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Pollination, a matter beyond the visible

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ABSTRACT.

Pollen viability is defined as a mechanism of male fertility, necessary for the reproduction of plants, since its mission is the fertilization of the ovules for the formation of seeds. Knowing this process is key to improving quality techniques and grain germination capacity, which are related to flower biology, used to refer to germination and viability, which directly or indirectly influence the fertilization process in plants. flowers. The development of the cocoa flower is subject to genetic, environmental, pathological, nutritional factors, the alteration between these factors affects fertilization and production in cocoa plantations. The heterogeneity in the crops, can present low yields, among them it can be associated with the reproductive development of the crop, size of the fruits, number of seeds, which could be associated with pollen viability, being these an important tool in the programs of genetic improvement.

Sexual compatibility increases the possibility of successful fertilization and is a key aspect for increasing productivity. Sexual incompatibility considerably limits the productivity of trees, since it cannot fertilize itself or with different material, making partial or total fertilization difficult due to inhibition due to chemical and physiological causes or lack of stimulating substances for the growth of the pollen tube.. The present study was carried out by the National Federation of Cocoa Growers with resources from the National Cocoa Fund, through the research program, where the objective was to evaluate the qualitative and quantitative differentiation of viable pollen grains to calculate the percentage of germination in 36 materials of cocoa, through in vitro tests in culture media, each one evaluated with five flowers, with counts in 250 pollen grains taken at random per flower, 50 per anther, for a total of 1,250 grains, the germination percentage is carried out two hours after sowing.

It was possible to make a classification of the evaluated materials according the in-vitro germination capacity into four groups: high (above 75%): FSA12, FLE3, FSV1, FTA2, FSA20, FCHI8, FGI4; intermediate-high (50-75 %): FTA4, FEAR26, FSV41, FSV30, CCN51, ICS1, FLE4, FBO1; intermediate-low (25-50%): ICS39, EET8, ICS95, FSV25, FTA7, FSA13, FSV155, FLE2, TSH565; and low (≤25%): FEAR5, FSV80, FMAC11, FYC2, FSA11, FEC44, FSV153, FLE28, FEC2, FEC7, FQUIP1.

Keywords: Pollination, germination capacity, Fedecacao, cocoa flower.

1. Introduction

Pollen viability is defined as a mechanism of male fertility, necessary for the reproduction of plants since its mission is the fertilization of the ovules for the formation of seeds (Pacini et al., 2019). The term viability has also been used to describe pollen grains capable of germinating on the stigma (Morse 1987, Preston 1991, Vaughton and Ramsey 1991, Niesenbaum 1992), germinating in vitro (Shchori, et al. 1992, Beardsell et al. 1993, Lindgren et al. 1995), picking up certain stains (Bernhardt, et al. 1980, Becker and Ewart 1990, Mione and Anderson, 1992, Nyman 1992), and effective seed set following pollination (Smith-Huerta and Vasek 1984).

Exposure of pollen grains to the environment is a critical step in the reproductive cycle of higher plants. Adverse environmental conditions affect all stages of male gametophyte development

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(Mesihovic et al., 2016). Because pollen development occurs inside the anther and flowers of the mother plant, negative effects of the environment on the sporophyte are also communicated to the gametophytes (Hinojosa et al., 2018). Anther and pollen desiccation are crucial to prepare pollen for dispersal when environmental conditions are optimal for pollen survival. When the mother plant experiences stress conditions, this signal will reach the pollen grains and affect preparation and timing of pollen dispersal. Release of pollen in the environment is therefore controlled by an equilibrium between the physiological state of the sporophyte, pollinators, and the atmospheric conditions (Begcy and Dresselhaus, 2018) (Figure 1).

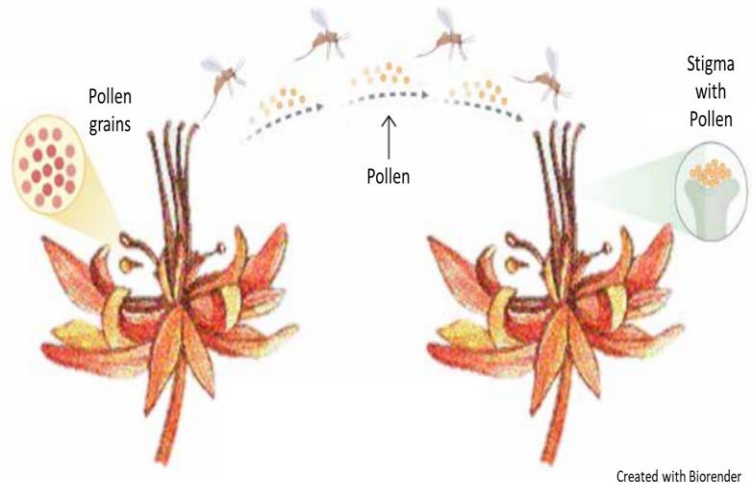

Figure 1. Main factors involved in T. cacao pollination. The tiny size and the intricate reproductive structure of the cacao flower, pollen viability and the specific kind of pollinators needed to complete the pollination process.

