anthocyanin is reduced or completely blocked have been identified in several plant species. Many of them affect enzymes in the flavonoid biosynthesis pathway, but some of them affect regulatory loci (Mol et al., 1998). In the WCP genotypes analyzed, the pigments may have lower importance in flowers because they are self-compatible. However, pollen grains of passiflora flowers are heavy and sticky, and are rarely taken by wind, and such characteristics, besides the self-incompatibility, are responsible for the failure of self-pollination in PCF genotypes.

4. CONCLUSION

The results of the in vivo pollination and meiotic behavior indicate that the WCF genotypes are self-compatible and produce normal gametes, thus being interesting genotypes to be used in breeding programs of yellow passion fruit, once genetic studies are carried out so that the self-compatibility of such plants is totally confirmed.

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