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# A Pollen Study of Two Local Cassava Genotypes (*Manihot esculenta*) in Sri Lanka

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## ABSTRACT

**Cassava (*Manihot esculenta*) is a perennial starchy root crop that is widely grown in the tropical regions of the world. Pollen viability and number of pollen grains per anther are two important parameters to get successful seed set from breeding programs and to increase the efficiency of recovery of doubled haploid plants from androgenesis. This study was undertaken to test the effect of location where the plants are grown, genotype and the position of the anther within the male bud on pollen viability and pollen count. Two locations, Makandura and Gannoruwa, two cassava genotypes, *kirikawadi* and MU51 and two whorls in the male bud, inner and outer were compared using 30 male buds for each treatment. The experiment was repeated two times. There was a location effect on the number of pollen grains per anther resulting higher value in Gannoruwa (200.9) over Makandura (189.2), whereas any effect was not observed on pollen viability. Tested two genotypes performed differently on both parameters. *Kirikawadi* resulted highest pollen viability (75.6%) and number of pollen grains per anther (209.2) over MU 51 revealing a genotype effect. Any significant difference was not observed in the values of both parameters between two whorls indicating the functional similarity of the two whorls.**

**KEYWORDS:** Cassava, Pollen count, Pollen grains, Pollen viability, Staining method

## INTRODUCTION

Cassava (*Manihot esculenta*) is the sixth world food crop for more than 500 million people in tropical and sub-tropical Africa, Asia and Latin America (FAO, 2008). It is the third most important carbohydrate food source in the tropics providing more than 60% of the daily calorific needs of the population in tropical Africa and Central America (Nartey, 1978). Cassava production is constrained by factors such as limited adoption of improved varieties in some areas, limited land and lack of good quality planting material, pest and disease incidences and unreliable rainfalls (Kiwanuka and Kintu, 2004). It grows well under marginal as well as favorable conditions of soil fertility and rainfall. It tolerates a wide range of soil pH, from four to eight (Howeler, 1978).

Cassava is a monoecious with male and female flowers found on the same plant where the female flowers usually mature earlier than the male flowers enhancing out-crossing (Ng and Ng, 2002). Female flowers are located on the lower part of the inflorescence whereas male flowers are concentrated on the upper part. Size of the male flower is approximately half of the female flower. The male flower consists with ten stamens which are arranged in two whorls. Five external stamens are free and longer than inner ones and the inner stamens unite at the top in some genotypes. The pollen grains of cassava are relatively large in size and are sticky. Cassava pollen shows size dimorphism within the same genotype (Perera *et al.*, 2013), the larger grains being approximately 130 to 150 μm in diameter. In some clones, the larger grains are more abundant, whereas in other clones the smaller grains are more common (Plazas, 1991).

It is pollinated by insects mainly bees, wasp and honeybees (Hahn *et al.*, 1979; Kawano, 1980). Pollination is required in order for the plants to reproduce. Even though, cassava is mainly propagated *via* vegetative means, in plant breeding programs the resulted progenies are grown by seeds.

Viable pollen and sufficient amount of pollen grains are the prerequisites for having successful fertilization process and the reproductive success (Dafni and Firmage, 2000). Therefore, assessing these parameters is important not only in artificial pollination and breeding experiments (Stone *et al.*, 1995) but also producing doubled haploids *via* androgenesis (Perera *et al.*, 2013). Thus, the present study was conducted to assess the pollen viability and to quantify the amount of pollen grains per anther in cassava.

## MATERIALS AND METHODS

### *Location*

This study was carried out at the Department of Horticulture and Landscape Gardening of the Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila, from December 2015 to May 2016.

### *Bud Collection*

Early stage (<0.5 mm) male flower buds

were collected from two genotypes, *kirikawadi* and MU 51 from two agro ecological zones Gannoruwa and Makandura. Thirty buds from each variety and each location were collected and fixed in Carnoy's fixative solution (absolute ethanol: glacial acetic acid, 3: 1).

***Assessing Pollen Viability***

Lugol's solution prepared by dissolving 2 g of iodine and 4 g of potassium iodide in 100 mL of distilled water was used to study pollen viability. The solution was stored in a dark bottle and kept in the refrigerator until use. Flower buds were dissected under a dissecting microscope and the anthers were extracted. Viability was assessed in two anthers from each whorl in each bud having a total of four anthers per bud. Each anther was taken on to a glass slide and pollen grains were released in to a drop of Lugol's solution. Cover slips were gently placed on the extracted pollen. The pollen grains turned into black color were counted as viable pollen whereas yellow color ones were the non-viable. Experiment was repeated two times.

***Assessing the Pollen Quantity per Anther***

Sampling the anthers was done in the same as described above. The number of pollen grains was counted by releasing the pollen grains carefully from each anther into a drop of water. Experiment was repeated two times.

***Data Recording***

The slides were observed under the light microscope. A randomly selected field was observed under the 10x objective and counts were taken. Percentage of pollen viability was calculated by dividing the number of stained pollen grains over total number of grains in the microscopic field multiplied by 100. The quantity of the pollen grains per anther was determined by counting all the pollen grains released to the microscopic slide.

***Data Analysis***

Data from the experiment were statistically analyzed using SAS statistical analysis package with GLM procedure (SAS, 2009).

