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SCREENING FOR SEED FERTILITY AND PLOIDY IN *MUSA* SPP

[1]Ukwueze, C.K., [2]Oselebe, H.O. and [1]Nnamani, C.V.

[1]Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria

[2]Department of Crop Production and Landscape Management, Ebonyi State University, Abakaliki, Nigeria.

Correspondence: ukwuezecalistus@gmail.com

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ABSTRACT

Musa species (spp) comprise genotypes with varying ploidy and fertility levels which limit seed production and damper its improvement. This study was aimed at assessing the ploidy and fertility of 11 *Musa* accessions in the germplasm held at Ebonyi State University, Abakaliki, through crosses, using artificial hand-pollination to generate hybrids. The ploidy levels of the accessions were determined by assessing their chloroplast density in pairs of stomatal guard cells. Fertility was assessed by crossing, following North Carolina II Mating Design, whereby males (Calcutta 4 and PITA 14) were used to fertilise females ('Agbagba', 'Efol red', 'Efol', 'Owom', 'Numbrantor', 'Atagafong', 'Nblepaul', 'Aging' and Sh3436). For ploidy determination, chloroplast numbers of accessions were significantly different at $p \leq 0.05$, and revealed that 18.18% were tetraploid (males), 72.73% were triploid females (except 'Efol red') and 9.09% were diploid ('Efol red'). For the fertility assessment, the crosses did not generate seeds. This could be attributed to triploid nature of most accessions, which favours nondisjunction during meiosis, hereby, producing sterile gametes. To enhance seed generation in *Musa* crosses, ploidy nature should be among the basic determinants in assigning parental roles to accessions.

Keywords: Fertility; *Musa*; North Carolina; mating design; hand pollination

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INTRODUCTION

Musa species which comprise plantain and banana (Pereira and Maraschin, 2015) are acceptable food which is sold in most commercial settings, and consumed either alone or combined with other foods, in delicacies by most households. *Musa* spp are perennial with a faster relative growth rate than other fruit crops and produce fruits throughout the year (Hinge *et al.,* 2022). This all-year-round food production contributes in their ranking above other staple food crops (Nayar, 2010). Medicinally, *Musa* spp can serve as anticancer (Vijayakumar *et al.*, 2017), antioxidant (Fahim *et al.,* 2019; Oyeyinka and Afolayan, 2020), antidiabetic (Arun *et al.,* 2017), antiulcer (Krishnan *et al.,* 2014), for wound healing (Vu *et al.*, 2018), for cure of gastrointestinal tract disorders (Karaket *et al.,* 2021), for treatment of abdominal pain (Abe and Ohtani, 2013) and antidepressant (Tavakkoli-Kakhki *et al.*, 2014). Nutritionally, presence of fat, moisture, carbohydrate at about 68%, fibre and minerals such as potassium, phosphorus, nitrogen, calcium, sodium, magnesium, and traces of elements like zinc, copper, iron and minute quantity of manganese were reported through proximate analysis of its flesh, peel and peel aqueous extract (Oyeyinka and Afolayan, 2019). Other additional applications as livestock feed and compost have been recorded (Agama-Acevedo *et al.,* 2016), as well as in biorefinery (Martinez-Ruano *et al.,* 2018).

With these important attributes of *Musa* spp, it is pertinent to breed it for better yield, agronomic and physiological traits as this will boost food security. According to Brown *et al.* (2017), those *Musa* spp cultivated for consumption are primarily seedless triploids, with their seed set being extremely limited, which complicates breeding effort. These reproductive barriers discourage sexual recombination, hence, hindering plant improvement. Ortiz (2015) and Ortiz and Swennen (2014) reported that difficulty in banana improvement is also compounded by parthenocarpy, reduced male fertility in some cultivars, low seed viability, irregular meiotic behaviour, long generation times and diverse genomic configurations. The limited progress that has been achieved in banana breeding has occurred through crossbreeding approaches to produce hybrids (Brown *et al.,* 2017). Since

Musa spp are clonally propagated in nature, some of the newly produced hybrids are usually accompanied by undesirable growth and fruit characteristics. Some successes have been recorded on crosses which produced desirable hybrids. In National Agricultural Research Organisation (NARO), Tushemereirwe *et al.* (2015) reported that tetraploids (AAAA) were synthesised from a cross between EAHBs (AAA) and the wild-seeded, fertile male parent Calcutta 4 (AA) which serves as a source of resistance to multiple pests and diseases. The selected hybrids were resistant to black sigatoka with an increased bunch size.

This study was aimed at testing for seed fertility of *Musa* accessions in germplasm, through crosses using artificial hand-pollination to produce improved hybrid.

MATERIALS AND METHODS

The experiment was carried out at Ebonyi State University (EBSU), Abakaliki, Ebonyi State, Nigeria. Ebonyi State is in Southeastern Nigeria, and bears coordinate of latitude 6° 19' N and longitude 8° 6' E. It has high temperature and rain fall with mean monthly temperature of 27˚C (Njoku *et al.*, 2015). Rainfall starts in April and ends in October, with a completely dry period between November and April. The rainfall is patterned bimodal, and peaks in the months of July and September. The total annual rainfall ranges from 1500 mm to 2000 mm, with a mean of 1,800 mm.

