

Hybridization of Castor Bean (*Ricinus communis* L.) in Morelos, México

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ABSTRACT

Objective: To determine the stigma receptivity and the pollen viability in order to make manual crosses and obtain viable progeny in castor bean (*Ricinus communis* L.).

Design/Methodology/Approach: Pollen viability tests were made on breeding materials by staining with acetocarmine solution. In elite materials, the receptivity of the stigma was evaluated with hydrogen peroxide. From the inflorescences, the male ones were removed and the female ones were preserved, which were covered with a glassine bag. After six days, they were checked and when they were considered receptive, manual pollinations were carried out between viable pollen materials and receptive stigma. The pollen of the male parent was impregnated in the stigmas and the inflorescence was covered again.

Results: The breeding materials exhibited viable pollen since they were stained red. Whereas, the application of hydrogen peroxide to the stigmas of the elite materials showed bubbling, indicative that they were receptive. In 2014, 400 inflorescences were pollinated with 8 flowers on average and a fruit pollinated percentage of 61.21. In 2015, 245 inflorescences with 12 flowers on average and 61.24% of fruit were pollinated.

Study Limitations/Implications: Temperature and relative humidity were fundamental factors for the success of castor bean fertilization.

Findings/Conclusions: The elite castor bean materials presented receptive stigmas and the improved viable pollen materials. Manual crosses produced fruits and seeds. The fruits pollinated were 61%.

Keywords: Pollen viability, stigma receptivity, fruits pollinated, hybridization, genetic improvement.

INTRODUCTION

The irreversible exhaustion of oil reserves globally led to the search for alternative sources of renewable energies such as bioenergy. Biofuels in their solid, liquid and gas forms are currently of great importance, and therefore their study, production and use have intensified in the last 15 years (Guo *et al.*, 2015). The search for new bioenergetics sources have centered on the production of ethanol and biodiesel from biomass of sugarcane

(*Saccharum* spp. hybrids), maize (*Zea mays* L.) and grain sorghum (*Sorghum bicolor* L.). Advances have also been made in the research and technological development of other crops such as castor bean (*Ricinus communis* L.). Castor bean belongs to the Euphorbiaceae family, with chromosome number $2n=2x=20$. Their area of distribution is extensive worldwide, from tropical and subtropical regions to temperate zones (Lu *et al.*, 2019). In Mexico, it is possible to find it throughout the country, and it is common for it to grow on the edges of paths and disturbed vegetation areas.

At the commercial scale, castor bean is cultivated in 1.5 million hectares in the world, where India, China, Brazil, Russia and Thailand stand out as main producers (Rukhsar *et al.*, 2018). The oil from the seeds has chemical and physical properties that are unique and exceptional for industrial use (Lu *et al.*, 2019; Rodrigues *et al.*, 2019). In Brazil its cultivation is considered for family subsistence, interspersed with maize and bean, using local varieties with long cycles whose seed maturation is heterogeneous (Milani and Nóbrega, 2013).

In Mexico there are native populations of castor bean with wide morphological, productive, genetic and adaptive variability (Barrios-Gómez *et al.*, 2018; García-Herrera *et al.*, 2019). However, the productive potential is limited because they are normally tall plants with abundant aerial biomass, lax bunches (sparse) of small size, sometimes indehiscent, with few fruits per bunch and seeds per fruit, and lower grain yield.

The commercial cultivation of castor bean in Mexico uses landrace and imported breeding materials. The landrace varieties are tall with low yield potential, but with tolerance to adverse factors, a useful trait to generate hybrid or improved varieties. These two groups of materials, together with the germplasm from native populations from Mexico, represent a valuable opportunity for genetic improvement of the species. Generating breeding materials that are adapted to particular production zones in Mexico require strategies that include the selection, mutagenesis, use of molecular markers and hybridization.

The success of hybridization in any plant species is sustained on understanding the moment when the stigmas are receptive and the time lapse when pollen grains are liberated during the day. In genetic improvement of cultivated species it is difficult to find

two parents that originate a progeny with the highest amount of desirable traits (Ferreira *et al.*, 2015). However, it should be considered that the quantitative traits of greatest economic importance are controlled by many pairs of genes.

