Study of pollination and floral biology of *Passiflora edulis* f. *edulis* Sims as a basis for pre-breeding

Estudio sobre polinización y biología floral en *Passiflora edulis* f. *edulis* Sims, como base para el premejoramiento genético

Juan Sebastián Rendón[1], John Ocampo[2], Ramiro Urrea[3]*

[1]Agricultural Engineer, Caldas University/Agrojar, Jardín, Antioquia, Colombia
[2]Agricultural Engineer, M.Sc., Ph.D., Faculty of Agricultural Sciences, Universidad Nacional de Colombia, at Palmira/Centro Internacional de Agricultura Tropical - CIAT/DAPA, Colombia.
[3]Agricultural Engineer, M.Sc., Faculty of Agricultural Sciences, Caldas University, Colombia.
*Corresponding autor: jaocampop@unal.edu.co

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Abstract

The purple passion fruit is the second economically most important species of the genus *Passiflora* L. as it is marketed as fresh and processed fruit in all continents. The objective of this research was to study the mechanism of natural and assisted pollination to understand gulupa reproductive system that enables the implementation of pre-breeding and conservation strategies. A total of 32 accessions from 10 Colombian departments under five pollination treatments (natural and assisted) were evaluated in Manizales, Caldas at 2340 m.a.s.l. Results show that 80% of the anthesis in purple passion fruit occurred between 6:00 and 8:00 hours, prevailing flowers with fully curved style (66.4%). The highest percentage of fruit formation was found in manual self-pollination and geitonogamy treatments (82 and 86%), and followed by cross-pollination (68%) with significant differences (Duncan, $P<0.05$). High precipitation during the anthesis phase (≥9 mm/day) generated a negative impact on pollination and on the activity of pollination agents. Fruit from manually and natural pollinated flowers showed an average of 151 to 157 seeds with no significant differences. The results show that the purple passion fruit is an autofertile species that depends on pollinator insects for genetic flow and productivity. The capacity of autocompatibility of the purple passion fruit (28 to 86%) permits producers conserve the genetic quality of elite plants or their use in genetic breeding programs to obtain greater cultivar robusticity and productivity.

Key words: Fruit production, *Passiflora*, pollination, purple passionfruit, self-compatibility

Resumen

La gulupa es la segunda especie en importancia económica del género *Passiflora* L., ya que se comercializa como fruta fresca y procesada en todos los continentes. El objetivo de esta investigación fue estudiar los mecanismos de polinización natural y asistida para comprender el sistema reproductivo de la gulupa, con el fin de implementar estrategias de premejoramiento genético y de conservación. Un total de 32 accesiones de 10 departamentos colombianos fueron evaluadas bajo cinco tratamientos de polinización natural y asistida en Manizales, Caldas, a 2340 m.s.n.m. Los resultados mostraron que 80% de la antesis en la gulupa ocurre entre 6:00 y 8:00 a.m., predominando las flores que poseen el estilo totalmente curvo (66.4%). Los mayores porcentajes de frutos formados se presentaron en los tratamientos de autopolinización manual y geitonogamia (82 y

86%), seguidos por la polinización cruzada (68%) con diferencias significativas (Duncan, P<0.05). La alta precipitación durante la fase de antesis (≥9 mm/día) tuvo un impacto negativo sobre la polinización y la actividad de los agentes polinizadores. Los frutos de flores polinizadas manualmente y en forma natural presentaron un promedio de 151 a 157 semillas, sin diferencias significativas. Los resultados mostraron que la gulupa es una especie autofértil, que depende de los insectos polinizadores para el flujo génico y la productividad. La capacidad de autocompatibilidad de la gulupa (28 a 86%) permite a los productores conservar la calidad genética de las plantas élite o su uso en programas de mejoramiento genético para la obtención de cultivares de mayor rusticidad y productividad.

Palabras clave: Autocompatibilidad, gulupa, *Passiflora*, polinización, producción de fruto, semilla

Introduction

Gulupa or round curuba (*Passiflora edulis* f. *edulis* Sims) is one of the main species of Passiflora by its high economic potential in the domestic and international markets (Ocampo and Wyckhuys, 2012). This species is native to southern Brazil, Paraguay and northern Argentina, and has been introduced in five continents as an ornamental plant and a commercial crop. The fruit is a spherical or ovoid dark purple berry when ripe and its interior yellow almost orange with pleasant aroma, rich in sugars, vitamins (A, B12, B3 and C) and minerals (Sims, 1818). Gulupa has a solitary flower, hermaphrodite, pentameric, with five white petals and sepals and a crown composed of two outer white sets and purple towards the base (Sims, 1818). Pollination is mainly performed by bumblebees the genus *Xylocopa* spp., which are attracted to the sweet nectar, where they are impregnated by the sticky pollen from the anthers (Nishida, 1963; Ángel *et al.*, 2011; Nates *et al.*, 2012).

