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Article

Availability of floral resources in yellow passion fruit cultivars

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Abstract

The reproductive strategies and resources available in the flower are characteristics that stimulate research studies on the genetic improvement and fruit yield of commercial cultivars. In this sense, this study aimed to study the floral biology aspects of passion fruit cultivars in Tangará da Serra region, Mato Grosso State, Brazil at different evaluation times. The experiment was carried out with eight cultivars of yellow passion fruit from an ex situ germplasm bank of the State University of Mato Grosso. The floral resources (pollen and nectar) were evaluated in a completely randomized, factorial arrangement (8 cultivars x 5 evaluation times), with five replications. Throughout anthesis, the pollen viability, stigma receptivity, and sugar volume and concentration in the nectar were evaluated. The evaluations were carried out in 1-h intervals, between 1:30 and 5:30 p.m. The highest nectar volume production was at 2:30 p.m., decreasing gradually thereafter at the later evaluation times, for all cultivars. Pollen viability increased from the first to the second evaluation time and then decreased gradually until the last evaluation. Stigma receptivity was higher than 90% in all cultivars, at all evaluation times. BRS Rubi do Cerrado and FB 200 were the most promising cultivars for nectar volume and concentration, pollen viability, and stigma receptivity characteristics.

Keywords: Nectar, stigma receptivity, pollen viability

Introduction

The Passifloraceae family has 150 species in Brazil distributed in all geographic regions (Bernacci et al., 2016). Among the commercial species, the most important is the yellow passion fruit (*Passiflora edulis* Sims), which is mainly consumed as fresh fruit and processed by juice industry (Meletti, 2011).

The yellow passion fruit has floral characteristics such as hercogamy (stylet located above the anthers), the protandry (maturing pollen grains before the stigma is receptive) and a self-incompatibility system (Angel-Coca et al., 2011; Dai & Galloway, Which makes cross-pollination indispensable for fruit and seed production (Yockteng et al., 2011).

However, for successful pollination, floral characteristics such as pollen viability, stigma receptivity and nectar secretion should be considered. The pollen viability may be affected by genetic factors such as abnormalities during microsporogenesis and climatic factors such as relative air humidity and air temperature that affects the pollen grain physiology (Ferreira et al., 2007).

The probability of a yellow passion fruit flower receive a sufficient number of pollen grains is favored by the time length that the stigma remains receptive. This receptivity may be influenced by several factors such as flower age,

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presence or absence of stigmatic exudate and daily climatic conditions (Marshall et al., 2010; Das et al., 2013).

The reproductive strategies and the available resources in the flower are characteristics that corroborate for studies that objective the genetic improvement and the fruit yield of commercial cultivars. In this sense, the objective of this study was to study the floral biology aspects of passion fruit cultivars in Tangará da Serra region, Mato Grosso State, Brazil at different evaluation times, based on the following hypotheses: 1) the sugar volume and concentration in the nectar vary among cultivars and evaluation schedules; 2) pollen viability decreases throughout the anthesis and 3) there is synchrony between stigmatic receptivity and pollen viability for the cultivars analyzed.

Material and Methods

The experiments were carried out with eight yellow passion fruit cultivars from the active germplasm collection (AGC) of the State University of Mato Grosso (UNEMAT), Tangará da Serra *Campus*, 14°30S, 57°25W, altitude of 321 meters. The region climate is characterized as tropical humid. The micro-region of Tangará da Serra presents two well-defined seasons: a rainy season that includes October to April months and a dry season from May to September, average annual precipitation from 1300 to 2000 mm, average annual temperature 16 to 36 °C (Martins et al., 2010).

An AGC was implemented in January 2013 and the yellow passion fruit cultivars evaluated were: BRS Sol do Cerrado, BRS Gigante Amarelo, BRS Ouro Vermelho, BRS Rubi do Cerrado, FB 200, FB 300, IAC 275 and IAC 277. The orchard management system was vertical, with a plain wire n. 12, at two meters height from the soil, with a distance of six meters between the stakes. The drip irrigation system was used.

