



Floral Biology and pollination requirements of sesame (*Sesamum indicum* L.)

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ABSTRACT. This study aimed to investigate the floral biology and pollination requirements of sesame (*Sesamum indicum* L.), cultivar CNPA G2. The study was carried out at Barbalha, State of Ceará, and consisted of two phases: floral biology and pollination requirements. Flowers were observed as for time of anthesis, lifespan and stigma receptivity, and were applied the following pollination treatments: open pollination, hand cross-pollination, hand self-pollination, pollination restricted with tulle bags and pollination restricted with paper bags. Significant differences ($p < 0.05$) were detected between treatments for the number of fruits harvested 30 days after pollination. It was shown that the sesame plant presents a mixed-pollination system, because it can produce fruit whatever the type of pollination tested. Flowers are able to autopolinate and do not depend on external agents. Pollinations restricted with paper and tulle bags produced fruit with greater number of heavier seeds. It is concluded that sesame crop is able to autopolinate and benefit from both self-pollination and cross-pollination.

Keywords: self-pollination, cross pollination, pollinators, fruit.

Biologia floral e exigências de polinização do gergelim (*Sesamum indicum* L.)

RESUMO. O trabalho objetivou investigar a biologia floral e as exigências de polinização do gergelim (*Sesamum indicum* L.), cultivar CNPA G2. O estudo foi conduzido em Barbalha, Estado do Ceará e constou de duas fases: biologia floral e exigências de polinização. As flores foram observadas quanto ao horário de abertura, tempo de vida e receptividade de estigma, e aplicaram-se os seguintes tratamentos de polinização: polinização livre, cruzada manual, autopolinização manual, restrita com saco de filó e restrita com saco de papel. Houve diferença significativa ($p < 0,05$) entre os tratamentos no número de frutos colhidos 30 dias após as polinizações. Foi demonstrado que o gergelim é uma planta de polinização mista, pois pode produzir frutos sob qualquer um dos tipos de polinização testados. Suas flores são capazes de se autopolinizar e não dependem de agentes externos. As polinizações restritas com papel e com filó produziram os frutos com sementes mais pesadas e uma maior quantidade delas dentro do fruto. Conclui-se que a cultura do gergelim é capaz de se autopolinizar e beneficia-se tanto da autopolinização quanto da polinização cruzada.

Palavras-chaves: autopolinização, polinização cruzada, polinizadores, frutos.

Introduction

Sesame (*Sesamum indicum* L.) belongs to the Family Pedaliacea, and some authors report that it is native to Asian and some African countries (BEDIGIAN, 2003; DESAI, 2004). It is believed that sesame is one of the oldest crops in the world, cultivated for over 4,300 years in Babylon and Assyria (HWANG, 2005). Its cultivation has great economic potential, because of great demand, both nationally and internationally. The seeds, which contain about 50% oil, are the main reason for its cultivation, and may be used in the food, pharmaceutical and chemical industries (BLAL,

2013; ELLEUCH et al., 2007; MORETTO; ALVES, 1986; NAMIKI, 2007).

Presently, sesame is explored in 65 countries across Asia, Africa, Europe and Central and South America. Asia and Africa hold about 90% of the planted area (BELTRÃO; VIEIRA, 2001). The main producing countries are Egypt, Central Africa, Israel, Peru, Saudi Arabia and Macedonia. The current world production is estimated at 7,725,706 tons, with yield of 390 kg ha⁻¹, being the ninth most cultivated oilseed crop in the world.

Sesame was introduced in Brazil by the Portuguese in the XVI century from their Indian colonies (ELLEUCH et al., 2007; KOCA et al., 2007; WIESS,

1983). The country is a small producer with only 6,000 tons of grain produced per year on 9,000 ha and a yield of around 640 kg ha⁻¹ (FAO, 2012).

Sesame has zygomorphic flowers with pendulous tubular corolla of 3-4 mm in length and coloring of various shades of purple white. They occur singly or in groups of two to three in the leaf axils and are hermaphrodite. The androecium consists of four stamens, two long (1.5-2.0 mm) and two short (1.0-1.5 mm) and the gynoecium has superior ovary, multicarpelar and a long style (1.5 - 2.0 mm) with bifid stigma. The flower produces nectar in a nectary disk surrounding the ovary and in a couple of extrafloral nectaries on both sides of the pedicel. Anthesis occurs early in the morning when the stigma becomes receptive and senescence can occur six to 12 hours later, depending on the variety and environmental conditions (FREE, 1993). These characteristics of floral biology refer to varieties cultivated especially in warm weather environments, but there is evidence that varieties adapted to tropical conditions behave differently.

