

Flowering phenology and floral biology in pumpkin cultivars¹

Fenologia da floração e biologia floral em cultivares de abóbora

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ABSTRACT - Phenological events, in particular, flowering phenology and aspects of floral biology help to understand the relationship of plants with their pollinators. Thus, this study sought to estimate, under semi-arid conditions, the relationship between different genotypes of pumpkin or winter squash (*Cucurbita moschata* Duch.) and the environment, under the aspects of reproductive biology. Four cultivars were evaluated in three productive cycles, measuring the variables daily flower emergence, stigma receptivity, anthesis time and the morphometry of pistil and staminate flowers. The production of male flowers was higher than female flowers with a higher proportion in the third production cycle characterized by higher air temperature and lower relative humidity. Among cultivars, 'Jacarezinho' and 'Sergipe' presented the highest and the lowest values of flower production, respectively. Female flowers remain open longer than male flowers and stigmas remain receptive until the flowers close. Considering the morphometric data, there is positive association between the petal length and the corolla diameter, and between access to the nectar and the anther size, with a difference between cultivars and cultivation conditions.

Key words: Reproductive biology. Flowering. *Cucurbita moschata*. Anthesis. Stigma receptivity.

RESUMO - Os eventos fenológicos, em particular, a fenologia da floração e os aspectos da biologia floral ajudam a compreender a relação das plantas com seus polinizadores. Assim, essa pesquisa buscou estimar, em condição semiárida, a relação entre diferentes genótipos de abóbora (*Cucurbita moschata* Duch.) e o ambiente, sob os aspectos da biologia reprodutiva. Quatro cultivares foram avaliados em três ciclos produtivos, mensurando as variáveis emissão diária de flores, receptividade do estigma, o tempo de antese e a morfometria das flores pistiladas e estaminadas. A emissão de flores masculinas foi superior às flores femininas com maior proporção no terceiro ciclo produtivo caracterizado por maior temperatura do ar e menor umidade relativa. Entre cultivares o 'Jacarezinho' e 'Sergipe' apresentaram a maior e a menor emissão, respectivamente. As flores femininas permanecem abertas em maior tempo em relação às flores masculinas e os estigmas permaneceram receptivos até o fechamento das flores. Considerando os dados morfométricos, há associação positiva entre o comprimento da pétala e o diâmetro da corola e entre o acesso ao néctar e o tamanho da antera, com diferença entre os cultivares e as condições de cultivo.

Palavras-chave: Biologia reprodutiva. Florescimento. *Cucurbita moschata*. Antese. Receptividade do estigma.

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INTRODUCTION

In Brazil, squashes and pumpkins (*Cucurbita* spp.) are a staple food for the Brazilian population, with socioeconomic and nutritional importance (BLANK *et al.*, 2013). Fruit of the pumpkin and squash vines are consumed in different ways, even before the complete stage of maturity, using their peel, pulp and seeds and are appreciated all over the world. Asia stands out with 61.5% world production, followed by Europe, with 15.8%, and America with 11.7%. Among the main producing countries are China (5,492,389 t = 19.86% world production), India (4,179,570 t = 15.11% world production) and Russia (959,276 t = 3.47% world production) according to data from the Food and Agriculture Organization of the United Nations (FAOSTAT, 2020). In Brazil, the states that stand out in the production of squashes and pumpkins are Minas Gerais (106,755 t), Bahia (75,649 t), Rio Grande do Sul (69,661 t), Santa Catarina (51,980 t) and Goiás (34,449 t) (INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA, 2020).