The cacao tree cannot produce cacao beans unless its flowers are pollinated, making pollination an essential part in the production of chocolate. In biological terms, the pollination of a plant's flowers is the transfer of pollen to the plant's ovules (to allow their fertilization. The cacao tree produces approximately 4554 ± 687 flowers every semester, but around 5% get pollinated (Gaibor, 2018). This means that 95% don't receive any or enough pollen, and is therefore aborted by the tree.

Knowledge of this different processes is key to improving quality techniques and grain germination capacity, which are related to flower biology, used to refer to germination and viability, which directly or indirectly influence the fertilization process in plants. flowers. The development of the cocoa flower is subject to genetic, environmental, pathological, nutritional factors, the alteration between these factors affects fertilization and production in cocoa plantations. The heterogeneity in the crops, can present low yields, among them it can be associated with the reproductive development of the crop, size of the fruits, number of seeds, which could be associated with pollen viability, being these an important tool in the programs of genetic improvement.

2. Materials and methods

Schematic representation of the experimental procedure is showed in figure 2.

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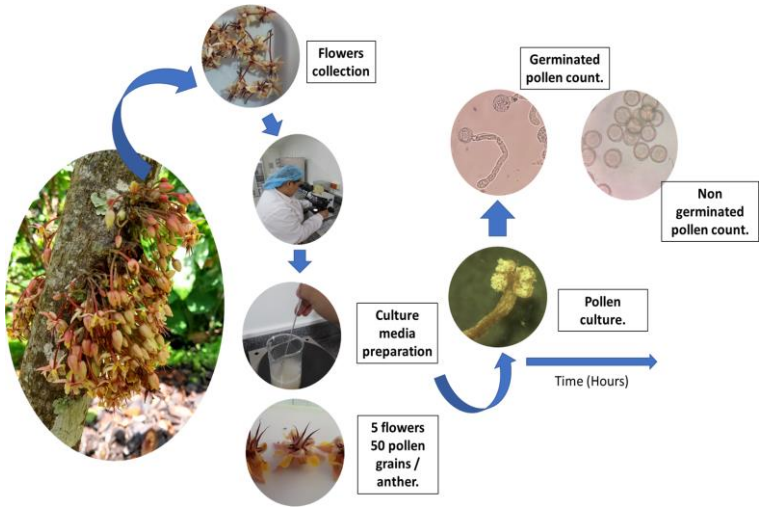

Figure 2. Schematic representation of the experimental procedure for pollen viability stimation.

Materials: To carry out the experiment, 35 cocoa clones were used, including regional materials from Colombia and introduced ones. The regional clones were selected through Participatory Varietal Selection - SVP, and later, evaluated by Fedecacao-FNC, coded as follows:: CCN 51, FSV30, FLE4, FQUIP1, FLE3, FSV80, FTA7, FSV1, FBO1, FTA4, FEAR26, FEC44, FSV153, FCHI8, FKE28, FSA20, FEC7, FEC2, FGI4, FYC2, FSV25, FMAC11, FSA11, FEAR5, FMA7, EET8, ICS1, ICS39, ICS95, FSA12, FTA2, FLE2, TSH565, FSA13, FSV41, FSV155.

Flower collection: For the development of the activity, it was necessary to collect flowers in advance of the genotypes of interest between 7:00 and 8:00 am. The flowers were transferred to the plant health laboratory of Fedecacao in San Vicente de Chucuri - Santander, for evaluation.

Culture medium: it was prepared based on agar, sucrose, B, Ca, poured into Petri dishes of 9.0 cm in diameter.

Pollen sowing: the pollen grains were sown with the help of a brush and incubated at °25C room temperature for two hours. After this time, the development of the pollen tube was observed through the stereoscope, in order to identify the pollen grains. germinated and non-germinated pollen.

Pollen count: visual stereoscopic counts were performed on 250 pollen grains taken at random per flower, 50 per anther, for a total of 1,250 pollen grains per material evaluated. The Petri dish was subdivided by means of a grid in order to sow the pollen of an anther for each square. The illumination setting of the stereoscope is very important for clear visibility of pollen grains and pollen tubes.

Germination percentage: the germination percentage was taken two hours after sowing, taking into account the emission of the pollen tube, to carry out the visual classification of viable and non-viable grains and calculate the percentage using the following formula:.

$$\text{Viability \%} = \frac{\text{Number of viable pollen grains}}{\text{Number of total pollen grains}} \times 100$$

A pollen grain is considered germinated when the pollen tube registers a length equal to or greater than the diameter of the pollen grain.

