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Pollen Viability in Taro (*Colocasia esculenta* (L.) Schott) in Cuba.

Yadelys Figueroa Aguila[1], Marilys D. Milián Jiménez[2], Yuniel Rodríguez García[3].

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ABSTRACT

In taro, pollen availability or variability, and the number of clones that produce inflorescences, are not worldwide problems today. However, in Cuba, the appearance of inflorescence is critical, since the high-yielding accessions are not the ones that produce the inflorescences; it rarely or never happens, but when it does, pollen is not viable in most cases. Therefore, the aim of this paper is to evaluate fertility and viability of pollen in taro accessions (*Colocasia esculenta*). The germplasm accessions of the species, preserved at the Center for Tropical Crops Research (INIVIT) were evaluated during the 2015-2016 period. Staining by aceto-carmine glycerol jelly or pollen viability test was performed to 19 accessions selected for their inflorescences in field conditions. The result was expressed as the percentage with respect to the total number of pollen grains, using a descriptor of variations of pollen viability to determine if the accessions could be used as male parents in crop breeding programs. Five of the nineteen accessions evaluated were observed to produce viable pollen, which may be used as potential male and female progenitors in the genetic breeding program by species hybridation.
Key words/: fertility, pollen, taro, *Colocasia esculenta*

INTRODUCTION

Taro (*Colocasia esculenta* (L.) Schott) is originally from southeast Asia, between India and Indonesia. The edible part of the plant is the underground corms (rhyzomes), which constitute an essential part in the diet of children and the elderly, because of their high nutritional values and properties when they are baked, fried, and mashed, or served in stews. Additionally, taro is commonly used to feed different animal species worldwide, including Cuba, as an alternative to imported foods, thus contributing to national food sustainability.

The utilization of improved clones introduced by INIVIT has contributed to increased production and technology without additional costs. However, few taro (*C. esculenta*) clones are available in Cuban agriculture today, particularly, due to the way the species is multiplied, which hinders the existence of broad sources of variability, both natural and induced. On one hand, spontaneous mutations are rare; on the other, the appearance of inflorescence is meager and unproductive with the use of inefficient pollinating agents that depend on the environmental conditions (Milián, 2016, personal communication). Therefore, the aim of this paper is to evaluate fertility and viability of pollen in taro accessions.

MATERIALS AND METHODS

[1] Agronomy Engineer, Associate Researcher, Center for Tropical Crop Research, INIVIT: geneticamc@inivit.cu
[2] PhD in Biological Sciences, Senior Researcher, Center for Tropical Crops Research, INIVIT: geneticamx@inivit.cu
[3] MSc in Sustainable Agriculture, Assistant Researcher, Center for Tropical Crops Research, INIVIT: geneticadioscorea@inivit.cu

This research was made at the Center for Tropical Crops Research (INIVIT), between December 2015 and December 2016. The clones were planted in a mulled carbonated brown soil (Hernández, Pérez, Bosch, *et al*., 2015).

The study comprised 19 accessions with inflorescence from the germplasm collection of the Center ('2000-21', 'Miyako', 'Surin', 'IND 178', 'C2-E11', 'Samoana', 'IND 231', 'IND 225', 'Takenokoimo', 'Samoa 13', 'Klang', 'Pa´akala', 'Pa´akala', 'Pauli', 'Sapapalii', 'Srisamrong', 'Saleapaga', 'Manu', 'Kluang', 'Laputara'), which were introduced from the Fiji Islands. Plots of 60 plants each were evaluated. The plants were in five rows, 0.90 m x 0.30 m from each other, separated 3m by boundary paths, and 2m between plots. Tilling was made according to the recommendations for taro (MINAG, 2008), and the field procedures established for germplasm management (Milián, Sánchez; Morales, *et al*., 2004).

Staining by aceto-carmine glycerol jelly or pollen viability test was run to evaluate pollen fertility (Marks, 1954). After 200 days of plantation, fully opened inflorescences bearing pollen were collected; 200 pollen grains were counted on the microscope slide. The grains were classified according to their shape and content. The result was expressed as the percentage with respect to the total number of pollen grains. The samples were observed through the microscope (Carl Zeiss Axiostar Plus 1169-149), with a 40x magnification.

