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POLLINATION AND ASPERMIA IN ANNONACEAE

M.C.T. PEREIRA[1], R.A.N. FARIA*[1], A.M.S.S. DAVID[1], J.F. SILVA[1], S. PIMENTA[1], S. NIETSCHE[2], B.R.A. RODRIGUES[1], A.M.L. PIMENTA[1], F.S. GOMES[1], F.C. OLIVEIRA[1], G.B. OLIVEIRA[1], A.R. SANTOS[1], E.A. PARAIZO[1], H.T.R. AMARO[3], J.B. BORGES[1], J.R.P SANTOS[1] AND H.K.C. FREITAS[3]

[1]State University of Montes Claros, Department of Agrarian Sciences, Janaúba 39440-000, Brazil
[2]Federal University of Minas Gerais, Institute of Agricultural Sciences, Montes Claros 39.404-547, Brazil
[3]State University of Montes Claros, Paracatu 38600-000, Brazil

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Abstract– This review presents advances in studies on pollination and aspermia in annonaceae of economic importance: custard apple, atemoya and soursop. The use of artificial pollination is essential for greater set rate, uniformity and fruit size. The condition of absence of seeds (aspermia) in species of the genus *Annona* occurs naturally by stenospermy, where the fruit partially contains formed seeds that were aborted after fertilization, or artificially, by the induction of parthenocarpy with the use of phytohormones. Stenospermy in *A. squamosa* is justified by the INO gene suppression, which causes lack of integument in the ovule and rupture of the integument adjacent to the perisperm. For parthenocarpy induction, the use of gibberellic acid has promoted good results in fruit set, quality and size, in addition to obtaining quality seedless fruits. Knowledge of parthenocarpy induction,stenospermy and seed development in fruits is relevant to ensure advances in breeding programs in the development of superior genotypes with seedless fruits, and quality parthenocarpic fruits.

INTRODUCTION

The genus *Annona* has more than 100 species, with emphasis on *Annonasquamosa* (custard apple, sweetsop or sugar apple), atemoya (*A. squamosa* x *A. cherimola* hybrid) and *Annonamuricata* (soursop), species of great economic importance in Brazil and in the world. Fruits have high nutritional value, aromatic and pleasant taste, and can be used for fresh consumption andproduction of ice creams, juices and pulps (Pereira and Borém, 2021).

Brazil, Thailand, India, Taiwan, Cuba and the Philippines stand out in the production of custard apple fruits. In Brazil, there are many commercial custard apple plantations, with high quality fruits and good economic expression. Cultivation areas are mainly concentrated in the Northeastern and Southeastern regions of the country, with emphasis on the states of Bahia, Alagoas, Paraíba, São Paulo, Minas Gerais, Pernambuco, Ceará and Rio Grande do Norte, with planted area of more than six thousand hectares and production of 36,654 $t.ha^{-1}$ (Lemos, 2021).

The leading countries in soursop production are Brazil and Mexico. In Brazil, the fruit gained prominence due to the good acceptance by consumers of pulp and processed products. Production is concentrated in the Northeastern, Northern, Midwestern and Southeastern regions. The Northeastern region is the largest producer, with emphasis on the states of Bahia, Pernambuco, Ceará, Alagoas and Paraíba (Lemos, 2021). Australia is the largest atemoya producer, but several countries cultivate this hybrid on a small commercial scale, including Brazil, where cultivation occurs mainly in the states of São Paulo, Minas Gerais, Paraná and Bahia (Lemos, 2021).

Atemoya and soursop trees have hermaphrodite flowers; however, they present protogynous dichogamy, in which stigmas are receptive before the release of pollen grains by stamens. Natural pollination is hampered by this phenomenon and by the scarcity of pollinating insects, requiring the use of artificial pollination to ensure high fruit set rates, in addition to uniform and large fruits (Pereira *et al.*, 2021). Artificial or natural pollination is also essential for fruit production in seedless custard apple varieties (Mendes *et al.*, 2012).