The planting spacing used was 3m between plants and 3.5m between rows. There were five plants of each access (cultivar) of AGC. Orchard management, pruning, fertilization, and cultural treatments followed the recommended methodology for passion fruit cultivation (Bruckner & Picanço, 2001). The experiments were carried out during the months of May to October in an established orchard, during the first cycle of cultivation.

The floral resources evaluations (pollen and nectar) were performed using a completely randomized experimental design, in a factorial arrangement 8 x 5 (cultivars x evaluation time) with five replications. The evaluations were performed at one-hour intervals, beginning at 1:30 p.m. until 5:30 p.m. because in such times most flowers are open (Cobra et al., 2015). The experimental plots consisted of twenty - five flowers per plant.

The pollen viability was evaluated by two dyes, acetic carmine (AC) (Kearns & Inouye, 1993) and Alexander triple solution (ATS) (Alexander, 1980). In the pre-anthesis, phase when the sepals of the flower buds began to open, the buds were marked and covered with paper bags to prevent pollen loss or mixing. At anthesis, twenty-five flowers per cultivar were collected, conditioned in a humid chamber (plastic container covered by a moist absorbent paper layer) and immediately sent to the Botany Laboratory (UNEMAT). The pollen grains were removed with a brush and placed on a histological slide, containing one drop of the dye and covered with coverslip. Up to 200 pollen grains were counted in each flower collected per hour (treatment).

For acetic carmine dye, pollen grains were considered viable when presented carmine color, sterile pollen grains were transparent and non-colored (Frescura et al., 2012). Under Alexander's triple solution the viable pollen grains exhibit purple cytoplasm and green color wall, whereas the non-viable ones present only the green-colored wall (Alexander, 1969).

For the stigmatic receptivity evaluation period, flower buds were bagged at pre-anthesis to prevent any substance deposit on the stigma to provide a false positive result. The collected pistils (N = 25) had the stigmas dipped in 3% hydrogen peroxide (HP) solution and other 25 in alpha naphthyl acetate (ANA). Immediately, they were taken to the laboratory and observed in a stereoscopic microscope. They were classified as receptive according to the following observations: (a) 3% hydrogen peroxide: receptive stigmas when there was immediate bubbles formation after immersion, indicating the peroxidase presence; (b) alpha-naphthyl acetate: receptive stigmas when stigmatic papillae were dark staining, indicating esterase enzyme activity (Dafni, 1992; Souza et al., 2004).

The nectar volume and nectar sugar concentrations were evaluated in the same plants of pollen viability and stigmatic receptivity. At pre-anthesis the floral buds were bagged and, at anthesis, the nectar secretion volume was measured with microcapillary graduated (precision ± 0.2 µL) and then discarded. The flowers were re-bagged for further evaluation. The nectar sugar concentration was measured using a 0-90% Brix portable refractometer.

The evaluated characteristics were submitted to analysis of variance and the means were compared by the Scott Knott test at 5% probability using the statistical program Sisvar (Ferreira, 2011).

Results

The evaluation of floral resources presented significant interaction for the nectar volume characteristic (Table 1). Stigmatic receptivity did not present a significant difference for the cultivar factor in both methods (Table 1). The nectar sugar concentration did not present significant difference as a function of evaluation schedules (Table 1).

Table 1. Summary of variance analysis for nectar volume (NV), nectar sugar concentration (NSC); pollen viability using Alexander triple solution (ATS), acetic carmine (AC); stigmatic receptivity using hydrogen peroxide (HP), in eight yellow passion fruit cultivars.

Variation source	Freedom degrees	Average square of variables studied				
		NV (µL)	NSC (%)	ATS (%)	AC (%)	HP (%)
Cultivar (C)	7	12.4**	223.2**	1015.2**	48.6**	84.8^{NS}
Time (T)	4	1413.7**	24.5^{NS}	1161.7**	648.1**	382.5**
C*T	28	3.0*	12.6^{NS}	4.4^{NS}	4.1^{NS}	55.6^{NS}
Residue	160	1.7	11.4	8.1	2.8	46.8
Average	-	11.75	45.85	81.74	91.2	98.13
CV (%)	-	11.3	7.4	3.5	1.8	6.9

^{ns}Non-significant. **and* Significant at 1 and 5% of probability error, respectively, by F test.