With regard to the pollination requirements of sesame, there is no consensus on the predominant type of pollination. According to Wiess (1983), this species is predominantly autogamous. Nevertheless, crossing rates reported in some studies ranged from only 1 to 68% (ABDEL ALL et al., 1976; ASHIRI, 2007; FREE, 1993; SARKER, 2004; YERMANOS, 1980), evidencing the need for further clarifications in this regard.

Due to the lack of information on the pollination of sesame in the literature and its contradictions, the present study was conducted to study the floral biology and determine its requirements for pollination in the state of Ceará.

Material and methods

The research was carried out at the Brazilian Agricultural Research Corporation - EMBRAPA Cotton, Experimental Field of Barbalha, State of Ceará, 405 km from Fortaleza, with the sesame cultivar CNPA G2. The experiments were developed from November to December 2008 and fruit were harvested in January 2009. The predominant climate in the region is Aw'i (humid tropical climate), according to the Köppen classification, and the average annual temperature is 24°C and mean annual rainfall of 1,200 mm, with the rainy season from December to May.

The floral biology was studied in the following aspects: the flowering period, morphology and color of the flower, time of anthesis, flower longevity, period of stigma receptivity, number of stamens and anther characteristics.

The observations concerning floral morphology, color of petals, stamen number and anther characteristics were performed on 30 randomly tagged flowers, while flower buds were monitored every hour from the time of anthesis until they fall from the plant.

To study the time of anthesis and flower longevity, 30 flower buds in pre-anthesis were tagged and randomly bagged with 20x20 cm nylon tulle bags 1 mm mesh. The next day, the flowers were observed from 6:30 to 15h, from which time the flowers begin to wither and fall. Time of anthesis and duration of flowers were recorded.

For the stigma receptivity test, we used the direct method of cross-pollination of flowers throughout the day, since according to Kearns and Inouye (1993) it represents the actual capacity of the flower to be fertilized at that time. For this, 120 flower buds were bagged and tagged and, in the next day, from 7h, when the flowers were already open and the stamens had released pollen, 30 flowers were debagged, hand-pollinated with pollen from flowers of another plant and bagged again. This procedure was repeated with groups of 20 different flowers every hour until 11h, when the availability of fresh pollen in flowers was minimal and stigmatic surface was dry and withered. After the flower fall the tulle bag was removed. Five days after pollination, the number of fruits set per treatment was checked to determine the periods of stigma receptivity, since the more receptive is the stigma at that time, the greater the percentage of fruit set (DELAPLANE et al., 2013; FREE, 1993).

For the experiment on pollination requirements, we randomly selected 813 flower buds, which were divided into the following treatments:

- T1: Free pollination - 140 sesame flowers were tagged with a ribbon in order to know the level of natural pollination of flowers by the activity of pollinators in the experiment site.

- T2: Pollination restricted with paper bag - 144 flower buds were monitored for the occurrence of autopolllination in sesame. The flower buds were isolated, bagged with paper bag and tagged the day before their anthesis. The bags remained in flowers for 3 days, preventing any contact with biotic and abiotic pollinators.

- T3: Pollination restricted with tulle bag - the same procedure of the prior treatment, except for the bag that was made of fine gauze. In this treatment we sought to estimate the effect of wind on pollination in sesame, bagging 174 buds.

- T4: Hand self-pollination (geitonogamy) - a day before the work of pollination, 185 flower buds that would open the next day were bagged with tulle bag and were protected from any possible pollinator.

The next day, at the moment of highest receptivity of stigmas (determined as described above), we removed the bag from each flower and then took the anthers of some flowers on the same plant and touched the stigma of the flower to be pollinated. Next, the pollinated flower was bagged again, aiming to isolate it, and properly tagged. The bag remained on the plant until the fall of the flower.

- T5: Hand cross-pollination - This type of pollination was conducted in 176 flowers by following the previous method, differing only in the origin of the pollen donor flower, which in this case were flowers of another plant. Before hand-pollination, the flowers were emasculated with the aid of fine tweezers, while their stamens were not releasing pollen.

The fruit set was assessed five days after the handling of flowers and fruit persistence was observed at harvest.