* **Corresponding author's e-mail:** renataapneres@gmail.com, marlon.pereira@unimontes.br

In Brazil, there is a selection of seedless custard apple called Brazilian seedless, which has been used in breeding programs with the objective of transferring this characteristic, the absence of seeds (Nassau, 2021; Nietsche *et al.,* 2021). Aspermic species are those that can produce fruits without seeds, with small number of seeds or that contain only remnants of aborted seeds (Varoquaux *et al.,* 2000). The characteristic of absence of seeds is of great importance for species with commercial potential due to the good acceptance of fruits for fresh consumption and easy processing into pulp (Lora *et al.,* 2011a; Panddolfini, 2009). The condition of absence of seeds in species of the genus *Annona* occurs naturally by stenospermy, where the fruit contains partially formed seeds that were aborted after fertilization, or by the induction of parthenocarpy with the use of phytohormones (Lora *et al.*, 2011b; Nassau *et al.*, 2011b; Nassau *et al.*, 2021; Pereira *et al.*, 2019a; Santos *et al.*, 2019).

The knowledge of parthenocarpy induction, stenospermy and seed development in the fruit is relevant to ensure advances in breeding programs in the development of superior genotypes with seedless fruits, and quality parthenocarpic fruits.

Pollination and seed development

The main cultivated Annonaceae are allogamous plants, with hermaphroditic flowers; however, they present the phenomenon of protogynousdichogamy, that is, when pollen grains are released, the female organs are no longer receptive, generally preventing self-pollination in the same flower (Pereira *et al.,* 2021). Furthermore, flowers of the same genotype usually open synchronously and, consequently, pollen transfer between different flowers of the same genotype is difficult (Lora *et al*., 2010). Mendes *et al*. (2017), confirmed the phenomenon of protogynous dichogamy in *A. squamosa*, observed the maturation of female and male organs separated in time, and classified phenological stages based on a system for standardizing the coding of the phenological stages of the species, the BBCH scale of Hack *et al.* (1992), in which flowers were pre-female at stage 60 and female at stage 61, when the stigma proved to be receptive and ideal for artificial pollination; the functional pistillate phase occurred at stage 65, when pollen was released.

Environmental conditions influence the length of the receptive phase, thus, stigmatic receptivity is extended with increasing humidity and decreasing temperatures and shortened with decreasing humidity and increasing temperatures and, under some conditions, stigma can be receptive even when flowers changed to the male stage (George *et al.,* 1989; Lora *et al.,* 2011). Pollen development in *A. cherimola* before anther dehiscence is greatly influenced by climatic conditions, temperature variations influence the release of bicellular or tricellular pollen, with viability and high sensitivity to desiccation, making pollen storage difficult (Lora *et al.,* 2006). In *A. squamosa*, the rainy season has effects on microsporogenesis and microgametogenesis, promoting higher fruit quality (Rodrigues *et al*., 2018). As climate influences fruit quality during fertilization, it can also influence seed development, and according to Carvalho and Nakagawa (2012), the environmental conditions during flower formation and fertilization may have an impact on the vigor of seeds formed.

Most Annonaceae species are pollinated by small beetles, especially those of the Nitidulidae family, such as *Carpophilus humeralis* and *C. mutilatus*, which reproduce and feed on decaying fruits or sap flows and are the main pollinators of commercial *Annona* species, mainly custard apple and atemoya. In *Annonamuricata*, beetles of the Scarabaeidae family, larger species, are considered efficient pollinators. However, there is shortage of these insects, in addition, fruits formed via natural pollination are malformed and defective, requiring artificial pollination (Peña *et al.,* 2002; Pereira *et al.,* 2021). Artificial pollination is performed using a brush or pollinator pump. Pollen grains can be removed at the time of pollination in the field or collected in advance; flowers at the female stage in late afternoon, and pollen grains extracted in the morning, at which time flowers will appear as male (Pereira *et al.,* 2021).

Pollen viability is one of the factors that directly influence the fertilization success in the pollination of species of the genus *Annona*; pollen has short viability after plant removal (Bonaventure, 1999; Pinto, 2003). Pollen conservation allows its use at any time due to the artificial synchrony between pollen dispersion and floral receptivity. In a study carried out in *A. cherimola* with pollen conservation at low temperatures, it proved to be viable after three months of storage, resembling fresh pollen (Lora *et al.,* 2006). According to Bettiol Neto *et al*. (2009), custard apple, cherimoya and atemoya pollen collected in the wet season shows greater fecundity. In *A. squamosa*, pollen grains stored under ambient conditions for 24 hours remain viable

(Araújo *et al.*, 2021). In the study carried out by Pereira *et al.*, 2014, with 'Lessard Thai' sugar apple and 'Gefner' atemoya, it was shown that their pollen grains can be stored in refrigerator for upto 24 and 48 hours, respectively. In *A. squamosa* L. cv. Balanagar, pollen stored at -196 °C tends to be viable for a period of two months (Chander *et al.*, 2019).