The scale for pollen viability was used to determine if the accessions fit the standards for male parents in crop breeding programs (Table 1).

Table 1: Scale for pollen viability

No.	**Status**
1	90-100 % very high
2	60-90 % high
3	40-60 % moderate
4	≤ 40% low

The influence of some climatic factors on the appearance of inflorescence, like temperature, relative humidity, and precipitations during the crop cycle was evaluated. The data were collected at the agrometeorological station No. 326, in INIVIT.

One-way analysis of variance was made to analyze the data for Duncan's multiple ranges.

RESULTS AND DISCUSSION

All the accessions evaluated (19) produced inflorescence, but few of them (5) had viable pollen (Fig.1.). Accessions 'Miyako', 'Manu', 'Samoana', '2000-21', and 'IND 225' showed significant differences in terms of pollen viability; 'Miyako' had the highest mean (81.75%), followed by 'Manu' (71.88%). The pollen grains from each accession were fully grown, and were abundant in the samples collected. Accessions 'Samoana', '2000-21', and 'IND 225' showed low pollen viability. Similar results were reported by González, Estévez, and Castillo *et al.* (2002), who stated that pollen viability allows for reliable estimations of fertility, and they can be used in cross-incompatibility studies.

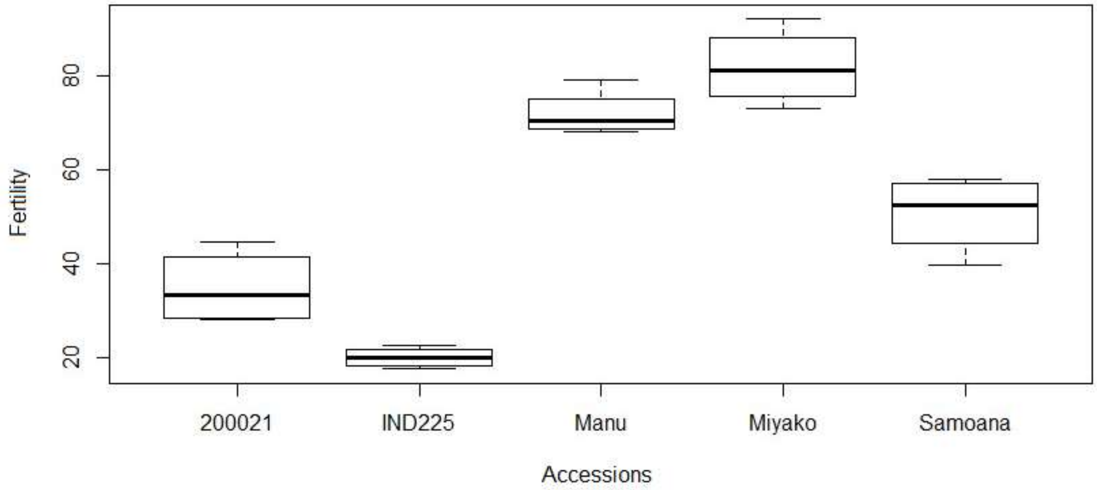

Fig. 1. Pollen viability (%) in the accessions evaluated

The presence of pollen may be considered abundant in the accessions with more than 70% pollen fertility (Miyako and Manu), and scarce for inflorescences with less than 50% (Samoana, 2000-21 and IND 225) (Fig. 2). The abundance of pollen and fertility are determining factors to increase the efficiency of the hybridation program. The viable pollen is often found in species of the Araceae family (Ramanchadran, 1978). According to Sharma and Das (1954), large quantities of viable pollen grains are expected to appear due to high percentages of normal tetrads that can fertilize mature ovules.