Table 2. Average nectar volume variation (µL) as a function of the interaction between yellow passion fruit cultivars and different evaluation times.

Cultivar	Evaluation times				
	1:30 p.m.	2:30 p.m.	3:30 p.m.	4:30 p.m.	5:30 p.m.
IAC 277	14.1aB	20.0aA	10.1bC	7.0bD	5.3aE
IAC 275	14.4aB	20.4aA	10.5bC	7.6bD	4.6aE
FB 200	15.2aB	20.5aA	11.1bC	7.3bD	4.8aE
FB 300	14.4aB	20.8aA	11.4bC	6.8bD	4.8aE
BRS Sol do Cerrado	14.4aB	19.9aA	10.1bC	7.0bD	4.6aE
BRS Gigante Amarelo	15.3aB	21.3aA	14.2aB	9.9aC	5.7aD
BRS Rubi do Cerrado	13.2aB	20.8aA	12.9aB	8.2bC	5.0aD
BRS Ouro Vermelho	13.1aB	18.1bA	12.9aB	7.6bC	5.1aD

Averages values followed by the same small letter in columns and capital letter in rows present no statistical difference by Scott-Knott test 5% probability error.

The nectar production peak for all cultivars was at 2:30 p.m., decreasing gradually to the last evaluated time (Table 2). The highest nectar volume rates replacement were observed in BRS Gigante Amarelo and BRS Rubi do Cerrado cultivars (Table 2). The sugar concentration in the nectar ranged from 41.3 to 49% in the evaluated cultivars, with higher mean values for IAC 277, BRS Giant yellow, BRS Rubi do Cerrado and BRS Ouro Vermelho (Table 3).

The cultivars FB 200 and BRS Rubi do Cerrado presented the highest pollen viability percentages for the two dyes used (Table 3). The viability percentage increased from the first to the second evaluation time, and, from this time a gradual reduction in the percentage was registered, highlighting the time of 17:30 h (Table 4).

The stigmatic receptivity recorded in this study was considered high. The eight cultivars evaluated presented stigmatic receptivity above 90%, for the test with hydrogen peroxide (Table

Table 3. Average percentage of nectar sugar concentration (NSC) and pollen viability in acetic carmine (AC) and Alexander triple solution (ATS) in yellow passion fruit cultivars.

Cultivares	NSC (%)	AC (%)	ATS (%)
IAC 277	48.2a	91.1c	82.0b
IAC 275	41.3c	90.7c	81.6b
FB 200	41.6c	93.0a	84.5a
FB 300	45.5b	91.1c	80.7c
BRS Sol do Cerrado	45.6b	89.5d	79.3c
BRS Gigante Amarelo	47.3a	89.5d	78.9c
BRS Rubi do Cerrado	49.0a	92.9a	84.1a
BRS Ouro Vermelho	48.2a	91.9b	82.7b

Averages values followed by the same small letter in columns present no statistical difference by Scott-Knott test 5% probability error.

Table 4. Average variation on pollen viability using acetic carmine (AC) and Alexander triple solution (ATS) and stigmatic receptivity using hydrogen peroxide (HP) in yellow passion fruit cultivars.

Times	AC (%)	ATS (%)	HP (%)
1:30 p.m.	86.3e	76.6d	100.0a
2:30 p.m.	96.3a	88.9a	100.0a
3:30 p.m.	93.7b	85.4b	100.0a
4:30 p.m.	91.2c	81.0c	97.7a
5:30 p.m.	88.3d	76.8d	92.9b

Averages values followed by the same small letter in columns present no statistical difference by Scott-Knott test 5% probability error.

4) and 100% of receptivity at all times and for all cultivars for the alpha naphthyl test.

Discussion

Variation in nectar volume, similar to that observed in this study was also recorded in a study conducted in Campos dos Goytacazes, Rio de Janeiro State, Brazil. Benevides et al. (2009) observed that the maximum nectar volume in *P. edulis* was 18μL at 2:30 p.m., after this period, a decrease in nectar production occurred to 5μL at 3:30 p.m. On the other hand, Varassin et al. (2012) in Brazilian south verified a 80μL mean nectar volume in *P. edulis* until 6:00 p.m., decreasing after this period.