In Colombia, gulupa is found in the hillside areas above 1500 m.a.s.l. in renatured or wild form and adopted by farmers in home gardens. On the other hand, its cultivation is relatively new for Colombia and in recent years there has been a growing demand in international markets, ranking third after bananas and gooseberry in the line of fruits exported to the European market. In Colombia there are still no reports of cultivars, and farmers obtain seeds from the best fruits of each harvest or from seedlings from commercial nurseries with no guarantee of genetic quality.

In the genus *Passiflora* L. there are several studies on floral biology, with emphasis on the mechanisms of pollination by different agents such as insects, birds and mammals (Kishore *et al.*, 2010). In *P. moorean*, Amela and Hoc (1998a) identified three phases of floral anthesis and determined that lasts 24 hours, depending on weather conditions. The same authors (Amela and Hoc, 1998b) also found that *P. foetida* L. can produce fruits by induced selfing and open pollination, indicating that the species is optional self-pollinated. In a study of floral biology in *P. cincinnata* Mast, Aponte and Jáuregui (2004), established that 75% of fruit formation depends on the mechanisms of cross-pollination by insects (*Xylocopa* spp.). In another study in *P. edulis* f. *edulis, P. quadrangularis L., P. foetida L.* and *P. edulis* f. *flavicarpa*, Degener mentions that the largest number of fruits formed (set) occurs when the flower styles (gynoecium) are completely curved, allowing greater pollen responsiveness (Kishore *et al.*, 2010). In passion fruit (*P. edulis* f. *flavicarpa*) pollen viability tests showed that it can remain viable for up to 24 h after anthesis, with percentages between 50% and 75% (Souza *et al.*, 2004). On the other hand, the success and reproductive capacity of these plants are directly related to the time of flower opening and the time it remains open, depending on the environmental conditions of each area. In some species of *Passiflora* the opening occurs with the appearance of sunlight, as in *P. foetida*

(Amela and Hoc, 1998b), *P. edulis* f. *edulis* (Ángel *et al.*, 2011). However, in other species such as *P. edulis* f. *flavicarpa* (passion fruit) anthesis starts after 12:00 hours in most production areas (Souza *et al.*, 2004).

In Colombia, information on studies of pollination and breeding systems in *Passiflora* are scarce. An initial study by Ángel *et al.* (2011) in commercial crops located in Buenavista (Boyacá) stated that gulupa is a self-incompatible species that needs pollinators to produce a harvest, as only 33% of the fruits are formed without access to these. On the other hand, in a study published by Medina-Gutierrez *et al.* (2012) found that differences in altitude (1657 and 2225 meters) in two crops gulupa established in Boyacá (Colombia) affected the composition, the number of visitors and pollinators. Knowledge of the reproductive biology is of practical importance in agriculture, allowing direct interspecific mating patterns and developing management practices aimed at promoting the plant species and its pollinators (Sage *et al.*, 2005).

The objective of this research was to study the mechanisms of natural and assisted pollination to understand the reproductive system of gulupa, which enables the implementation of strategies for pre-breeding and conservation of superior genotypes.

Materials and methods

Study area. The research was conducted at the Tesorito farm in the municipality of Manizales (Caldas, Colombia), at 2340 masl (5° 01' 49'' N -75° 26' 13'' W), average temperature 17 ° C, annual rainfall of 1800 mm, relative humidity of 78% and 1215 h-light of solar brightness per year (weather station, University of Caldas).

Plant material. The study was conducted with plants of 32 accessions of gulupa, 15 months of age (Table 1) from 10 Colombian departments and evaluated between July 2010 and September 2011. Each accession was composed of five plants, set at a distance of 4 m between rows x 3 m between plants and manages with agronomic practices, which included monthly soil fertilization (NKP), natural rainfall irrigation, growth and sanitary pruning, as recommended by Ocampo and Wyckhuys (2012).

Table 1. Accessions evaluated in the study of the National Collection of Gulupa *Passiflora edulis* f. *edulis* Sims, Colombia.