The inflorescence of castor bean is a monoecious bunch with female flowers in the higher part and male in the lower part (Merkouropoulos *et al.*, 2016). It is considered to be a crossed-pollination species (Ramesh *et al.*, 2017) where wind is the main pollinating agent (Anjani *et al.*, 2018). The liberation period of the pollen grains lasts 1 to 2 days, with optimal temperature conditions between 26 and 29 °C and relative humidity of 60%. Meanwhile, the stigma is completely receptive for a period of 5 to 10 days depending on environmental conditions (Milani and Nóbrega, 2013). With the aim of establishing the bases of hybridization in castor bean in Mexico this study had the objectives of determining the receptivity of the stigma and viability of pollen, and making manual crosses to obtain viable progeny.

MATERIALS AND METHODS

This study was carried out in the Zacatepec Experimental Field of the National Institute for Forest, Agricultural and Livestock Research (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, INIFAP), located in the Zacatepec-Galeana Highway km 0.5, on coordinates 18° 39' 16" LN, 99° 11' 54.7" LW and an altitude of 911.8 m. The zone presents warm sub-humid climate with summer rains (Aw0), where average annual precipitation is 800 mm and average annual temperature 24 °C (Ornelas *et al.*, 1997).

The genetic material used consisted of six elite materials of castor bean collected in many states of the country, which were selected for having greater size, weight and seed yield (Barrios *et al.*, 2013), and three commercial varieties liberated by foreign seed companies. Receptivity tests were made on the stigma of elite materials, and pollen viability tests were applied on the breeding materials.

In 2014, pollen viability tests were made with the method proposed by Dempsey (1993), which consists in staining with acetocarmine (Meyer[®] coloring solution) and observing the change of color in the stereoscopic microscope. The stigma receptivity tests were applied following the method proposed by Osborn *et al.* (1988), which consists in placing a drop of hydrogen peroxide

at 3% on the stigmas; if bubbles are produced in the reaction then they are considered as receptive.

The genetic materials that resulted with viable pollen and receptive stigmas were used as parents to make manual crosses in November 2014 and October 2015. From the inflorescences, 10 female flowers were chosen that were in the same phenological state and the male flowers were eliminated (Figure 1a); then the inflorescence was covered with a glassine bag. The state of development of the stigmas was checked six days later and, when they were considered receptive, manual pollination impregnating the male parent's pollen on the stigmas was done; then the inflorescence was covered again (Figure 1b). Pollination was carried out from 9:20 a.m. to 11:30 a.m. in 2014 and from 10:00 a.m. to 1:30 p.m. in 2015. In the Zacatepec Experimental Field, in Morelos, the values of mean maximum, mean minimum, and mean daytime temperatures were 30.8, 15.9 and 27.4 °C in October and 30.8, 12.1 and 26.7 °C in November, respectively. The monthly average precipitation was 67.2 mm in October and 10.4 mm in November. These means come from records that cover from 1961 to 2003 (Díaz et al., 2008). For each cross the variables that were found were the number of pollinized flowers, number of harvested fruits, and percentage of fruit pollinated.

RESULTS AND DISCUSSION

In the pollen viability tests in breeding materials, pollen grains stained with red color were observed, which indicated that they were viable so they could be used as male parents. Likewise, the application of hydrogen peroxide to the stigmas of the elite materials showed bubbling, indicative that they are receptive (Figure 1c). The results of pollen viability and stigma receptivity indicated that the environmental conditions in the zone were optimal to carry out the pollination and later fertilization. Studies about the determination of pollen viability and stigma receptivity are essential activities to carry out genetic recombination. These tests have been useful in hybridization processes in species of the *Echeveria* genus (Rodríguez-Rojas et al., 2015) and in poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) (Canul-Ku et al., 2015; Rodríguez et al., 2017).

In the year 2014, 400 inflorescences were pollinated, with 8 flowers on average each and fruit pollinated was 61.21%. In 2015, 245 inflorescences were pollinated, with 12 flowers on average and fruit pollinated of 61.24% (Figure 1d).



Figure 1. Hybridization process in castor. a) Emasculated inflorescence. b) Manual pollination. c) Receptive stigma. d) Fruit development.

In the evaluation of the behavior of fruit pollinated in function of the time of pollination in 2014, it was observed that the highest percentage was obtained from pollinized flowers at 9:20 in the morning and the lowest at 10:30 (Figure 2). These results show that the minimal differences of time to do the manual pollinations are dependent on climate conditions.

In the year 2015 the pollinations performed at 10:20 in the morning presented the lowest percentage of fruit pollinated with 33% and at 12:30 the highest with 77%. In that year, the lowest fruit pollinated was during the period of 10:20 to 10:50 (Figure 3).

