SOP24:

Standard Operating Procedures (SOP) for cross pollination using complete pollen germination media (CPGM)

	Crop: Banana **Function:** Improving plantain seed rate through use of complete pollen germination media during cross pollination	**SOP #**	IITA-BP-SOP24
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1. Introduction

The seed set rate in plantain breeding program is very low and several factors are known to contribute to this trend including pollen quality, weather conditions, agronomic practices and microenvironment at the stigma (Waniale et al. 2021). Weather conditions and microenvironment factors influence the receptivity of the stigma to pollen affecting pollen tube germination, fertilization and embryo development. Previous studies showed that use of diluted nectar and glucose improved pollen germination and seed set rates in bananas (Nyine and Pillay, 2007; Waniale et al. 2021). To improve the rate of genetic gain in the plantain breeding program, generation of large number of hybrids is necessary to increase the chances of identifying the best candidate hybrid for selection and advance into a new variety. This SOP provided an innovative approach to increase pollination success in plantain and banana breeding by applying complete pollen germination media on the stigma after pollination to facilitate pollen germination.

2. Purpose

The purpose of this SOP is to provide step-by-step procedures on the use of complete pollen germination media (CPGM) during cross pollination to improve seed set rate in plantain and banana crossbreeding.

3. Scope

The SOP covers the preparation, use and storage of CPGM.

4. Definition of terms

- **Complete pollen germination media (CPGM)**: A mixture of glucose, magnesium sulfate heptahydrate, potassium nitrate, calcium nitrate and boric acid dissolved in water.
- **Pollen:** Powdery grains produced on the male parts of flower called anthers.
- **Stigma:** The sticky female part of the flower that receives pollen grains to initiate the process of fertilization.

5. Roles and Responsibilities

The Research Technician is responsible for preparing and storage of the PGM.

The Field Assistants are responsible for application of PGM during cross-pollination.

6. Procedure/Protocols

Preparation of the PGM (Research Technician)

Requirements

- Electronic weighing balance
- Weighing boats/papers
- Spatula
- 1Litre measuring cylinder
- 500 ml mist spray bottles
- Paper towels
- Parafilm

- Water
- Glucose
- Magnesium sulfate heptahydrate ($MgSO_4.7H_2O$)
- Potassium nitrate (KNO_3)
- Calcium nitrate ($Ca(NO_3)_2$)
- Boric acid (H_3BO_3)
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Procedure

1. Place the electronic weighing scale on a flat and firm surface where there is no wind interference and power it ON.
2. Place a clean dry weighing boat or paper on an electronic weighing scale.
3. Tare off the weight of the boat or paper.
4. Using a clean spatula weigh 30 g glucose, 0.25 g $MgSO_4.7H_2O$, 0.25 g KNO_3, 0.4 g $Ca(NO_3)_2$, and 0.1 g H_3BO_3 and transfer into a 1L measuring cylinder. Clean the spatula using a paper towel after weighing each chemical.
   Note: For incomplete pollen germination media, only glucose is used.
5. Add 500ml of water to the measuring cylinder, cover with parafilm and mix by inverting the cylinder up and down until the chemicals dissolve.
6. Add water to make the final volume of 1000ml and mix to get a homogenous solution.
7. Divide the solution into two spray bottles.
8. Label the bottles with PGM as the content and the date of preparation.

1. Application of CPGM during pollination

1. After dusting the pollen on the stigmas of the female flower, shake the bottle containing the CPGM.
2. Place the bottle nozzle at ~30cm away from the pollinated hand, press the trigger to mist the stigmas three times.
3. Cover the pollinated inflorescence with the bag and proceed to the next plant.

3. Storage of CPMG before and after use

1. Store the CPGM solution at 4-10^oC if it is not used immediately.
2. If the bunches for pollination are few, aliquot the CPGM into small amounts and store the rest for up to one week. If sterile distilled water is used, the solution can be stored up to two weeks.

7. References

Nyine, M., & Pillay, M. (2007). Banana nectar as a medium for testing pollen viability and germination in Musa. African Journal of Biotechnology, 6(10).

Waniale, A., Swennen, R., Mukasa, S. B., Tugume, A. K., Kubiriba, J., Tushemereirwe, W. K., ... & Tumuhimbise, R. (2021). Seed set patterns in east African highland cooking bananas are dependent on weather before, during and after pollination. *Horticulturae*, *7*(7), 165.

8. *Annex I: In vitro banana pollen germination using complete pollen germination media*

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