Departament	Code	No. accessions
Boyacá	BoyEdu	6
Antioquia	AntEdu	4
Caldas	CalEdu	2
Cundinamarca	CunEdu	7
Huila	HuiEdu	4
Quindío	QuiEdu	2
Tolima	TolEdu	3
Putumayo	PutEdu	1
Valle del Cauca	ValEdu	1
Risaralda	RisEdu	2
Total	–	32

Floral studies

Floral biology. The time of anthesis was determined by randomly marking 25 flower buds in pre-anthesis in different accessions from 0:00 pm, followed every hour until 12:00 h counting the number of open flowers. The type of flower in relation to the curvature of the style (no curvature, partially curved and fully curved) was established by scoring 20 flowers per accession (two plants) and counting the frequency of each type present in the flower (Photo 1).

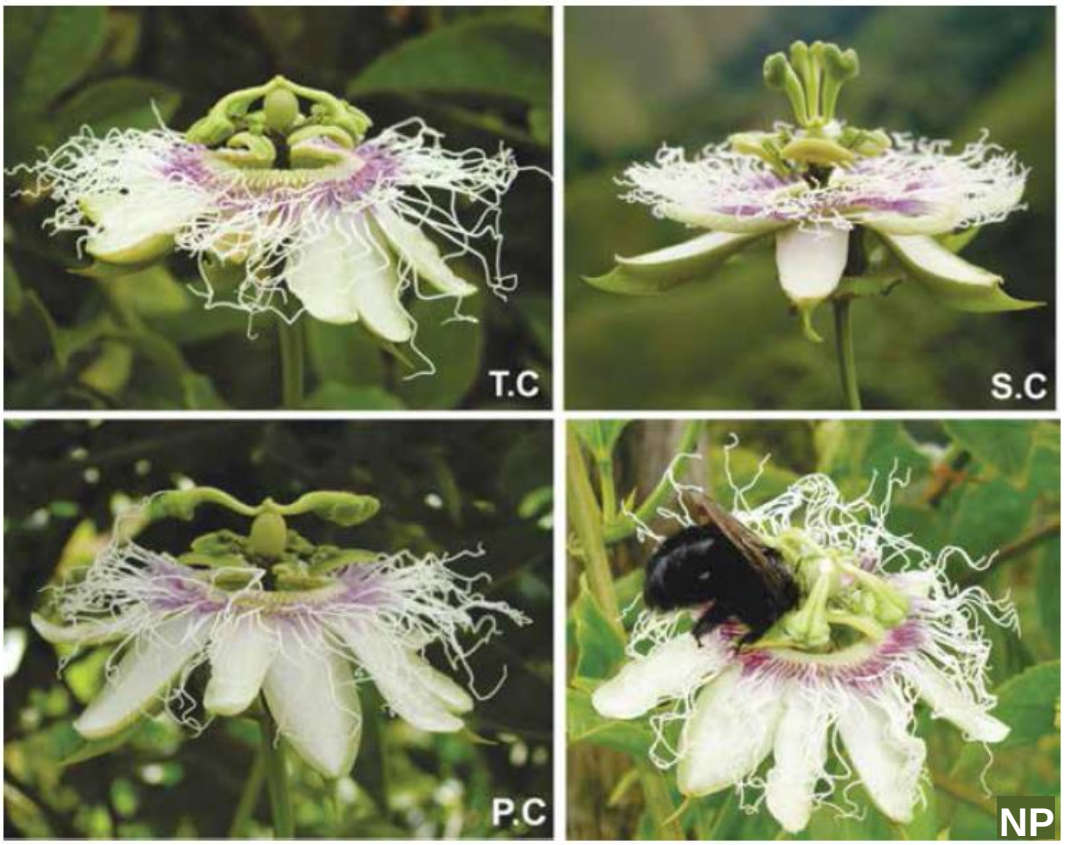

Photo 1. Types of flower in gulupa, *Passiflora edulis* f. *edulis* Sims, according to the position of the stigmas (T.C. = curved, S.C. = no curvature and P.C. = partially curved) and pollinator bumblebee genus *Xylocopa* spp.

Pollination. Knowledge of aspects of reproductive biology in gulupa was performed by natural and controlled pollination, evaluating the effect of different treatments on the production of fruits and seeds, following the methodologies Ángel *et al.* (2011) and Medina-Gutierrez *et al.* (2012). These experiments were conducted by marking and bagging 25 buttons (pre-anthesis stage) every week in three plants per accession for one month and three repetitions in time (periods), for a total of 300 flowers per accession; pollination treatments were: (1) Self-pollination (SP). For natural self-pollination, the flower buds were marked and protected with muslin bags 10 x 20; (2) Natural pollination (NP). For this method flowers were labeled at anthesis stage and left exposed to natural pollinators; (3) Manual self-pollination (MSP). In this method, the flowers in pre-anthesis were marked and covered with bags and pollinated when they opened using a brush, before covering them again; (4) Geitonogamy (G). Flowers in pre-anthesis stages were marked and bagged; when they opened were emasculated and pollinated manually with pollen from another flower belonging to the same plant, and finally bagged again; (5) Cross-pollination (PC). In this method, anthers from flowers from different accessions were removed during anthesis; these were mixed to obtain the pollen that was used to pollinate flowers previously emasculated in other plants.