The best behavior of fruit pollination was obtained in 2014 (Figure 2). The next year increases and decreases were observed during the pollination period (Figure 3). This response is probably due to the environmental conditions that were present. Historical data show that the mean daytime temperature in the Zacatepec Experimental Field is 26.7 to 27.4 °C (Díaz *et al.*, 2008) during the time period when the pollinations were performed. If it considered that pollen grains from castor bean are liberated in optimal conditions of temperature between 26 and 29 °C for 1 to 2 days and the stigma is completely receptive 5 to 10 days (Milani and Nóbrega, 2013), it is deduced that the environmental conditions of the zone favored fertilization.

In the pollination of five species from the *Echeveria* genus, 100% of fruit pollinated was obtained, although the viable seeds only formed in intra and interspecific crossed pollination (Rodríguez-Rojas *et al.*, 2015). In

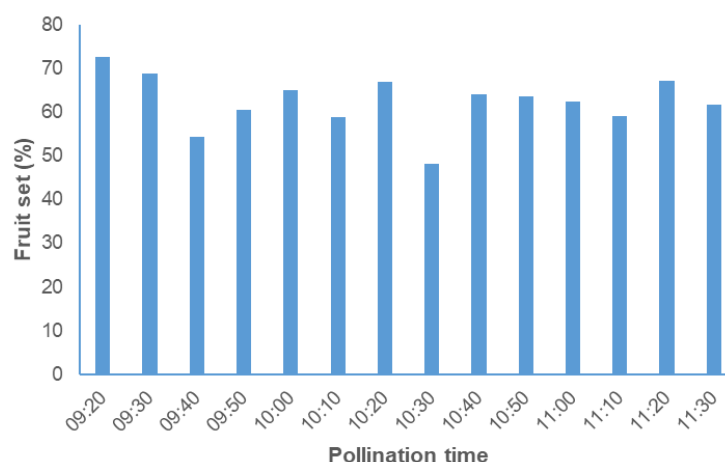


Figure 2. Percentage of fruit set as a function of pollination time. Zacatepec Experimental Field. 2014.

poinsettia it was reported that fruit pollinated is 68% with the technique of modified emasculation and it is crucial for hybrid generation. The process consists in cutting two thirds of the bract from the apex to the base of the inflorescence, eliminating male flowers, leaving between three and five non-receptive female flowers with the same phenological state covered with a waxed bag (Canul-Ku *et al.*, 2015).

The knowledge generated about pollen viability, stigma receptivity and fruit pollinated in these castor bean materials establish the bases that will allow generating genetic variants. With this information it would be possible to undertake the genetic improvement of castor bean and to obtain varieties and hybrids for specific environmental conditions in Mexico. The main goal of this genetic improvement will be to reduce the varietal dependency on imported breeding material.

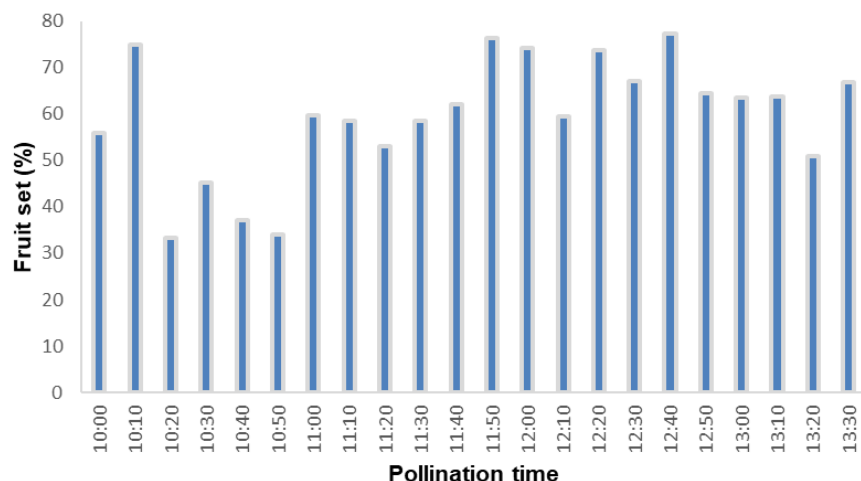


Figure 3. Percentage of fruit set as a function of pollination time. Zacatepec Experimental Field. 2015.

CONCLUSIONS

The elite materials of castor bean presented receptive stigmas and the breeding materials viable pollen. Manual crosses produced fruits and seeds. Fruit pollination was 61%. This indicates that there is genetic potential to generate new combinations through hybridization in castor bean.

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