The five domestic species of the genus *Cucurbita* are grown in Brazil *Cucurbita moschata*, *Cucurbita ficifolia*, *Cucurbita maxima*, *Cucurbita argyrosperma* and *Cucurbita pepo*, of which *Cucurbita moschata* is the most prominent in the Brazilian territory (PRIORI *et al.*, 2018). It is an annual species with decumbent growth habit, like the other species of the genus *Cucurbita*, is monoic, presenting pistillate and staminate flowers in the same plant (SAMPATH; KRISHNAMOORTHY, 2017; SOUSA; GERVASIO, 2018). Species with this type of sexual system need a vector for the transfer of pollen grains from male flowers to the stigma of female flowers. In Brazil, several types of insects are considered as possible pollinating agents of different squash and pumpkin species. However, two bee species, *Apis mellifera* and *Trigona spinipes*, are more frequently observed on flowers and are identified as efficient pollinators of the crop (KLEIN *et al.*, 2020; SERRA; CAMPOS, 2010).

The relationship between plants and their pollinators is mediated by several means, in which the visual characters, those related to floral architecture, assist the bees in locating the flower during the search for food resources (VARASSIN; AMARAL-NETO, 2014). Thus, investigating aspects of floral biology becomes essential to understand the interactions between the plant and its pollinator (ORBÁN; PLOWRIGHT, 2014). On the other hand, the phenology studies the seasonal rhythm of the life cycle events of plant species, and the flowering phenology can be regulated by endogenous characteristics associated with climate variations and biotic factors that can be reasons for selective pressure (CARADONNA; ILER; INOUE, 2014).

Investigating the reproductive phenology of plants helps to understand how ecological and evolutionary factors influence the temporal variation in flowering and the relationship with its pollinators (ELZINGA *et al.*, 2007). The cultivation of squash and pumpkin species occurs, in general, in the rainy season or in irrigated systems, given the water requirement for the ideal development of the fruit, still needing a fertilized soil and adequate soil correction for the productive success. Under ideal conditions, gene expression is decisive in production, but climatic factors, soil nutrition and pollination can affect the productive patterns of the species.

The objective of this study was to estimate the interaction of pumpkin cultivars with the environment, under the aspects of reproductive biology. To meet this objective, the following questions were asked: (1) What is the flowering pattern? (2) Is there an overlap in flowering between flower types in different cultivars and different environmental conditions? (3) Does floral biology change between different genotypes?

MATERIAL AND METHODS

Three different experiments with *Cucurbita moschata* Duch. were conducted in the Experimental Field of Embrapa Tropical Agroindustry (4°10'S and 38°27'W, 60 meters above sea level) located in the municipality of Pacajus, state of Ceará, which has an average annual rainfall of 1,020 mm. The first occurred in the period from October 2017 to February 2018, in the rainy season; the second, from November 2018 to March 2019; and the third, from July to November 2019. In the second and third experiments, the soil was amended and fertilized, according to the recommendations for the crop, respectively, in the rainy and dry season.

Soil was amended with dolomitic lime. In pre-planting fertilization, we applied 320 g organic fertilizer per pit and 600 g simple superphosphate per pit. Nitrogen fertilization was carried out by applying, per plant, 66 g urea before flowering and 132 g urea during fruiting. For potassium fertilization, 50 g potassium chloride were added to each plant before flowering and 50 g potassium chloride during fruiting. Post-planting fertilizations were carried out via fertigation.

Cultivation was carried out with seedlings, when twelve days after sowing, they were transplanted into the field in 3.0 meters spacing between plants and 4.0 meters between rows, under drip irrigation. This was a 4x3 factorial randomized block design, with four pumpkin cultivars and three cultivation cycles, with six plants per plot, three blocks for the first experiment, and four blocks for the second and third experiments.

The commercial cultivars 'Jacarezinho' and 'Baiana tropical', a local variety from the state of Sergipe and a progeny developed by Embrapa Coastal Tablelands (CPATC) were analyzed for flowering phenology, monitoring eight female flower buds and twenty flower buds of male flowers. In the pre-anthesis stage, for each variety studied, flowers were marked with cotton threads at the base of the pedicel in different plants distributed at random. The evaluation was carried out visually, following the moment of petal opening until their total closure, when the petals touched each other and the corolla united, and there was no longer the possibility of receiving any visits.