When manual pollination was performed in treatments 3, 4 and 5, the amount of pollen was distributed evenly on each flower, using a brush, placing an approximately equal amount in each of the stigmas. The efficiency of the different treatments was assessed 8 days after

pollination and when the fruits reached harvest maturity (formed or set fruits and number of seeds per fruit). Moreover, insect vectors were collected (11 individuals), photographed and identified with the taxonomic keys from González *et al.* (2009) as species of the genus *Xylocopa* spp. (Hymenotera: Apidae: Xylocopini).

Data analysis

The data obtained from the different treatments were tabulated and analyzed with SAS v. 9 software with parameters of descriptive statistics: mean, standard deviation, coefficient of variation and Duncan's means comparison test ($P < 0.05$). Additionally, the treatment of natural pollination (NP) was correlated to climate information of precipitation (mm) recorded during the course of the study, using Pearson's correlation (r).

Results and discussion

Floral biology

Gulupa flowers began the anthesis process in the presence of sunlight, at 6:00 AM until 10:00 h (Figure 1). The highest percentage of open flowers (56%) occurred between 6:00 and 7:00 pm, which is consistent with the study of Kishore *et al.* (2010) in India, where 54.5% anthesis occurred on this same interval. Later, at 8:00 pm, the flowers reached 24% anthesis, down to 4% at 10:00 h. This accumulated percentage (80%) between 7:00 and 8:00 pm is similar to that found by Ángel *et al.* (2011) Buenavista (Boyaca, Colombia), where they recorded that anthesis in gulupa occurs between 6:00 and 8:00 h. Anthesis began at 6:00 h registering a percentage of floral opening during this period of only 8% and completed 5 hr later. The duration of the flower can last up to 24 h after anthesis, a period that depends on the climatic conditions of each area and mainly from precipitation. These results are key to the management of the crop, since the chemical applications should be made in the afternoon to avoid coinciding with the highest percentages of anthesis and activity of pollinating insects.

According to the curvature of style in floral opening or anthesis three types of flowers (heteromorphous) were identified, that may occur in the same plant with different frequencies, which influences at the moment of pollination by insects. These types were (Figure 1): (1) T.C. flower with fully curved stigmas, below the anthers and with an occurrence between 51 and 55%. This type favors self-pollination; (2) S.C. flower with non-curved

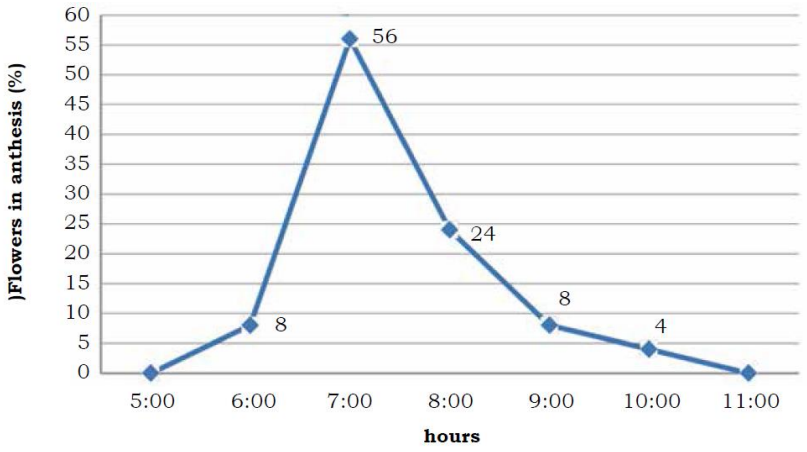

Figure 1. Behavior of flower opening (anthesis) in gulupa, *Passiflora edulis* f. *edulis* Sims, farm Tesorito, Manizales, Colombia.

stigmas (erect), above the anthers at an angle of 70° to 90° and with an occurrence of 12% to 18%. This type favors flower sterility; and (3) P.C. flower with partially curved stigmas, above the anthers at an angle between 0° and 45° and with an occurrence of 20% to 27%.

