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IN-SITU METHOD OF SCREENING PARENTAL FERTILITY OF *MUSA* SPECIES

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ABSTRACT*:* *Conventional plant breeding is the development or improvement of cultivars using conservative tools for manipulating plant genome within the natural genetic boundaries of the species which is aimed at producing plant that are superior in terms of quantity and quality than the pre-existing ones. However, the success of conventional plant breeding depends completely on the fertility status of the two parent plants involved in the hybridization program. Plantain and Banana are monoecious, sterile and parthenocarpic which leads to the production of fruit without seeds. The genetic breeding of plantain and Banana through hybridization is limited by the occurrence of sterility in most cultivars resulting in low production or absence of seeds. Studies are needed to better understand the processes involved in both fertility and sterility. Cultivated Musa species are seedless, Seed set after pollination is an indication of fertility of both male and female parents which can be used to develop hybrids of commercial varieties and wild relatives. The main objective of the present research which was investigated at the Teaching and Research Farm of the Rivers State University, Port Harcourt, Nigeria to identify the fertility status of seven (7) USTPX plantain hybrids which includes USTPx/01/01, USTPx/01/02, USTPx/01/03, USTPx/01/05, USTPx/01/06, USTPX/01/07 and KM5 using in-situ method of screening for fertility. Different interspecific crosses were made. Result indicates that seven (7) species were fertile apart from USTPX/01/07 as they produced seeds after such different interspecific crosses. Out of the seven cultivars pollinated, six cultivars produced a total of 1748 seeds as female parents except from USTPx/01/07 which do not produce neither seeds nor pollen grains. Out of 1748 seeds, the highest numbers of seeds (1346 and 379 seeds) were obtained from USTPx/01/02 and USTPx/01/01 with a mean of 134.6 and 37.9 as female parents respectively in which USTPx/01/02 shows significant difference (P>0.05) from the other cultivars. The highest and the least number of seeds (1076, 412 and 2 seeds) were obtained using pollens from USTPx/01/01, USTPx/01/02 and USTPx/01/06 as male parents respectively*

KEYWORDS: pollination,*in-situ*,fertility, hybridization, hybrid-seeds.

INTRODUCTION

Plantains and bananas (Musa sp.) are perennials which grow to produce generations of ratoon crops. They represent the world's second largest fruit crop with an annual production of 129,906,098 metric tons. They rank as the fourth most important global food commodity after rice, wheat and maize in terms of gross value of production. They are originated from South east Asia. Plantain and Banana are reliable sources of energy and fibre. Plantain and banana store carbohydrate reserves in the form of starch and sugar respectively. Bananas provide a starch staple across some of the poorest parts of the world in Africa (with consumption up to 400 kg per person per year) and Asia, while dessert bananas are a major cash crop in many countries (FAOStat, 2007).

Cultivated bananas and plantains are giant herbaceous plants within the genus Musa. Bananas (including plantains) grown today worldwide are cultivars derived from the interspecific hybridization of *Musa acuminata* and *Musa balbisiana*. There are over a thousand Musa cultivars embracing a wide genetic diversity, which reveal their multiple origins through hybridization from these two ancestral diploid species (Heslop Harrison and Schwarzacher, 2007). Inter- and intra-specific hybridizations have led to parthenocarpic diploid and triploid cultivars. However, the difficulty for generating hybrids and sterility of many cultivars showing parthenocarpy makes Musa cross-breeding challenging.

The main objective for the genetic improvement of banana is to breed sterile triploid hybrids through the recombination of fertile cultivars and species that meet farmers' needs and consumers' demands (Ortiz and Vuylsteke, 1996). Although sterility and parthenocarpy are im-portant factors that contribute to the desirability of banana fruits, sterility has impeded progress in breeding programs. Through natural somatic (vegetative) mutation, hybridization, and selection over many thousands of years, considerable genetic variability has arisen within the cultivated bananas, giving rise to more than 1000 varieties worldwide.

Musa breeding started about one century ago in Trinidad, aiming to develop disease- and pest-resistant cultivars for the export trade. Even so, all banana export cultivars grown today are still selections from somatic mutants of the group Cavendish and have a very narrow genetic base (Perrier *et al*., 2011). As noted by the late Dirk R. Vuylsteke, "A broad-based, improved Musa germplasm with pest/disease resistance will be a major component to achieve sustainable production of this vegetatively propagated, perennial crop. Such germplasm can be produced through conventional cross-breeding, enhanced by the utilization of innovative methods for the introduction of additional genetic variation. Also, the increased use of molecular markers will accelerate the process of recurrent selection of improved Musa germplasm and, hence, facilitate the development of new hybrids. The prospects of banana and plantain breeding are unlimited and increased efforts will at once initiate a new phase of *Musa* evolution" (Ortiz, 2001).