As a way of assessing the receptivity of stigmas of pistillate flowers, a hydrogen peroxide test (H_2O_2 - 10 volumes) was performed weekly on 10 flowers, two per evaluation, at intervals of 60 min, from 05h00min to 11h00min in all cultivars evaluated, thus covering the entire flowering period. The stigma was considered receptive when there were bubbles formed due to the release of oxygen, after the breakdown of hydrogen peroxide molecules, caused by peroxidase and catalase present in the receptive stigmas.

Pistillate and staminate flowers were daily counted in all cultivars under study, from the onset of flowering until fruit formation, allowing to evaluate the emergence of flowers, the relationship between pistillate and staminate flowers and flowering days. To analyze flower morphometry, flowers without defects were collected in all cultivars, and, using a digital caliper, corolla diameter, calyx diameter, petal length, fillet length, anther length, sepal length, and access to nectar, were measured in twenty staminate flowers; and corolla diameter, calyx diameter, petal length, ovary diameter, ovary length, sepal length, stigma diameter, in eight pistillate flowers.

At the Agrometeorological Station of the Embrapa Experimental Field - Pacajus-CE, daily data on air temperature ($^{\circ}C$), relative humidity (%) and the rainfall index (mm) were collected throughout the experiment. Rainfall in the experimental period was 132.9 mm in the first cycle, 728.9 mm in the second and 6.7 mm in the third cycle. The average relative humidity and the average air temperature were, respectively: 78.9% and 27.5 $^{\circ}C$ in the first cropping cycle; 82.1% and 27.3 $^{\circ}C$ in the second cropping cycle and 76.5% and 27.9 $^{\circ}C$ in the third cropping cycle.

Data on daily flower emergence were tested by analysis of variance followed by Scott-Knott's test for comparison of means, at 5% significance level. For variables related to morphometry, multivariate principal component analysis was used, using the correlation matrix between the variables, and clustering analysis, using the

Euclidean distance matrix between cultivars. Analyses were performed with the aid of the statistical software SISVAR 5.6 and R Development Core Team. Data related to the time (hour: minute) of flower anthesis were transformed into decimals for application of the test.

RESULTS AND DISCUSSION

Flowering started 40 days after transplanting, with the emergence of staminate flowers, and after 43 days, the first pistillate flowers appeared (Table 1). The length of the flowering period, between the cultivars studied, varied between 36 and 41 days for staminate flowers, and between 25 and 34 days for pistillate flowers. This variation in the flowering period between the two types of flowers was also observed in other cucurbits, such as melon and watermelon grown in semi-arid conditions (BOMFIM *et al.*, 2012; DUARTE *et al.*, 2015; SIQUEIRA *et al.*, 2011).

The mean number of flowers emerged per plant daily was 3.06 male flowers and 0.13 female flowers. The interaction between cultivar and cycles was not significant ($p > 0.05$). On the other hand, the simple effects were significant for both factors. For the two floral types, 'Jacarezinho' and 'Sergipe' cultivars had the highest and the lowest emergence, respectively, and the highest mean value for female flower emerged per plant ($p < 0.05$) occurred in cycle II (Table 1), in which the necessary soil amendments and fertilizations were carried out during the rainy season, showing that this is the best condition for planting the pumpkin (*Cucurbita moschata*), since female flowers will give rise to fruits. In addition, the attractiveness of a given plant species to its pollinators is partially determined by the number of flowers available, which may be associated with the pollinators ability to learn and memorize (SCHIELTL; JOHNSON, 2013).

There was a predominance of male flowers over female flowers, in the three productive cycles and in the four cultivars (Table 01). This greater number of male flowers is commonly observed in most of the planted species of the family Cucurbitaceae (BOMFIM *et al.*, 2012; SIQUEIRA *et al.*, 2011; TSCHOEK *et al.*, 2015). The highest proportions between male and female flowers were found in the third production cycle, similarly to that observed in cucumber (*Cucumis sativus*), also belonging to the family Cucurbitaceae, where high air temperature favors the emergence of male over female flowers (NICODEMO *et al.*, 2012). The high proportion found in this study may be the pattern of *Cucurbita moschata*, as it was observed in different cultivars and in different soil and climate conditions.