In *P. cincinnata*, Aponte and Jáuregui (2004) consider that in T.C.-type flowers the stigmas are in the most favorable position to make contact with pollinating insects when seeking nectar. Ruggeiro *et al.* (1976) indicate that passion fruit flowers with curved styles generated greater amount of fruits (between 70% and 75%). Furthermore, Ishihata (1991) and Ángel *et al.* (2011) mention that like S.C.-type flowers produce viable pollen and serve as donor parents, since they can only self-pollinate between 6% and 13%.

Pollination

The efficiency of the five treatments evaluated on natural and assisted pollination was negatively affected by rainfall. The presence and intensity of rainfall in the morning prevented fruit formation in most treatments, mainly in natural pollination (NP) with a correlation of r = -0.50 (Figure 2). In the latter, with the influence of precipitation in the phase of anthesis (≥ 9 mm / day), the percentage of fruits formed decreased between 25% and 27% and caused the abortion of the floral structures. The effect of precipitation on natural and artificial pollination in gulupa was reported by Ishihata *et al.* (1984) who found a range in the reduction of fruit formation between 64% and 75%. These numbers support the results of this study and confirm that precipitation during anthesis affects the percentage of fruit set, due to the decrease in the activity of pollinators and the stigmatic receptivity due to excess moisture.

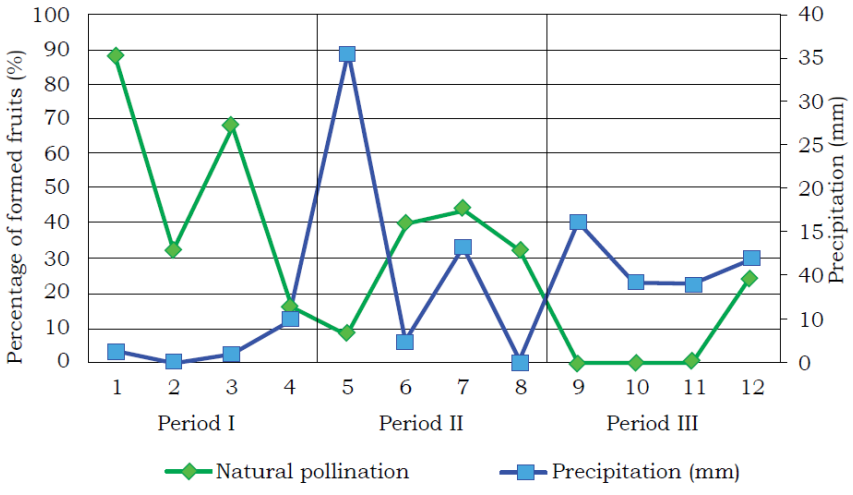

Figure 2. Relationship between the percentage of gulupa fruits *Passiflora edulis* f, *edulis* Sims, formed by natural pollination (PN) and precipitation (mm). Number of flowers per period (hours), n =100. Correlation r = -0.50.

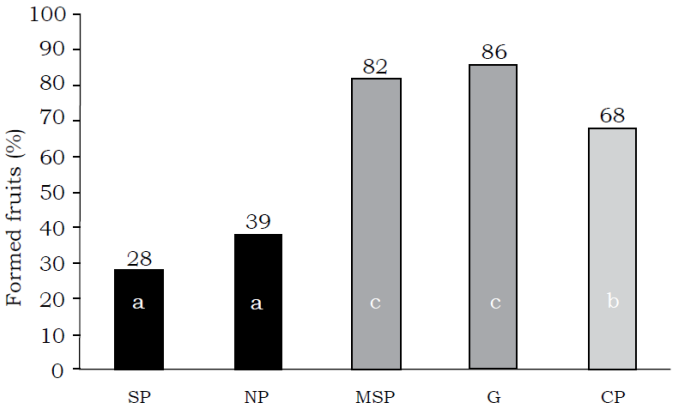

Figure 3 Percentages of fruits formed with different pollination systems in gulupa, *Passiflora edulis* f. *edulis* Sims, (*n* =300). **SP:** Self-pollination; **NP:** Natural pollinatión; **MSP:** Manual self-pollination; **G:** Geitonogamy and **CP:** Cross pollination.
*Differents letters indicate significant differences between means (Duncan, P < 0.05).

Table 2. Range of values obtained in the formation of gulupa fruits, *Passiflora edulis* f. *edulis* Sims.