Eleven accessions, which include eight landraces (‘Agbagba’, ‘Efol red’, ‘Efol’, ‘Owom’, ‘Numbrantor’, ‘Atagafong’, ‘Nblepaul’ and ‘Aging’), two hybrid varieties (PITA 14 and SH3436) and Calcutta 4 obtained from EBSU germplasm were used for the experiment.

Ploidy Determination of Field-Established Accessions based on Chloroplast Density in Stomatal Guard Cells

The density of chloroplast in stomatal guard cells of the accessions studied was determined following Compton *et al.* (1999) with modification. Second fully expanded leaf samples of accessions from *Musa* germplasm were collected. Three evenly distributed sections were made along the middle part of the leaves. Forceps were used to remove the lower epidermis of the sections before being transferred to a microscope slide, where they were immersed in a drop of 100 % iodine solution. Cover glasses were used to cover the preparation and allowed to stain for 5 min. The entire slides were observed under bright field illumination using a LeitzDiaplan binocular microscope at 400x magnification. Ploidy level of each accession was estimated by counting the number of chloroplasts per guard cell pair of 20 stomata of leaf sections. Accessions in germplasm were grouped following Krishnaswami and Andal (1977), which grouped genotypes of *Gossypium* spp into different ploidy levels with an interval of four chloroplasts between ploidy levels. The total number of chloroplasts was assessed, analysed and the mean recorded. Analysis of variance (ANOVA) and Least Significance Difference (LSD) analysis were carried out at $p \leq 0.05$ with the help of statistical software SPSS version 25.

Screening for Seed Fertility

An experimental population was generated by crossing the accessions using North Carolina II Mating Design (Table 1). Here, a group of *Musa* accessions used as males (Calcutta 4 and PITA 14) were individually crossed to a group of accessions used as females (Agbagba, Efol red, Efol, Owom, SH3436, Numbrantor, Atagafong, Nblepaul and Aging). Pollination was carried out following the method by Ortiz *et al*. (1998). Here, pollen of the male parents was collected around 7:30 a.m. from male flowers, which was firstly covered with bags sown with calico material to prevent pollen from other accessions contaminating the pollen of interest due to animal and wind activities. Likewise, inflorescences of the female parents were bagged until the last female flower was pollinated to avoid natural crossing with unidentified pollen sources. Artificial hand-pollination was done between 7:30 and 10:30 a.m. in exposed female flowers by rubbing a cluster of male flowers onto the female flowers. Pollinated bunches were tagged, indicating parents and date of initial pollination.

At maturity, after about 90 days from the day of first pollination, bunches were harvested and stored for four days in ripening rooms, after which the fruits were assessed for presence of seeds.

Table 1: Possible crosses of the *Musa* accessions using North Carolina II Mating Design

Female group	Male Group	Female x Calcutta 4	Female x PITA 14
Agbagba	Calcutta 4	Agbagba x Calcutta 4	Agbagba x PITA 14
Efol red	PITA 14	Efol red x Calcutta 4	Efol red x PITA 14
Efol	-	Efol x Calcutta 4	Efol x PITA 14
Owom	-	Owom x Calcutta 4	Owom x PITA 14
Sh3436	-	SH 3436 x Calcutta 4	SH 3436 x PITA 14
Numbrantor	-	Numbrantor x Calcutta 4	Numbrantor x PITA 14
Atagafong	-	Atagafon x Calcutta 4	Atagafon x PITA 14
Nblepaul	-	Nblepaul x Calcutta 4	Nblepaul x PITA 14
Aging	-	Aging x Calcutta 4	Aging x PITA 14

RESULTS

Ploidy Levels of *Musa* Accessions

The result of chloroplast count was significant at $p < 0.05$. The ploidy distribution showed that the chloroplast in guard cell pairs of the accessions range was wide (Table 2). Least mean value of 9.45 ± 0.61 was observed for 'Efol red' with very high values of 16.00 ± 0.73 and 16.70 ± 0.92 observed for Calcutta 4 and PITA 14, respectively (Table 2). Means of 'Numbrantor', 'Aging', 'Agbagba' and 'Atagafong' were comparatively similar, ranging from 11.30 ± 0.57 to 11.80 ± 0.41, while means of 'Nblepaul', 'Efol' and 'Owom' showed similarity, ranging from 12.30 ± 0.47 to 12.90 ± 1.17. SH3436 had a mean value of 13.30 ± 1.72. The results showed that 'Efol red' was diploid, 'Agbagba', 'Efol', 'Owom', SH3436, 'Numbrantor', 'Atagafong', 'Nblepaul' and 'Aging' were triploid while Calcutta 4 and PITA 14 were tetraploid.