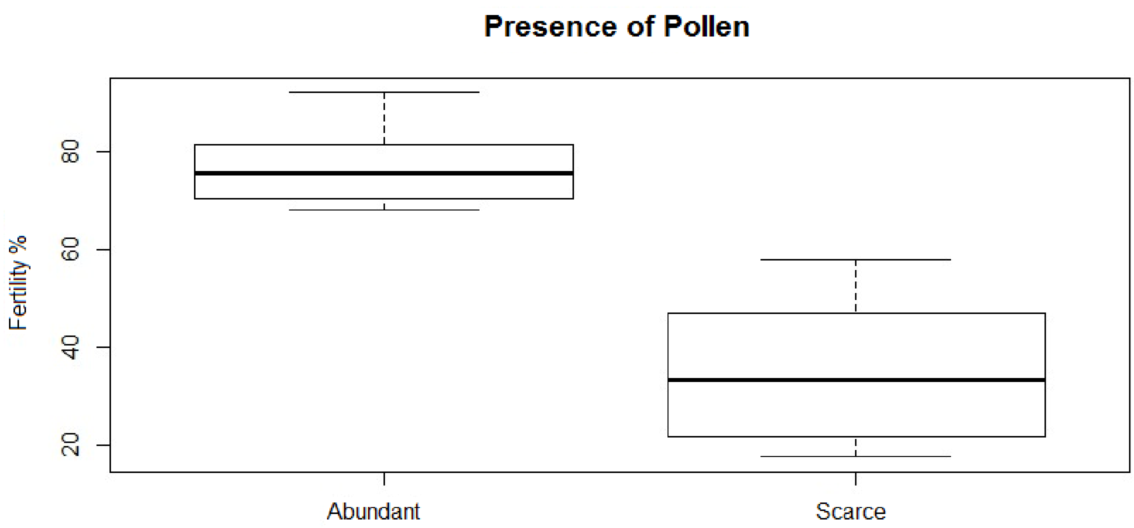

Fig. 2. Pollen fertility in the taro (*C. esculenta*) accessions evaluated

The grains of viable pollen from the accessions of 'Miyako', 'Manu', 'Samoana', '2000-21', and 'IND 225' observed through the microscope were intense red; whereas the non-viable were deformed, colorless, or light rose color (centroids) (Fig. 3). The findings of this research coincided with the criteria of Srinivasan and Gaur (2012), who noted that pollen viability can be identified by the round red grains; whereas the non-viable grains are shrunken and colorless.

Taro (*C. esculenta)* is reproduced by cross-pollination, and it is highly heterozygous. Therefore, breeders must perform a high number of hybridizations every year to detect the best heterozygous combinations for the crop (Bradshaw, 2010). It also coincided with the results achieved by Lagos, Creuci, and Vallejo *et al.* (2005), who found deformed sterile grains in a comparison study with viable pollen grains, and added that the quantity and quality of the pollen produced by one inflorescence is one of the most important aspects in evolutionary studies, and adaptation of genotypes. Hence, the determination of viability, and characterization of pollen in species is a necessary task.

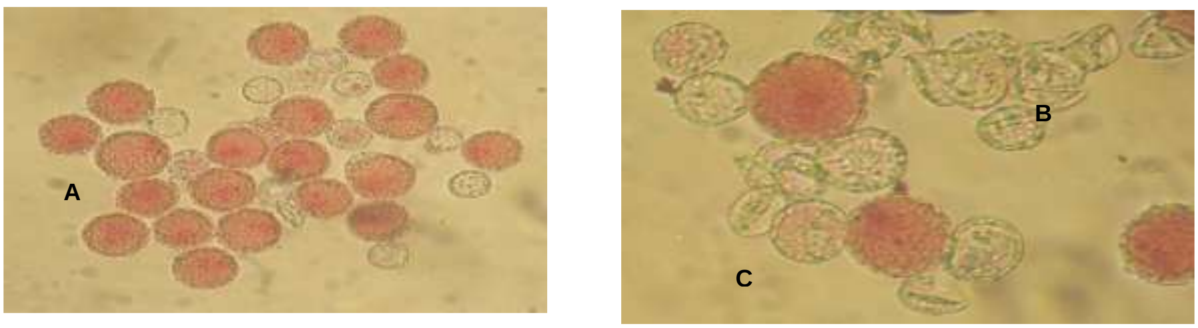

Fig. 3. Viable pollen grains of taro (*C. esculenta)* (A); non-viable deform pollen grains (B); colorless or pale rose pollen grains (C) (staining with aceto-carmine glycerol jelly at 40X).

Precipitations (Fig.4) is an important climatic factor, considering this crop demands high levels of moisture to achieve optimum growth and guarantee quality inflorescence. The monthly precipitation values were 298.7 mm (highest), 4.8 mm (lowest), and 24.32 mm (mean), throughout the crop cycle. According to Ramírez (2009), the occurrence of rainfall correlated positively to the number of species with flowers.