Factors such as day length, air temperature, sun radiation, latitude and rain precipitation promote variations on nectar volume(Kenoyer et al., 2006; Ataíde et al., 2003). The different climatic conditions may explain the variation results for the different Brazilian regions.

The continuous nectar replacement of yellow passion fruit cultivars, allows the pollinators visitation during a longer period and favors the pollen transfer. This uninterrupted nectar availability ensures the retention of pollinators. This is an important reproductive strategy because during the pollinators visit looking for nectar, it is possible to contact the floral stigma and then transfer of pollen. The pollen amount transferred to the stigma has a direct influence on fruit seed number, pulp amount and, consequently, on fruit weight (González et al., 2006; Hafez et al., 2015).

The nectar sugar concentration results for *P. edulis* cultivars were similar to those observed for the same species in cultivated areas in the North of Rio de Janeiro State, that is, between 38% and 42% (Benevides et al., 2009). In Paraná state, the nectar average sugar concentration was 57% (Varassin et al., 2012) and 47% in Bahia State (Siqueira et al., 2009).

The nectar available to pollinators appear to be available at sufficient concentrations for pollinators constantly visit yellow passion fruit flowers (Kim et al., 2011), and associated with the energy requirements of large bees (Varassin et al., 2001).

The results obtained in the present study showed a high pollen viability rate for the two staining methods used. In a study carried out by Souza et al. (2002) with the same species, the pollen viability recorded was 88.70% using lugol solution, and Costa et al. (2009) for *P. alata* (Curtis), recorded 71.95% with Alexander triple solution.

Pollen viability similar to those presented in this study were recorded for the same species in Campos dos Goytacazes (Brazil), where the viability percentage was 95.36% at anthesis beginning and less than 90% at the end of the afternoon (Souza et al., 2002). Our results corroborate with the results of the aforementioned studies demonstrating that the pollen viability period is a limiting factor for the reproductive success of Passifloraceae.

The pollen viability loss throughout floral anthesis may be related to environmental conditions such as air temperature and relative air humidity, because these factors may lead to grain pollen desiccation when they remain exposed to these environmental factors after anther dehiscence (Pacini et al. 1997, Ge et al., 2011).

Desiccation of pollen grains is of particular concern in species with flowers with dry stigma (Nepi et al., 2001), such as Passifloraceae. The absence of exudate secretion on the stigmatic surface may impair the pollen grains hydration (Souza et al., 2006).

Pollen viability may vary depending on dye specificities. The acetic carmine dye indicates chromosomal integrity (Munhoz et al., 2008), whereas Alexander triple solution differentiates the aborted pollen grains from the non-aborted pollen grains, since the latter do not have the nucleus and only the cellulose contained in the wall is evidenced (Alexander, 1980). However, regardless of the dye used, a pollen grain viability above 70% is considered high (Soares et al., 2013).

For the cultivars evaluated in the present study, the time of 2:30 p.m. is the most indicated to perform manual pollination, due to the higher viable pollen percentage. The higher deposition of viable pollen grains on the flower stigma guarantees the fruit production with important characteristics for commercialization (Rendón et al., 2013).

The stigmatic receptivity observed in this study was similar to the study carried out in Rio de Janeiro State, where viable pollen where stigmatic receptivity was higher than 80% for yellow passion fruit (Souza et al., 2004). The presence of receptive stigma during floral anthesis increases the time for the pollen grains.

The evaluations carried out in this study generate subsidies for yellow passion fruit crop management, providing practical information about the pollination period based on floral resources.

Conclusions

The highest nectar production volume was at 2:30 p.m., this volume decreases gradually for the other schedules, in all cultivars.

Pollen viability increases from the first to the second evaluation time and gradually decreases until the last evaluation time.

The stigmatic receptivity is above 90% for any cultivar evaluated, at all times of evaluation.

The cultivars BRS Rubi do Cerrado and FB 200 were the most promising for the nectar volume and nectar sugar concentration, pollen viability and stigmatic receptivity.

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