Table 2: Ploidy distribution of accessions through chloroplast count

Serial Number	Accession	Mean ± Standard deviation	Ploidy level
1	'Efol red'	9.45 ± 0.61^{b}	2X
2	'Numbrantor'	11.30 ± 0.57^{a}	3X
3	'Aging'	11.55 ± 0.51^{a}	3X
4	'Agbagba'	11.60 ± 1.76^{a}	3X
5	'Atagafong'	11.80 ± 0.41^{ad}	3X
6	'Nblepaul'	12.30 ± 0.47^{cd}	3X
7	'Efol'	12.65 ± 0.88^{c}	3X
8	'Owom'	12.90 ± 1.17^{cf}	3X
9	SH3436	13.30 ± 1.72^{ef}	3X
10	Calcutta 4	16.00 ± 0.73^{g}	4X
11	PITA 14	16.70 ± 0.92^{h}	4X

Mean values were significantly different at $p \leq 0.001$. Least significant difference was determined at 0.05. X represents ploidy. Means with the same superscripts are not significantly different

Fertility of *Musa* Accessions

The crosses carried out using North Carolina mating design (Table 3) did not generate seeds.

Table 3: Result of crosses of the *Musa* accessions using North Carolina II Mating Design

Female x Calcutta 4	Number of seeds generated	Female x PITA 14	Number of seeds generated
'Agbagba' x Calcutta 4	0	'Agbagba' x PITA 14	0
'Efol red' x Calcutta 4	0	'Efol red' x PITA 14	0
'Efol' x Calcutta 4	0	'Efol' x PITA 14	0
'Owom' x Calcutta 4	0	'Owom' x PITA 14	0
SH3436 x Calcutta 4	0	SH3436 x PITA 14	0
'Numbrantor' x Calcutta 4	0	'Numbrantor' x PITA 14	0
'Atagafong' x Calcutta 4	0	'Atagafong' x PITA 14	0
'Nblepaul' x Calcutta 4	0	'Nblepaul' x PITA 14	0
'Aging' x Calcutta 4	0	'Aging' x PITA 14	0

DISCUSSION

This study assessed the accessions for seed fertility through crosses with the aid of hand- pollination. This is due to the need to improve *Musa* spp, a feat which has proven too difficult to achieve. According to Brown *et al.* (2017), the greatest constraint to banana genetic improvement is the limited production of viable seeds due to polyploidy and female sterility, among other factors. Various ploidy levels result from continuous natural crossing between wild species and the effect of the environment (Sumardi and Wulandari, 2010). For a successful breeding programme in *Musa* spp, production of viable seeds through hybridisation is very critical. Ploidy level influences fertility of *Musa* spp (Chandra Das *et al.*, 2018). Knowledge of ploidy level in *Musa* accessions is vital because breeding success is affected by ploidy (Suman *et al.*, 2012). There is a great challenge in recognising the ploidy status of *Musa* accessions. Tang *et al.* (2010) reported that chloroplast count is an effective method for identifying ploidy.

The crosses carried out using North Carolina II mating design did not generate seed, even though the same mating design has been used in other crops to generate progenies. According to Agaba *et al.* (2021), same design was used to carry out crosses on *Pachyrhizus spp* and studies were carried out on F_3 generation of the offspring. Even for groundnut, Oppong-sekyere *et al.* (2019) used the same design to generate offspring which they studied. The absence of seed generation could be attributed to the tetraploid nature of accessions which served as male parents, because they have less probability to produce fertile gametes when compared to diploids. Therefore, tetraploid status of Calcutta 4 and PITA 14 may have contributed to the non-generation of seeds by the crosses, should the pollen used during pollination be probably sterile. Similarly, sterile gamete could also be produced by other triploid accessions used as female. Edible triploid bananas are invariably seed-sterile (Shepherd, 1954). Triploidy is one of the best ways to obtain seedless cultivars (Ahmed *et al.*, 2020). Report by Frost and Soost (1968) showed that triploidy results as unbalanced meiosis, leading to male and female sterility, which promote the production of seedless fruits, besides parthenocarpy (Ollitrault *et al.*, 2008; Navarro *et al.*, 2015). Nondisjunction leads to production of infertile gametes with irregular chromosome content, resulting in banana and plantain fruits without seeds.

Although seeds were not produced in this study, Brown *et al.* (2017) reported that through crossbreeding process which involved hybridisation, limited progress has been made in *Musa* breeding. An auto-tetraploid banana (AAAA) has been generated from similar crosses (Tushemereirwe *et al.*, 2015). Also, Noumbissie *et al.* (2016) reported segregation of the endogenous banana streak virus (eBSV) sequences in the hybrid derived from crosses between the tetraploid hybrid CRBP 39 (+eBSV) and the AA male parent Pahang (−eBSV), from which resulted triploid eBSV-free offspring. This work which did not generate hybrids used tetraploids as male parents; the previous work generated hybrids, by using diploids as male parents (Tushemereirwe *et al.*, 2015; Noumbissie *et al.*, 2016).

CONCLUSION

The ploidy level of plants contributes to their fertility. This informed the analyses of the ploidy levels of the *Musa* accessions at EBSU field germplasm bank. From ploidy distribution, the males were tetraploid and eight female accessions were triploid, hence, they are prone to producing sterile gametes. Such crosses wherein triploids are used for crosses rarely lead to fertilisation; therefore, seed generation is not guaranteed. To achieve productive crosses among accessions, ploidy nature should be the basis for assigning parental roles across the accessions.

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