The variables evaluated are: number of pollen grains and determination of the percentage of germinated and non-germinated pollen.

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3. Results

Below are the evaluation results of 35 materials obtained from the in vitro germination of pollen under laboratory conditions. Pollen viability was evaluated by counting 250 randomly taken pollen grains per flower, 50 per anther, observing germinated and non-germinated pollen grains, determining the percentage of viable grains.

Through the evaluation of pollen germination during a period of two hours, it was possible to observe that the germination of pollen in some materials did not exceed 25%, which may be associated with the season, rainy season and temperature, since some of these materials were collected under these conditions in which the climate plays an important role in the phenological development of the plant, decreasing its viability and fruit set. Plants being sessile photoautotrophic organisms, and, therefore, must constantly adapt to environmental factors, for their development and optimal growth (Duarte., 2019).

Even when self-incompatible and self-compatible materials are found in the evaluation, this parameter does not guarantee that the pollen germination percentages are <25% or >75% respectively, since their response may be influenced by environmental conditions, nutrition, among others. .

Germination >75% ensures that the pollen grains are viable and metabolic processes are occurring between them. Another relevant factor for obtaining good results in pollen germination is the quality of the culture medium and commercial brand. The FGI 4 material showed the highest level of germination using the proposed methodology. It was possible to classify the materials evaluated according to the in vitro evaluation according to the germination capacity classified into four groups: high (>75%): FSA12, FLE3, FSV1, FTA2, FSA20, FCHI8, FGI4; intermediate-high (50-75%): FTA4, FEAR26, FSV41, FSV30, CCN51, ICS1, FLE4, FBO1; intermediate-low (25-50%): ICS39, EET8, ICS95, FSV25, FMA7, FSA13, FSV155, FLE2, TSH565; and low (≤25%): FEAR5, FSV80, FMAC11, FYC2, FSA11, FEC44, FSV153, FLE28, FEC2, FEC7, FQUIP1 (Figure 3).

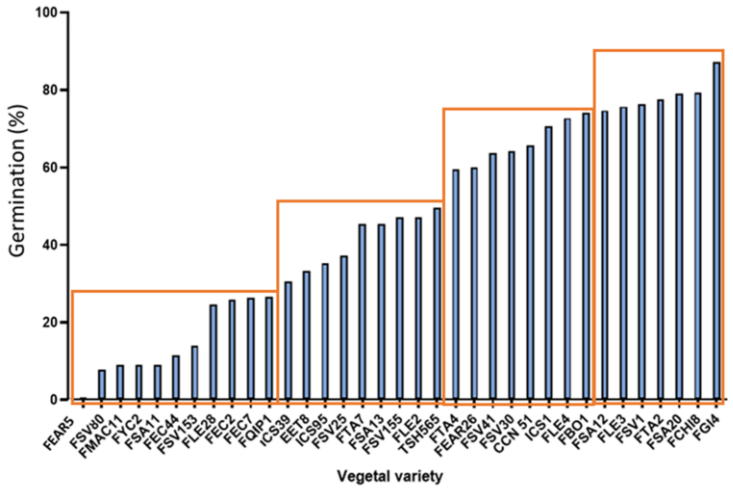

Figure 3. Classification of Colombian cacao clones regarding germination percentage after in vitro pollination.

The results allowed us to identify differences between the evaluated materials with respect to the pollen germination capacity.

The percentage of pollen germination, evaluated through the laboratory method, is reliable for its evaluation of viability and could contribute to the processes in the tests to determine the sexual compatibility of the materials.

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4. Conclusions

The regional material FGI4 showed the highest level of germination using the proposed methodology.

It was possible to classify the materials evaluated according to in vitro germination capacity into four groups: high (>75%): FSA12, FLE3, FSV1, FTA2, FSA20, FCHI8, FGI4; intermediate-high (50-75%): FTA4, FEAR26, FSV41, FSV30, CCN51, ICS1, FLE4, FBO1; intermediate-low (25-50%): ICS39, EET8, ICS95, FSV25, FTA7, FSA13, FSV155, FLE2, TSH565; and low (≤25%): FEAR5, FSV80, FMAC11, FYC2, FSA11, FEC44, FSV153, FLE28, FEC2, FEC7, FQIP1.

Taking into account the range of variability that can occur during the evaluation, it is important to define the time of flower collection, pollen sowing and the culture medium (agar) to be used, to avoid loss of quality and therefore the results. .

Finally, analyzing the information on pollen viability activity is important to identify possible factors that contribute or hinder the processes during fertilization, pollen germination, and pollen tube length.

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