Table 1 - Flower emergence in four cultivars of *Cucurbita moschata*

Cultivar	Cycle			Mean
	I	II	III	
Staminate				
Flower emergence per plant per day				
Baiana Tropical	2.10	3.38	2.89	2.79 b
CPATC 10	2.36	3.24	3.37	2.99 b
Jacarezinho	2.88	4.02	4.68	3.84 a
Sergipe	1.87	2.91	3.17	2.64 b
Mean	2.30 B	3.39 A	3.53 A	3.06
Flowering days				
Baiana Tropical	40	40	38	39.3
CPATC 10	40	41	37	39.3
Jacarezinho	37	38	36	37.0
Sergipe	39	40	38	39.0
Mean	39	39.7	37.2	38.7
Number of flowers emerged per plant				
Baiana Tropical	86.05	138.58	109.96	111.53
CPATC 10	97.05	133.17	128.21	119.48
Jacarezinho	117.94	164.79	177.92	153.55
Sergipe	76.61	119.33	120.42	105.45
Mean	94.41	111.57	134.13	118.59
Pistillate				
Flower emergence per plant per day				
Baiana Tropical	0.08	0.22	0.10	0.14 b
CPATC 10	0.07	0.18	0.09	0.11 c
Jacarezinho	0.10	0.28	0.13	0.17 a
Sergipe	0.06	0.12	0.06	0.08 d
Mean	0.08 B	0.20 A	0.10 B	0.13
Flowering days				
Baiana Tropical	25	34	29	29.3
CPATC 10	25	30	27	27.3
Jacarezinho	26	36	25	29
Sergipe	28	33	26	29
Mean	26	33.2	26.7	28.7
Number of flowers emerged per plant				
Baiana Tropical	3.39	8.96	3.92	5.42
CPATC 10	2.67	7.37	3.58	4.54
Jacarezinho	4.22	11.46	5.12	6.93
Sergipe	2.5	5.12	2.21	3.28
Mean	3.19	8.23	3.71	5.04

Continuation Table 1

	Pistillate (♀) : Staminate (♂)			
Baiana Tropical	1:16.54	1:13.31	1:20.64	-
CPATC 10	1:22.09	1:13.50	1:26.61	-
Jacarezinho	1:19.94	1:13.59	1:24.11	-
Sergipe	1:21.78	1:19.87	1:39.62	-

* Different lowercase letters, in the same column, and uppercase letters, in the same row, indicate a significant difference ($P < 0.05$) by Scott-Knott test

In the evaluation of anthesis time, the staminate flowers open, on average, at 4:40 h and close at 10:20 h in the morning, remaining open for 5:40 h. In the evaluation between cultivars and cycles, the interaction was significant ($p < 0.05$) in staminate flowers. In general, in the third cycle, the flowers opened earlier, being a uniform behavior among all cultivars, except for 'Baiana tropical'. Assessing the moment when the flowers close, it is clear that this was later in cycle II and, among the cultivars, 'Jacarezinho' was the only one that differs from the others, with early closing (10:16 h) (Table 2). Considering the longevity of the flower, that is, the time that it remains open and can be visited by a possible pollinator, in cycles II and III, the longest longevities are present. Cultivars 'CPATC' and 'Sergipe' remained open for the longest time.

For pistillate flowers, there was no effect of the interaction between cultivars and production cycles. In general, flowers opened at 4:36 h and closed at 10:53 h in the morning, with a duration of the opening of 6:17 h. In cycle III, although the flowers opened earlier than in the other cycles, they also closed earlier, so that in this cycle the shortest longevities were observed (Table 2). The early closure in the third production cycle (dry season) may be related to the local humidity (76.5%) and the average air temperature (27.9 °C), which can facilitate water loss and the consequent petal withering. According to Nepi and Pacini (1993), the variation in the opening and closing time of the flowers in the different seasons in *Cucurbita* is mainly due to the climatic conditions, in which the low humidity and high air temperatures favor the closing of flowers.