Fruits were harvested when ripe, and evaluations of seed weight and number of seeds per fruit were used for comparison between treatments.

Data related to the requirement for pollination in sesame, i.e., fruit set depending on the type of pollination were analyzed using the nonparametric chi-square test (ZAR, 1984). For this, we used the software SAS, FREQ procedure, because the data had binomial character (fruit set = 1 X fruit not set = 0), thus not meeting the assumptions for analysis of variance. Additionally, the treatments were compared one by one, at the 5% significance level.

Data of seed weight and number of seeds per fruit were analyzed by analysis of variance followed by Tukey's test.

Results and discussion

Floral biology of sesame

The flowering period was from November to December, starting the ripening of fruit after 60 days of flowering. At the end of December, the flowering decreased, ceasing completely in January.

Unlike other varieties of sesame which have one or two flowers per leaf axil, the CNPA G2 variety has three flowers. The flowers are complete, gamopetalous, zygomorphic and with a short stalk. The calyx has five fused sepals. One of the petals serves as a landing platform for the visiting insects. The tubular corolla is white, with a lobe upwards and the other downwards.

The androecium is didynamous with four stamens, in pairs, one lower than the other, epipetalous, fused at the base of the upper lip of the corolla tube, and anthers with rimosa or longitudinal dehiscence. Anthers are yellowish and 1 mm in length. The pollen grain is yellowish; gynoecium is

bicarpelar, with bilocular ovary and axile placentation. The observations showed that the ovary is superior and green, and the style is filiform, ending in a bifid stigma. The fruit is dehiscent, and the dehiscence starts at the apex toward the base. These features are apparently common to many varieties of sesame, considering the similarity with that observed in other varieties by several authors (FONT QUER, 1970; PRATA, 1969; YERMANOS, 1980).

With respect to the floral development, floral buds had a green corolla slightly rigid, which with the passing of time was growing and changing from green to white. At this stage, while the flower was developing, the anthers of the longer stamens were located below the height of the stigma, but still closed, while those of the shorter stamens, in turn, were located well below the anthers of the longer stamens. Two hours before anthesis, the four filaments of stamens elongated rapidly, so that at the time of anthesis (6:30 to 7h), anthers of longer stamens reached the stigma height, while those of shorter stamens were positioned just below the stigma. At that point, all four anthers opened up longitudinally and released pollen grains and the two lobes of the stigma opened in Y, coming into contact with the anthers and receiving a large amount of pollen on the inner surface. In this way, flowers of sesame autopollinate around the time of anthesis.

The stigma was receptive from the moment of lobes opening until the late morning, i.e., from 7 to 11 hours, the hours between 7 and 9 o'clock were those which provided a higher percentage of fruit set (Table 1). Around 12h, the stigma had become dry and withered, lobes were closed again.

Table 1. Number and percentage of fruit set in sesame flowers (*Sesamum indicum* L.), for different times of hand cross-pollination in Barbalha, State of Ceará, 2009.

Time of pollination (h)	Number of tagged flowers	Number and percentage (%) of fruit set
7:00 – 8:00	30	22 (73.33%) ab
8:00 – 9:00	20	17 (85%) a
9:00 – 10:00	20	11 (55%) b
10:00 – 11:00	20	10 (50%) bc

Values followed by the same letter in the column are not significantly different at $p < 0.05$, Tukey's test.

Considering the hand pollination, there was initial fruit set of flowers at all times tested (7, 8, 9 and 10h). However, a significant difference ($p < 0.05$) was found in the comparison of fruit set rates at different times. At 7h, when the flower opens, the rate of fruit set was 73.33%, not different ($p > 0.05$) from 8 hours, which was 85%, with the highest percentage of fruit set when compared to other times (7, 9 and 10h), suggesting a peak of receptivity, and therefore of fruit set at that time. Then there was a drop at 9h, which was even

more pronounced at 10h, although these two times were not significantly different to each other. The last pollination was held at 10h, showing the lowest number of fruit set and significantly different ($p < 0.05$) from the time of 8h. After 10h, pollen grains were no longer observed with the naked eye and stigmas had withered up.