Treatment	Minimum (%)	Maximum (%)	Average (%)	S. D.	VC (%)
Self-pollination (SP)	4	60	28 a*	16.3	58.4
Natural pollination (NP)	8	88	39 a	25.2	64.4
Self-pollination (MSP)	60	96	82 c	10.9	13.3
Geitonogamy (G)	64	96	86 c	11.2	13.1
Cross-pollination (CP)	36	100	68 b	21.3	29.2

* Different letters indicate significant differences between averages (P > 0.05), accordin to Duncan's test

The percentage of fruits formed in the different treatments are shown in Figure 3 and in Table 2, excluding zero values (0) due to precipitation, as these can skew the average for each treatment. The results show that flowers naturally self-pollinated (SP) produced the lowest percentage of formed fruits (28%), although with no significant differences (P> 0.05) when pollinated naturally (NP, Photo 1) by insects (39%). In contrast, the highest percentages of fruits formed were obtained by manual self-pollination (MSP) and geitonogamy (G) without differences between them (P> 0.05), with 82% and 86%, respectively. In cross-pollination (PC) with pollen from the plants of different accessions the percentage of fruits formed decreased 17.8% in relation to MSP and G treatments, where pollen from the same plant was used. The high coefficients of variation found in the treatments of natural pollination (SP, CV = 58%; NP, CV = 64%) can be explained by the extreme variability in weather conditions during the course of the study, as seen in Figure 2. the differences between the treatments and the high percentage of fruits formed confirm that gulupa is a self-fertile plant that depends on cross pollination for increased production of fruits (Table 2).

SP treatment. In selfing a value of 28% of fruits formed was achieved, similar to the results found by Ángel *et al.* (2011) in Boyacá, where they obtained 33% of fruits formed for this treatment. These observations are also consistent with the results of the studies conducted by Knight (1972) and Nishida (1958) in Florida (USA), which highlight the self-fertility in gulupa with respect to passion fruit a self-nonfertile species (97%). In the formation of interspecific hybrids, gulupa due to its self-fertility, shows an acceptable receptivity with related species such as *P. alata* Curtis, *P. caerulea* L, *P. cincinnata, P. edulis* f. *flavicarpa* (Ocampo and Wyckhuys, 2012).

NP treatment. In natural pollination, the percentage of fruits formed was 39%, in

contrast with the results of Ángel *et al.* (2011) and Nates *et al.* (2012) who obtained 93% and 77% of fruits formed. The low percentage obtained by this method is explained by the negative effect of precipitation on the activity of pollinators at anthesis stage. This influence of rainfall has been recorded in various studies by Akamine and Girolami (1957) and Free (1993) who identified the same trend. Furthermore, Da Silva *et al.* (1999) confirmed that the increase in relative humidity of 55% to 80% results in a reduction in the population density of pollinators of the genus *Xylocopa* spp. In addition, these climatic factors may also adversely affect the germination of pollen during pollination (Free, 1993). Ruggiero *et al.* (1976) consider that when the percentage of fruits formed in passion fruit (*P. edulis* f. *Flavicarpa*) is <30%, a lack of pollinators occurs. Other important factors influencing the reduction in population size and activity of pollinators are the lack of conservation of habitats (wooded areas) where they reproduce and application of cultural practices unsuitable for crop protection (Calle *et al.*, 2010).

MSP and G treatments. The averages of the percentage of fruits formed by manual selfing (MSP) and geitonogamy (G) were equal with values of 82.3% and 85.75%, respectively. Similar results were found Ángel *et al.* (2011) who obtained values for MSP of 93% and 87% for G. By applying both methods the highest efficiency was achieved by increasing between 33% and 46% fruit formation, compared to SP and NP treatments. It should be mentioned that the method of hand pollination in gulupa is complex, compared with passion fruit or granadilla as pollen extraction should be performed with the help of a brush to open the anthers. According to Jimenez *et al.* (2009) there are no studies in Colombia on pollination of gulupa, nor cross-pollination is usually performed with a brush in this species; contrary to what happens in countries like Japan, where there are no natural pollinators and hand pollination is used with great success (Ishihata, 1991). This practice generates additional labor costs for producers and is not considered necessary when sufficient pollinator populations exist, as these ensure the formation of fruits. For this reason, the MSP and NP methods are estimated strategic to implement breeding programs, in order to preserve the genetic purity and characteristics of elite material due to the high percentage of fruits formed and the amount of seed produced.

PC treatment. In the manual cross-pollination a percentage of 67.9% of formed fruits was obtained using mixed pollen from different plants of the 32 accessions evaluated. This value is lower by 25% when compared with that found by Ángel *et al.* (2011) and can be explained by the influence of rainfall during the development of the study and the genetic variability of pollen employed, coming from different genotypes. However, this result is consistent with that found by Souza *et al.* (2004) in passion fruit in areas where anther opening coincides with high relative humidity that reduces and affects pollen viability. According to Jimenez *et al.* (2009) during the flowering period the rain should be minimal, since the pollen gets wet and loses its function generating floral abortions. The differences found in the percentages of each treatment in this study and those reported by Ángel *et al.* (2011) are explained by the agro-ecological characteristics in the study areas and the genetic variability of the accessions tested, which may influence the compatibility between related genotypes.