The flowers opened in the early hours of the day, even before the sunrise and the time they remained open was relatively short, which requires an efficient pollinator when visiting to ensure reproduction. Female flowers remained open 37 minutes, on average, more than male flowers (Table 2), giving the possibility of receiving a higher number of visits and potential pollinators.

In the assessment of the stigma receptivity by the indirect test with hydrogen peroxide (H_2O_2 - 10 volumes), the stigmas were receptive at all times of assessment, which started at 5:00 h a.m and were repeated every hour

until the total closing of the flower. The receptive stigma throughout the period of anthesis with the same result for all cultivars in the three cycles indicates that this is a characteristic inherent to *Cucurbita moschata*. This is essential, since the flower has a short anthesis, which may increase the possibility of fertilization if, when receiving visits from a potential pollinator, pollen is deposited and these are viable (KLEIN *et al.*, 2020).

Considering data related to morphometry, for the two floral types (male and female), there was no difference between the groups formed in the non-hierarchical (k-means) and hierarchical clustering analyses, even varying, in the latter, the clustering methods ("single", "complete", "average" and "Ward"), showing consistency in the clusters formed.

Basically, for staminate flowers, cultivar 'Sergipe' presented the greatest distance from the other cultivars, which were grouped together. This separation is consistent with the characterization by principal component analysis (Figure 1). In this characterization, only cultivar 'Sergipe' showed high values in the first component and, with the exception of the sepal length, this represents high values for all the variables analyzed in this component, which explains more than 83% variability of data related to morphometry of staminate flowers.

Considering the second principal component, cultivar 'Jacarezinho' was spatially separated from the other cultivars in its group, with the highest values of sepal length. This separation, however, is relative, since this component accounts for just over 11% data variability. Values of the cophenetic correlation coefficient were high, regardless of the clustering method adopted, which attests to a good degree of fit between the original distance matrix and the simplified matrix generated by the cluster analysis.

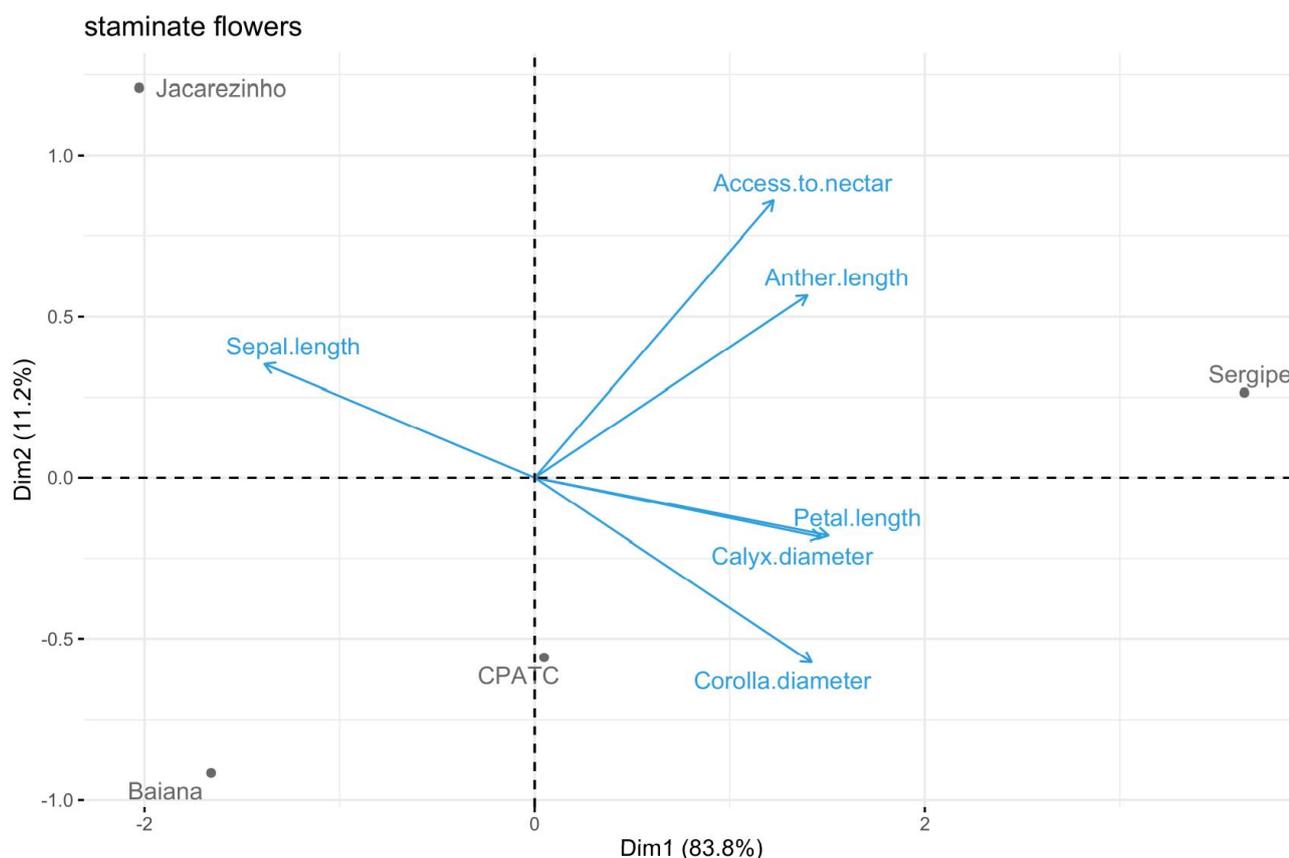
Observing the relationship between the variables, it is noticed that there was a certain positive association between the corolla diameter and the petal size. Larger petals increase the attractiveness for floral visitors, being an important visual signal for potential pollinators (FAEGRI; VAN, 1979).