Thus, sesame pollination can occur at any time from anthesis to 10h, but is most effective between 7 and 9h. Considering the stigma receptivity, studies in the literature are very divergent. While Yermanos (1980) stated that the receptivity of the stigma of the sesame flower is lost within 14 hours after flower opening, Abdel All et al. (1976) have suggested that it is receptive before flower opening, remaining viable for 24 hours after anthesis. On the other hand, Free (1993) states that the stigma of sesame flowers only becomes receptive from two hours after flower opening. Yermanos (1980) further argues that the temperature between 24 and 27°C is optimal for flowering of sesame and that under these conditions, the pollen remains viable for 24 hours. This variation in floral biology was probably due to different environmental conditions observed by these different authors in different locations and/or the variety studied is formed from crossing different varieties, producing a cultivar with high genetic variability. Langham (2007) described the development of sesame in 11 countries over 40 years and concluded that different genotypes can develop very differently under the same conditions.

After 10:00 am, it was observed a marked decrease in the number of pollen grains available in anthers and, at 11 hours, it was no longer verified pollen in anthers of flowers. Then the anthers dried up, changed color, darkened, and from white became yellow and brown. Around 11h, the flowers began to show signs of senescence, mainly by withering of anthers and drying of the stigma. Nevertheless, the stage of sesame flower senescence was characterized by wilting of the petals, which started from 12h, followed by the almost immediate fall of the corolla. When the corolla of the flower falls, the style remains in the flower until the end of the day, falling in late afternoon, including in those that were hand pollinated and later generated fruits. These observations were also reported by Yermanos (1980) and Mazzani (1983). However, Free (1993) in a study in India, reported that anthesis of flowers occurs earlier, at 4 hours, and the fall occur only after 12 hours. Probably, these variations are due to factors related to the differences in sesame cultivars, temperature, and/or relative humidity and luminosity at different hours of the day, among the sites studied.

Pollination requirements

The number of fruit set, five days after anthesis of flowers, and harvested showed significant differences ($p < 0.05$) between treatments of pollination in Barbalha, State of Ceará (Table 2).

The free pollination set significantly more fruits at five days, followed by hand pollination, pollination restricted with tulle, hand cross-pollination and restricted with paper (Table 2). Despite showing the lowest rate of fruit set and differ significantly ($p < 0.05$) from all other treatments, the pollination restricted with paper set fruits suggesting that sesame accomplish autopolination. This evidence becomes stronger because the manual self-pollination presented the second best rate of initial fruit set, implying the existence of a strong autogamy in this species. The pollination restricted with tulle, in turn, did not differ significantly ($p > 0.05$) from hand self-pollination, indicating the importance of wind to transfer pollen between anthers and stigma in the same flower, in sesame, mainly because this treatment was also significantly superior ($p < 0.05$) to the other in which pollinators were prevented from visiting the flowers (pollination restricted with paper). Finally, the free pollination resulted in the best fruit setting rate, which was significantly different ($p < 0.05$) from all other treatments, indicating that the visit by biotic pollinators is important for sesame pollination, whether increasing the efficiency of pollen transfer within the same flower or even bringing pollen from other flowers, providing cross-pollination, which was also able to set fruit in sesame (Table 2).

Data on fruit harvest showed different results of the initial fruit set. At harvest, a significant difference was detected between treatments ($p < 0.05$), but only the hand self-pollination differed from the others (Table 2). This result indicates that despite the hand self-pollination increased initial fruit set, it does not necessarily mean that the flower has been fertilized and the fruit can be aborted in its early stage of development, as registered by Holanda Neto et al. (2002) for the cashew (*Anacardium occidentale*).

The results of fruits harvested show that sesame is a plant with mixed pollination, which produce fruit by both autogamy (auto and self-pollination) and crossbreeding (cross-pollination), like most higher plants (DELAPLANE et al., 2013). The significantly higher ($p < 0.05$) fruit yield as a function of the cross-pollination in relation to self-pollination contradicts Weiss (1983), who stated that sesame is a predominantly autogamous species, which could only and possibly present allogamy

above 10%. The best performance of cross-pollination is probably due to exchange of genetic material promoted by this type of pollination, increasing the heterozygosity of the embryo formed in the seeds, which according to Mahfouz et al. (2012), enhances the quality and quantity of seeds produced, and anticipates the period of ripening and harvest. For the same reason, the treatment of open-pollination, which promotes cross-pollination, was also superior to others and was not different from that of hand cross-pollination (Table 2).