According to Bewley *et al.* (2013), in pollination, coupling, copulation, zygote formation and seed development occur. After fertilization, the ovule undergoes modifications and gives rise to the mature seed, which must have maximum size, dry weight, germination power and vigor due to the accumulation of photoassimilates, which are transformed into new cells and tissues, including reserve tissues (Carvalho and Nakagawa, 2012). *A. squamosa* flowers are pendant, with trimerous calyx and corolla, fleshy, with green color in the external phase and cream color in the internal phase. The gynoecium is composed of more than 100 carpels, with superior ovary and simple style, each carpel with a single anatropous ovule that can develop into a single seed; the androecium is located below the gynoecium forming a helical structure with more than 100 stamens, with short filaments, bithecal anthers and terminal connective (Kiill and Costa, 2003; Gupta *et al.*, 2015).

According to Gupta *et al.* (2015), knowledge of the initial phase of fruit development is important, since after fertilization, molecular and biochemical pathways occur, which determine number of seeds, fruit size and other fruit quality characteristics, such as accumulation of sugars and organic acids.

The initial development of the embryo and endosperm in *A. squamosa* occurs 24h after pollination, with the presence of starch associated with polar nuclei; and 72 h after pollination, seed growth is observed due to cell expansion in the nucellus (Santos *et al.*, 2014). In *A.cherimola*, eight days after pollination, the initial cell division in the zygote was observed, which leads to the formation of the embryo (Lora *et al.*, 2010). Santos *et al.* (2019) evidenced fertilization in atemoya at seven days after anthesis, when verifying embryos at the beginning of the micropyle area development.

Parthenocarpy and stenospermy

Parthenocarpy is a phenomenon that occurs naturally or induced, in which fruits are formed without the occurrence of fertilization. This phenomenon is desirable in fruits, due to the potential to improve fruit quality and the absence of seeds, a characteristic appreciated by consumers (Knapp, 2016). Phytohormonesauxins, gibberellins and cytokinins are responsible for coordinating the fertilization of ovules and seed and fruit development (Gillaspy *et al.*, 1993). The onset of fruit growth is under the control of phytohormones, and fruit development is commonly controlled by immature seeds (Taiz and Zeiger, 2010). In parthenocarpic fruiting, endogenous phytohormones are high, which allows us inferring that phytohormones produced outside developing seeds can regulate fruit growth (Gustafson, 1936). Thus, parthenocarpy can be induced through the exogenous application of phytohormones. Many studies have been successfully carried out using phytoregulators, effectively inducing fruit development in the absence of fertilization and increasing productivity (Acciarri *et al.*, 2002; Gillaspy *et al.*, 1993; Ficcadenti *et al.*, 1999; Ferrara *et al.*, 2014; Pereira *et al.*, 2014; Santos *et al.*, 2016; Tang *et al.*, 2015; Mahmood *et al.*, 2016).

Annonaceae fruits normally do not develop by parthenocarpy; the known aspermic genotypes have fruits developed by stenospermy, in which the fruit contains partially formed seeds that were aborted after fertilization (Bouquet and Danglot, 1996; Lora *et al.*, 2011; Mendes *et al.*, 2012). The known seedless varieties are of *A. squamosa: Cuba seedless* from Cuba, *Thai seedle*ss from Thailand and *Brazilian seedless* from Brazil (Araújo *et al.*, 1999; Pinto *et al.*, 2005; Lora *et al.*, 2011; Nietsche *et al.*, 2021). The accession called Brazilian seedless is originated from a natural mutation identified in Brazil in the year of 1940, and produces seedless fruits, but fruits are small, uneven and highly perishable, little used commercially (Nietsche *et al.*, 2021).