Table 2 - Opening time (hour: minute) of male and female flowers in four cultivars of *Cucurbita moschata*

Cultivar	Cycle			Mean
	I	II	III	
Staminate				
Opening				
Baiana Tropical	04:51 bA	04:51 bA	04:46 bA	04:49
CPATC 10	04:37 aB	04:38 aB	04:30 aA	04:35
Jacarezinho	05:05 cB	05:02 cB	04:34 aA	04:54
Sergipe	04:36 aB	04:41 aB	04:27 aA	04:34
Mean	04:47	04:48	04:34	04:43
Closing				
Baiana Tropical	10:18 bA	10:29 bC	10:23 bB	10:23
CPATC 10	10:25 cA	10:58 cC	10:33 cB	10:39
Jacarezinho	10:10 aA	10:16 aA	10:13 aA	10:13
Sergipe	10:19 bB	10:28 bC	10:13 aA	10:20
Mean	10:18	10:33	10:20	10:24
Longevity				
Baiana Tropical	05:27 bB	05:38 cA	05:37 cA	05:34
CPATC 10	05:48 aC	06:21 aA	06:02 aB	06:03
Jacarezinho	05:04 cC	05:13 dB	05:39 cA	05:19
Sergipe	05:43 aA	05:45 bA	05:46 bA	05:45
Mean	05:30	05:45	05:45	05:40
Pistillate				
Opening				
Baiana Tropical	04:25	04:25	04:27	04:25 a
CPATC 10	04:31	04:31	04:32	04:31 b
Jacarezinho	04:53	04:54	04:56	04:51 d
Sergipe	04:39	04:39	04:28	04:35 c
Mean	04:37 B	04:37 B	04:33 A	04:36
Closing				
Baiana Tropical	10:54	10:54	10:53	10:51 b
CPATC 10	11:01	11:02	10:48	10:57 c
Jacarezinho	11:04	11:04	10:49	10:06 c
Sergipe	10:54	10:55	10:31	10:46 a
Mean	10:58 B	10:59 B	10:43 A	10:53
Longevity				
Baiana Tropical	06:29	06:28	06:16	06:25 a
CPATC 10	06:30	06:31	06:16	06:25 a
Jacarezinho	06:11	06:10	06:03	06:08 b
Sergipe	06:15	06:15	06:02	06:11 b
Mean	06:21 A	06:21 A	06:09 B	06:17