In general, the efficiency of the five treatments evaluated on natural and assisted pollination was adversely affected during the course of this investigation. High values of precipitation generated extreme values of zero in the percentage of fruits formed. By eliminating these values, the efficiency of the treatments in fruit production increases considerably; For this reason, it is necessary to consider the historical information of climatic factors of each agro-ecological zone, for the selection of the best areas for the establishment of

crops and mitigate the effect of rainfall in the pollination process.

Number of seeds per fruit

The number of seeds per fruit between treatments with different methods of pollination (MSP = 153, G = 157; PC = 152; NP = 151) showed no significant differences (P> 0.05). In the treatment of self-pollination (SP) it was not possible to determine the number of seeds, since, due to high rainfall, the fruits showed a strong attack of the fungus *Cladosporium cladosporioides* (Fresen.) GA de Vries, which generated the abortion of all structures. The results obtained are related to the values presented in the study of Ángel *et al.* (2011) who found significant differences only with the treatment of self-pollination (SP) which produced 62.5 seeds/fruit. The results in this study are similar to those obtained by Akamine and Girolami (1957) who, in fruits from manual pollination, found larger, heavier and more numerous fruits than those obtained by self-pollination (SP). This natural pollination method allows obtaining pure seeds of elite plants with a success of 28%, and for 1000 seeds it is necessary to bag 57 flower buds undergoing preanthesis, equivalent to about 16 fruits.

Natural pollination

In gulupa, insects of the genus *Xylocopa* spp. show an ecological role in this study (ecosystem service), both from the point of view of production as in maintenance of genetic diversity due to genetic exchange during pollen transport. These components must be integrated into crop management plans, where the implementation of good agricultural practices (GAP) contributes to its conservation. It is also necessary to encourage and increase populations of pollinators providing sites where they can build nests, such as poles of red and light wood, near crops. On the other hand, the environmental component has a noticeable effect on the plant/pollinator interaction, because the plant phenology (flowering) can be altered by extreme climatic changes and intervenes in the temporal coupling with pollinating insects. In this sense extreme precipitation (≥ 9 mm/day) during the anthesis had a negative effect on the activity of the pollinator and therefore in the number of flowers pollinated.

Conclusions

- In the accessions tested, flowers with fully curved style had the highest incidence rate (51% to 55%) and its structure is designed for self-pollination. Moreover, the flowers with partially curved styles reached incidences between 20% and 27%. This kind of flower is adapted for pollination by *Xylocopa spp.*, allowing gene flow between plants and the increment in fruit formation.
- Treatments that included hand pollination showed the highest percentages of fruits formed (68% and 86%), confirming that the gulupa is a self-fertile plant. However, the results obtained with natural pollination (by pollinating insects) showed that the percentage of fruits formed (39%) exceeded those obtained by self-pollination (28%), suggesting that gulupa has a tendency to allogamy.
- The phenomenon of self-fertility reported in this study for gulupa is critical for the processes of breeding for the species due to the high degree of inbreeding that can support without compromising the production of fruits. Also, the results obtained on its reproductive biology can contribute significantly to the implementation of conservation strategies.

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References

Akamine, E. K.; y Girolami, G. 1957. Problems in fruit set in yellow passion fruit. Hawaii Farm Sci. 5:3 - 5.

Amela, M. T.; y Hoc, P. S. 1998a. Floral biology and reproductive system of *Passiflora mooreana* (Passifloraceae). Darwiniana 35:9 - 27.

Amela, M. T.; y Hoc, P. S. 1998b. Biología floral de *Passiflora foetida* (Passifloraceae). Rev. Biol. Trop. 46(2):191 - 202.

Ángel, C.; Nates, P. G.; Ospina, T. R.; and Melo, O. D. 2011. Biología floral y reproductiva de la gulupa (*Passiflora edulis* Sims f. *edulis*). Caldasia 33(2):433 - 451.

Aponte, Y.; and Jáuregui, D. 2004. Algunos aspectos de la biología floral de *Passiflora cincinnata* Mast. Revista Facultad Agronomía 21(3):211 - 220.

Calle, Z.; Guariguata, M. R.; Giraldo, E.; and Chará, J. 2010. La producción de maracuyá (*Passiflora edulis*) en Colombia: perspectivas para la conservación del hábitat a través del servicio de polinización. Interciencia 35(3):207 - 212.