As for the data concerning the weight and number of seeds per fruit, significant differences ($p < 0.05$) were observed in the number and average weight of seeds per fruit between the different treatments. The pollination restricted with tulle showed a significant difference ($p < 0.05$) between the hand self-pollination and hand cross-pollination, but pollinations restricted with tulle and paper were not significantly different (Table 3). According to these results, wind pollination had no effect on the number or the weight of seeds per fruit.

Pollinations restricted with paper and with tulle produced fruits with heavier seeds and a larger amount of seeds in the fruit (Table 3). Probably, these treatments had greater deposition of pollen directly on the stigma, enough to fertilize all the ovules present in the ovary. This indicates that possibly autogamy resulted in an advantage to the plant's reproductive success.

The treatments of hand self-pollination, hand cross-pollination and free pollination yielded fruits with fewer seeds.

The results suggest that the type of pollination affect the weight and number of seeds per fruit. Although the variables weight of seeds per fruit and

number of seeds per fruit presented statistical difference, they were relatively similar for all treatments. Nevertheless, the greater fruit set by cross- and free- pollination fruit led to fewer and lighter seeds per fruit, although meaning a greater total output value (VAISSIÉRE et al., 2011).

A higher number of seeds per fruit is a desirable feature for sesame both from the commercial and ecological point of view, being an important indicator of plant reproductive success, since a greater number of seeds produced will increase the chances of perpetuation of species (ROUBIK, 1989). In this experiment, the number of seeds produced per fruit in sesame indicated that the pollination requirements of sesame flower were met. In nature, self-pollination is less advantageous because it does not favor new genetic combinations and thus the production of more vigorous seeds and plants, so that some plant species have mechanisms to prevent the occurrence of self-pollination (CONSOLARO, 2005; RAVEN et al., 2007). On the other hand, self-pollination ensures the perpetuation of the species when one partner is not nearby or promoters of cross-pollination agents are absent or scarce. In this sense, although many plant species get more benefits by cross-pollination also accept a certain percentage of self-pollination. This becomes especially clear in species from harsh habitats with few biotic pollinators (FREE, 1993; FREITAS; PAXTON, 1998; HOLANDA-NETO et al., 2002). Nevertheless, sesame seems to have evolved to favor self-pollination, but also developed mechanisms to attract biotic pollinators, such as differently flower, showy color, odor and nectar secretion, in order to promote some percentage of cross-pollination (FAEGRI; VAN DER PIJL, 1979).

Table 2. Initial fruit set and persistence of sesame fruit (*Sesamum indicum*) according to the type of pollination in Barbalha, State of Ceará, 2009.

Type of pollination	Number of fruits	Number of fruits Set at five days	Number of fruits harvested
Free pollination	140	135 (96.43 %) a	115 (82.14 %) a
Hand pollination	179	163 (91.06 %) b	130 (72.63 %) b
Pollination restricted with tulle	174	149 (85.63 %) bc	136 (78.16 %) ab
Hand cross pollination	176	147 (83.52 %) c	142 (80.68 %) a
Pollination restricted with paper	144	116 (80.55 %) d	115 (79.86 %) ab

Values followed by the same letter in the column are not significantly different at $p < 0.05$, Tukey's test.

Table 3. Number and average weight of seeds of sesame (*Sesamum indicum*) produced from five types of pollination in Barbalha, State of Ceará, 2009.

Type of pollination	Number	Number of seeds/fruit (g)	Number of seeds
Hand self-pollination	130	0.192 ± 0.02 c	61.32 ± 5.05 c
Hand cross-pollination	142	0.206 ± 0.01 bc	62.36 ± 5.23 bc
Pollination restricted with tulle	136	0.240 ± 0.02 a	70.75 ± 5.97 a
Free pollination	115	0.203 ± 0.01 bc	58.75 ± 5.47 c
Pollination restricted with paper	115	0.223 ± 0.02 ab	65.15 ± 6.07 ab

Mean values followed by the same letter in the column are not significantly different at $p < 0.05$, Tukey's test.

Conclusion

Under the conditions of the Brazilian Northeast, the flower of sesame presents lifespan of 4 hours, and pollination must occur between 7 and 11 hours to ensure a greater fruit set.

Sesame is a mixed pollination plant, able to self-pollinate and reach levels above 50% fruit set, but with potential for significant increase in fruit production in the presence of biotic pollinators that promote cross-pollination.

The highest fruit set per plant due to cross-pollination by biotic pollinators produces fewer and lighter seeds per fruit, but a greater yield per plant.

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