* Different lowercase letters, in the same column, and uppercase letters, in the same row, indicate a significant difference ($P < 0.05$) by Scott-Knott test

Figure 1 - Biplot for the principal component analysis of four pumpkin cultivars based on six variables related to the morphometry of staminate flowers, considering the average of the three cultivation cycles



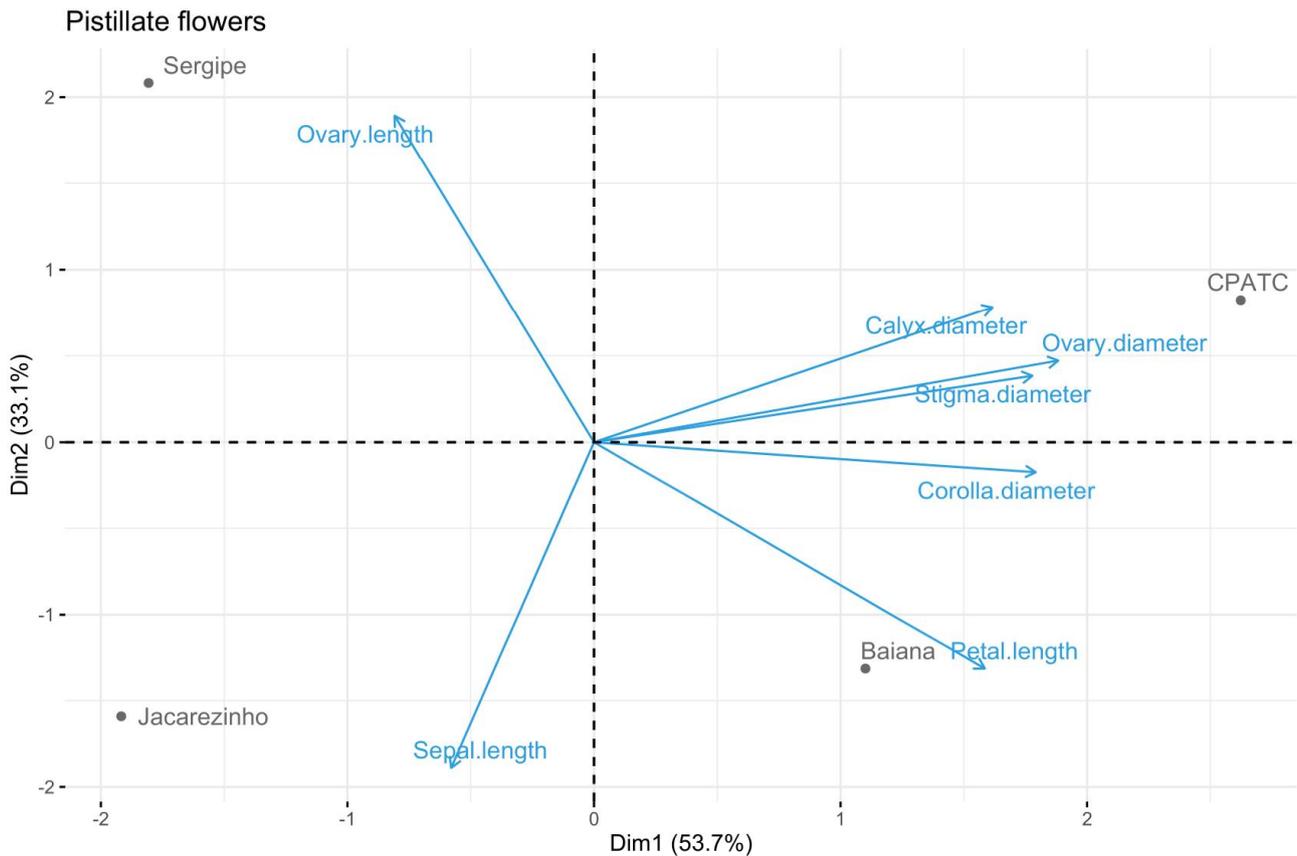
The staminate flower of all the cultivars analyzed contained five stamens with merged filaments and anthers, the nectary being inside the filament tube. The access to the nectar occurs through the holes formed between the filaments, and, for evaluation purposes, the average size of these holes was considered. This variable is strongly correlated with the anther size. In a way, the more accessible the nectar is to a floral visitor, the less time will be spent collecting the floral resource, which can decrease the amount of pollen to be adhered to the insect. On the other hand, larger anthers can maximize contact with the pollinator, which often touches the anther during the collection of floral resources. In this respect, the cultivar ‘Sergipe’ would potentially be more favorable to a greater and more prolonged contact of the anthers with pollinators.