## RESULTS AND DISCUSSION

The pollen viability could be affected by the genotypic and the environmental factors. Two genotypes those are currently used for androgenesis induction were evaluated to find any genetic effect on the two parameters of quantity and quality of the pollen grains that are important components of reproductive biology. Materials collected under two environmental conditions were evaluated to check any environmental impact on the tested parameters.

***Pollen Viability***

Pollen viability is considered to be an important parameter of pollen quality (Dafni and Firmage, 2000). It is defined as the ability to live, develop or to germinate when conditions are favorable (Lyra *et al.*, 2011). Iodine and potassium iodide in the Lugol's solution turn the viable pollen into black color by staining the starch in the viable pollen grain.

Pollen viability of two genotypes collected from two locations, Makandura and Gannoruwa did not show any significant difference (Table 1) indicating the environmental conditions prevailed in the selected locations did not show any effect for the pollen viability. During pollen development of cassava, the concentration of total soluble sugars gradually increased in the pollen grains, reaching maximum at anthesis (Perera *et al.*, 2013). Pressman *et al.* (2002) reported that continuous exposure of the plants to high temperatures (32/26 °C) prevented the transient increase in starch concentration and led to decrease in the concentrations of soluble sugars in the pollen grains. However, any difference in starch availability was not observed in the pollen grains collected from Makandura and Gannoruwa although the temperatures ranged between 31-33 °C (Anon, 2016b) and 25–28 °C (Anon, 2016a) respectively.

**Table 1. Average pollen viability and number of pollen grains of in two different locations**

| Location | Pollen viability | Number of the pollen grains |
|---|---|---|
| Makandura | $71.88^{a} \pm 1.17$ | $189.23^{b} \pm 1.46$ |
| Gannoruwa | $69.12^{a} \pm 0.93$ | $200.85^{a} \pm 3.41$ |

*Means with the same letter are not significantly different at $P<0.05$ confident levels; $n=240$; $\alpha=0.05$*

The viability of both genotypes were considerably higher, however, a genotype difference was observed among the genotypes tested (Table 2). Viability of *kirikawadi* pollen grains (75.6%) was greater over MU 51 (63.4%). It has been reported that the pollen viability is generally high in cassava, however, with a variation among the different genotypes (Perera *et al.*, 2013).

Any significant difference was not observed between the viability of pollen grains extracted from inner and outer whorl of the cassava buds (Table 3).

**Table 2. Average pollen viability and number of pollen grains of two cassava genotypes**

| Variety | Pollen viability | Number of the pollen grains |
|---|---|---|
| MU 51 | $65.39^{b} \pm 0.10$ | $180.91^{b} \pm 1.653$ |
| Kirikawadi | $75.61^{a} \pm 1.02$ | $209.18^{a} \pm 2.89$ |

*Means with the same letter are not significantly different at P<0.05 confident levels; n=240; α=0.05*

**Table 3. Average pollen viability and number of pollen grains extracted from inner and outer whorl of male buds**

| Anther whorl | Pollen viability | Number of the pollen grains |
|---|---|---|
| Inner whorl | $70.45^{a} \pm 1.11$ | $194.64^{a} \pm 2.73$ |
| Outer whorl | $70.56^{a} \pm 1.00$ | $195.44^{a} \pm 2.64$ |

*Means with the same letter are not significantly different at P<0.05 confident levels; n=240; α=0.05*

***Quantity of Pollen Grains***

A significant difference could be observed in the average number of pollen grains per anther collected from two locations of Makandura and Gannoruwa (Table 1). Irrespective to the genotype anthers collected from Gannoruwa contained a higher number of pollen grains (200.9) over Makandura (189.2) indicating the effect of the agro ecological conditions for initial differentiation into the pollen mother cells. It has been reported that the pollen quantity in an anther is influenced by climate conditions (Hu, 2005; 1997, Ren *et al.*, 2007). Devlin *et al.* (1992) reported that the environmental condition can negatively affect to the pollen production through male function by decreasing the amount of pollen.

Comparing the two genotypes, a significant difference was resulted between genotypes (Table 2). *Kirikawadi* contained a significantly higher number of the pollen grains (209.2) over MU 51 (180.9). Perera *et al.*, (2013) reported a difference observed in three different cassava genotypes, however, the number of pollen grains per anther fall into the same range as observed in the present study. Number of pollen grains of inner and outer whorl did not show any difference (Table 3).

Based on the current results and the previous reports, Cassava produces a very low quantity of pollen grains. Being a vegetative propagated crop it may not require a natural sexual reproduction that needs a large number of pollen grains for successful mass seed production. Furthermore, having higher pollen viability few pollen grains may play a sufficient role for its reproduction. Therefore pollen quantity is correlated with fruit production for two reasons, they are; A higher quantity of pollen can enhance fertilization and competition among male gametophytes. Other is the higher number of pollen grains demonstrate a higher quantity of total inflorescences (Reale *et al.*, 2006).

## CONCLUSIONS

Results revealed that the pollen viability of the tested genotypes are different, however, the agro ecological zone did not effect on viability. However, pollen quantity was varied among the genotypes collected from different locations. *Kirikawadi* performed better in both quality and quantity indicating a genotype effect. Pollen grains in inner and outer whorls of the male buds did not show any difference in the tested parameters showing the functional similarity of the two whorls.

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