Some studies were carried out on annonaceae with the aim of obtaining larger and seedless fruits. Encina *et al.* (2014) evaluated induction to obtain new high quality genotypes and worked in the cherimoya breeding program (*A. cherimola*), in the ploidy manipulation to obtain haploid, tetraploid and triploid (seedless) plants. Pereira *et al.*, (2014), Pinto *et al.* (2018) and Santos *et al.* (2019) carried out studies with the gefner atemoya hybrid aiming at obtaining parthenocarpic fruits, that is, without the need for pollination. These authors used gibberellic acid (phytohormone that plays a prominent role in fruit growth coordination and seed development) applied to flowers and fruits to induce parthenocarpy and associated with artificial pollination to obtain larger fruits, and demonstrated that gibberellic acid promotes the growth of pollinated fruits by stimulating cell division and expansion and induces parthenocarpy, maintaining division and stimulating cell expansion; however, parthenocarpic fruits were smaller than those obtained by pollination due to the absence of seeds. Pereira *et al.* (2019a), also with the aim of evaluating the effect of application of gibberellic acid (GA3) with and without artificial pollination on the set and quality of 'Red', 'Lessard Thai' custard apple and 'Gefner' atemoya fruits, observed significant increases in the length and weight of 'Red' and atemoya fruits, which showed reduction in the total number of seeds. They also observed that natural pollination associated with GA3 (1000 mg l^{-1}) was effective in the production of high quality 'Gefner atemoya seedless fruits.

Recent studies have been carried out with the aim of explaining stenospermy in *A. squamosa* and for further development of superior seedless cultivars. Nassau *et al*., (2021) concluded that the presence/absence of seeds in *A. squamosa* is governed by the ino gene, and that inheritance is possibly monogenic with complete dominance-type allelic interaction. Lora *et al*. (2011) in a study with the 'Thai seedless' mutant variety, demonstrated that stenospermy is due to the ino gene suppression, which leads to the loss of the outer integument of the ovule, thus affecting seed development. Likewise, Santos *et al*., (2014) reported that the aspermic nature of Brazilian seedless accession fruits is related to the lack of integument in the ovule and rupture of the inner integument adjacent to the perisperm and that stenospermy may be related to the ino gene suppression. Stenospermy in Brazilian seedless was also confirmed by Mendes *et al*. (2012), when evaluating fruit formation and germination of pollen grains, and concluded that these are viable, and artificial or natural pollination is essential for fruit production.

The absence of seeds in fruits is a characteristic appreciated by consumers for the practicality of fresh consumption, and the absence of seeds is also associated with the best gain due to the added value and easy fruit processing (Lora *et al*., 2011a; Pandolfini, 2009). Therefore, breeding programs are interested in developing seedless custard apple and atemoya genotypes. Pereira *et al*., (2019b) reported that the availability of improved custard apple, soursop and atemoya genetic materials is very limited in several countries, which reinforces the need for genetic improvement of the main commercial annonaceae. The Brazilian seedless selection has been used in breeding programs with the aim of transferring the seedless characteristic to an interspecific atemoya hybrid (Nassau, 2021; Nietsche *et al*., 2021). The knowledge of ino gene suppression in seedless custard apple species allows molecular marker-assisted selection using specific primers in subsequent generations and backcrosses with the Gefnercultivar (Nassau, 2021), as well as in future studies with intraspecific *A. squamosa* crosses with and without seeds.

CONCLUSION

Knowledge of pollen viability and seed development in Annonaceae, as well as climatic conditions at the time of pollination, is of great importance in the adoption of practices aimed at increasing fruit productivity and quality.

For a better understanding of parthenocarpy in *annona* fruits, further studies are needed to elucidate this mechanism; however, the practice of inducing parthenocarpy in atemoya has great potential for the production of seedless fruits. The use of phytohormones with methodology adjustments to the field, taking into account the financial return to the producer, can be considered a new practice to improve the quality of custard apple and 'Gefner'atemoya fruits.

The phenomenon of stenospermy in *A. squamosa*, due to the ino gene suppression, allows breeding programs to optimize the time of work with annonaceae, taking into account that they are perennial species with a long juvenile period. The use of specific ino gene primers, used in genotyping, allows the detection of the absence of seeds yet at the seedling stage.

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