Da Silva, M.; Bruckner, C.; Picanço, M.; and Molina, A. 1999. Número floral, clima, densidad poblacional de *Xylocopa* spp. (Hymenoptera: Anthophoridae) y polinización del maracuyá (*Passiflora edulis* f. *flavicarpa*). Rev. Biol. Trop. 47(4):7 - 10.

Free, J. B. 1993. Insect pollination of crops, 2 ed. Academic Press. 684 p.

González, V. H.; González, M. M.; and Cuellar, Y. 2009. Notas biológicas y taxonómicas sobre los abejorros del maracuyá del género *Xylocopa* (Hymenoptera: Apidae, Xylocopini) en Colombia. Acta Biológica Colombiana 14(2):31 - 40.

Ishihata,K.; Hayashi,M.; and Ikeda, M. 1984.Improvement of fruit set and fruit quality by artificial pollination in purple passion fruit, *Passiflora edulis* Sims. Bulletin of the Faculty of Agriculture, Kagoshima University 34:9 - 16 p.

Ishihata, K. 1991. Studies on pollen germination and tube growth from normal and upright style flowers in purple passion fruit (*Passiflora edulis* Sims) using various artificial media. Japanese J. Trop. Agric. 35(2):98 - 103.

Jiménez, Y.; Carranza, C.; and Rodríguez, M. 2009. Manejo integrado de la gulupa (*Passiflora edulis* Sims). In: Miranda, D.; Fischer, G., Carranza, C., Magnitskiy, S., Casierra, F., Piedrahíta, W. and Flórez, L. (eds.). Cultivo, poscosecha y comercialización de las pasifloráceas en Colombia: maracuyá, granadilla, gulupa y curuba. Sociedad Colombiana de Ciencias Hortícolas, Bogotá, Colombia. 121 - 158 p.

Kishore, K.; Pathak, K. A.; Shukla, R.; and Bharali, A. 2010. Studies on floral biology of passion fruit (*Passiflora* spp.). Pakistan J. Bot. 42(1):21 - 29.

Knight, R. J. 1972. The potential for Florida of hybrids between the purple and yellow passionfruit. Florida State Hort. Soc. 288 - 292.

Medina-Gutiérrez, J.; Ospina-Torres, R.; and Nates-Parra, G. 2012. Efectos de la variación altitudinal sobre la polinización en cultivos de gulupa (*Passiflora edulis* f. *edulis*). Acta Biológica Colombiana
17(2):379 - 394.

Nates, G.; Amaya, M.; Ospina, R.; Ángel, C.; and Medina, G. 2012. Biología floral, reproductiva, polinización y polinizadores en gulupa (*Passiflora edulis* var. *edulis*). In: Melgarejo, L. M (editor). Ecofisiología del cultivo de la gulupa (*Passiflora edulis* Sims). Universidad Nacional de Colombia. 115 - 121 p.

Nishida, T. 1958. Pollination of the passion fruit in Hawaii. *Journal of Economic Entomology* 51:146-149.

Nishida, T. 1963. Ecology of the pollinators of the passion fruit. Hawaii Agricultural Experiment Station, Honolulu. Techn. Bull. 55.

Ocampo, J.; and Wyckhuys, K. 2012. Tecnología para el cultivo de la gulupa (*Passiflora edulis* f. *edulis* Sims) en Colombia. Centro Bio-Sistemas Universidad Jorge Tadeo Lozano, Centro Internacional de Agricultura Tropical (CIAT), Ministerio de Agricultura y Desarrollo Rural (MADR). Bogotá, Colombia. 68 p.

Ruggiero, C.; Banzatto, D.; and Lam-Sánchez, A. 1976. Studies on natural y controlled pollination in yellow passion fruit (*Passiflora edulis* f. *flavicarpa* Deg.). Acta Hort. 57:121 - 124.

Sage, T. L.; Husband, B. C.; and Routley, M. B. 2005. Plant breeding systems and polen dispersal. In: A. Dafni; P. G. Kevan and B. Husband, (eds.). Practical pollination biology. Eviroquest Ltd., Ontario, Canadá. 27 - 55 p.

Sims, J. 1818. *Passiflora edulis*. Botanical Magazin. Fellow of the Royal and Linnean Societies. London. 45: 1989-1991.

Souza, M.; Pereira, T.; Viana, A.; Pereira, M.; Amaral, A.; and Madureira, H. 2004. Flower receptivity and fruit characteristics associated to time of pollination in the yellow passion fruit *Passiflora edulis* Sims f. *flavicarpa* Degener (Passifloraceae). Scientia Hort. 101:373 - 385.