The access to nectar differs between staminate and pistillate flowers and the nectary structure is important to facilitate the collection of the resource. Unlike the staminate flower, the pistillate flower nectary forms a circular ring at the base of the stylet that supports the three-lobed stigma. This can influence the collection time of potential pollinators between female

and male flowers. In zucchini (*Cucurbita pepo*), the time of nectar collection by bees is variable between male and female flowers, with longer time in staminate flowers (CANE; SAMPSON; MILLER, 2011). This reinforces the understanding that the more difficult it is to collect the nectar, the longer the time spent by a visitor in this structure, since the pumpkin produces nectar in abundance and compensates for the effort (FREE, 1993).

As for pistillate flowers, the cluster analysis, with a cophenetic correlation coefficient of around 75%, resulted in the formation of three groups that can be easily described according to the principal component analysis (Figure 2). ‘CPATC’ and ‘Baiana tropical’ formed the first group, characterized by high values of corolla diameter, stigma diameter, petal length, ovary diameter and calyx diameter. ‘Jacarezinho’, with high values of sepal length and with the lowest values for calyx diameter, ovary diameter and stigma diameter, was classified as the second group. Finally, cultivar ‘Sergipe’ was the third group, individualized with the highest values of ovary length, and the lowest values of corolla diameter and petal length.

Figure 2 - Biplot for the principal component analysis of four pumpkin cultivars based on seven variables related to the morphometry of pistillate flowers, considering the average of the three cultivation cycles



The size pattern of the sepals maintained a certain constancy among the four cultivars, for the two types of flowers, with cultivar ‘Jacarezinho’ showing the highest values of this characteristic. The same did not happen for petal length. Cultivar ‘Sergipe’, which had the longest petal length in staminate flowers, showed the lowest value of this variable in pistillate flowers.

Ovary diameter and length, which give the shape of the fruit to be generated, do not seem to be correlated with each other; cultivar ‘Sergipe’ had longer ovaries, and cultivar ‘CPATC’, wider ovaries. As mentioned, this relationship between the dimensions of the ovary remains until the fruit ripens, and it is possible to conclude on the final shape of the fruit before fertilization even occurs.

CONCLUSIONS

1. The study allows to conclude that the general flowering pattern of the pumpkin does not depend on the genotype

and the planting conditions, being characterized by the initial emergence of male flowers, followed a few days later by female flowers when there is the concomitant production of the two types of flowers. The production of a much smaller number of female than male flowers, and for a shorter period of time, are characteristics that favor cross-pollination mediated by pollinating agents;

2. The floral biology of the pumpkin vine with dioecious, large and showy flowers, longer anthesis in female flowers and receptive stigmas during the entire anthesis, demonstrates the need to attract biotic pollinators efficient in transferring a large amount of pollen between the flowers in a short time;

3. Although the general characteristics of flowering and floral biology are constant for the species, the pumpkin has numerical, morphometric and temporal variations in flowering according to the genotype and/or the cultivation conditions, which may or may not favor the attraction and/or action of pollinators. These differences in cultivars and cultivation conditions must be taken into account in the management of the crop in order to favor adequate pollination.

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