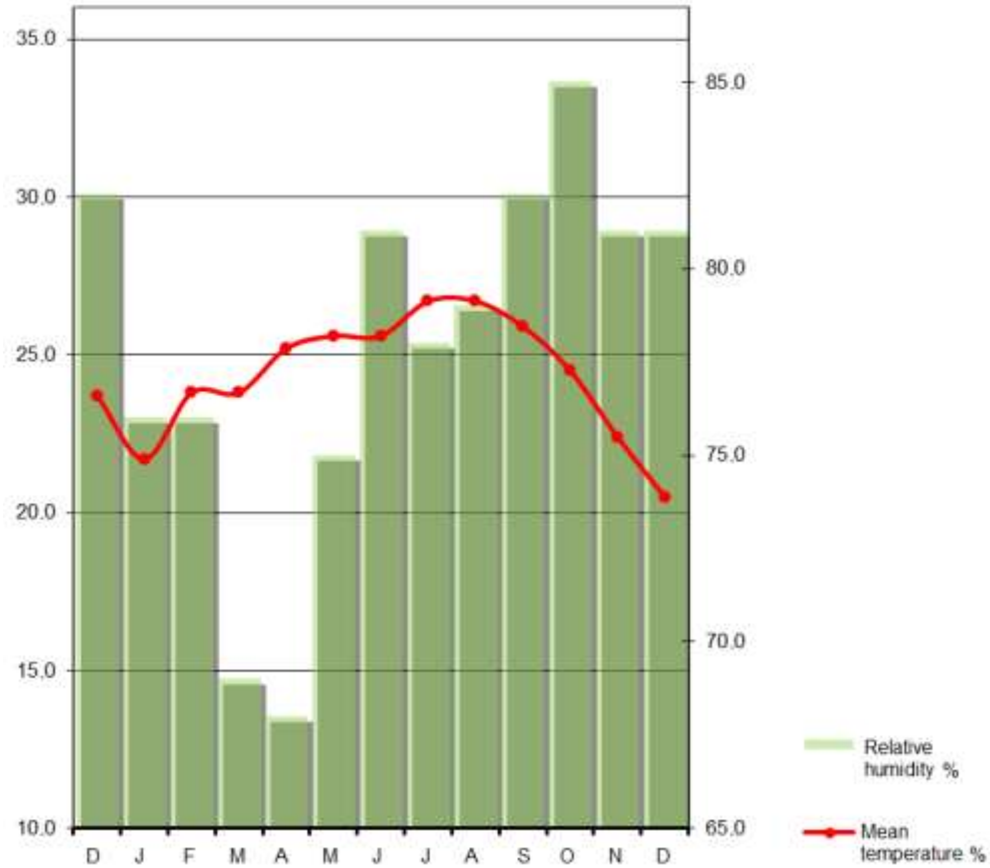

Fig. 4. Climate diagram based on the relative humidity and temperature data collected in the period evaluated.

The correlation between reproductive technology and the climatic variables associated with water availability was positive; however, correlation was negative when these variables were associated to water deficiency (Ramírez, 2009).

The values of monthly relative humidity were 85% (highest), 68% (lowest), and 93% (mean). Increased relative humidity from July to October (78-85%) coincided with the appearance of inflorescence; however, a decline (85-80%) was observed in October-November, when the crop flowering period ends.

The temperature values in the period were 26.7 ºC (highest), 21.7 ºC (lowest), and 24.32 ºC (mean). A gradual decline was produced in the air temperature (27 ºC-22 ºC) when the plant produced the inflorescence. According to Ivancic (2011), taro (*C. esculenta*) needs high temperatures to grow properly and stimulate the appearance of inflorescence, but then temperatures should decrease to ensure the production of viable pollen.

Lagos, Creuci, and Vallejo *et al*., (2005) noted that most changes in flower structure are associated to the agroclimatic conditions of the area where the plantation is located, particularly, temperature and relative humidity.

CONCLUSIONS

Five of all the accessions evaluated produced pollen, and three of the five were more than 50% viable. This study concluded that five of the nineteen accessions of taro (*C. esculenta*) that produced inflorescence may be used either as male or female progenitors.

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