MATERIALS AND METHODS

The experiment was conducted in the Teaching and Research Farm of the Rivers State University, Port Harcourt. Rivers State has a landmass of 19420 sq.km, and lies within tropical rainforest zone of Nigeria, located in latitude 4°-6°N and longitude 6°-8°E. Rivers State has a landmass of 19420 sq.km. The rainfall pattern is essentially bimodal with peaks in June and September, while in April and August there are periods of lower precipitation (Ukpong, 1992). The long rainy season is between April and October. The dry season lasts from November to March with occasional interruption by sporadic down pours. Annual rainfall is average of 2000mm to 4500mm (Anderson, 1967).

The mean monthly temperature ranges between 28^0C and 33^0C, while the annual monthly minimum is between 20^0C and 23^0C. The highest temperatures are experienced during the months of December through March and coincide with the overhead passage of sun (Enwezor, *et al*., 1990).

The project site is located between latitude 04.510N and longitude 07.010E with an elevation of 1.8m above sea level (FAO, 1984). The site has been used for breeding of *Musa* species for several years. The site is acquired by the University for various agricultural operations which has a well drain soil with secondary vegetation that is made up of weeds, annuals and perennial grasses. It is completely a secondary vegetation consisting of broadleaf weeds such as *Chromolena odorata, Calosopogum mucunoides, Centrosema pubuscens, Sida acuta* (Broom weed), wild shrubs and some grasses in abundance particularly *Pennisetum purpureum* (Elephant grass).

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At maturity of the plant, i.e. +90 days after shooting pollen grains of fleshly exposed male flowers were excised on daily basis within the morning hours of 7- 10 in the morning using ladder to reach the tall plants. Different interspecific crosses were carried out. At the end of the period, seeds were produced in some of the pollinated plants which indicate their inherent fertility to be selected as male and female parent any breeding program though; fertility level varies from variety to variety. The rate of genetic improvement in cultivated Musa depends on the reproductive fertility (Vuylsteke *et al*., 1993b).

RESULTS AND DISCUSSION

Hybrid Seed Set

The *Musa* accessions used for the experiment were seven (7) USTPx plantain hybrids. These putative plantain and banana hybrids of the USTPx series developed by the Department of

Crop/Soil Science of Rivers State University represent Du-novo genetic resources that could be utilized in any breeding and genetic research (Davids, 2001, Orluchukwu and Ogburia, 2014, Ogburia, 2017), they include USTPx/010/01, USTPx/01/02, USTPx/01/03, USTPx/01/05, USTPx/01/06, USTPx/01/07 and KM5 which were growing *in-situ*. During ripening and consequent squashing of the fingers, it was discovered that all hand pollinated stands produced seeds expect from USTPx/01/07, though some were slightly fertile such as USTPx/01/05, USTPx/01/06 and KM5. Among other factors, seed set in *Musa* was highly influenced by the genetic composition of the cultivar. Thus some cultivars are sterile while others are slightly fertile and, they set seeds less frequently (Stover and Simmonds, 1987).
Seed set percentage were calculated based on each cultivar. Formula for the calculation is: No. of seeds produced per cultivar /Total no. of seeds x 100.

Table 1. Seed production by different cultivars

Cultivar	No. of Mats Per Cultivars	No. of Mats Pollinated	% Seed Set	No. of Seeds obtained	No. of Mats Producing Seeds	No. of Ripe Bunches
USTPx/01/01	10	9	8.87	87	4	10
USTPx/01/02	10	5	88.79	871	5	10
USTPx/01/03	10	10	1.83	18	4	10
USTPx/01/05	10	6	0.10	1	1	6
USTPx 01/06	10	2	0.10	1	1	2
USTPx 01/07	10	10	0	0	0	10
KM5	10	10	0.31	3	3	10
TOTAL	70	52	100	981	18	58

The highest numbers of seeds were obtained from USTPx/01/02 in which more than two seeds were found in all fingers shown below;

Fig 1: Opened finger of USTPx/01/02 cultivar showing seeds (brown and black)

While the others produced only few seeds per bunch. The black spots on the finger in fig 1 indicate the presence of seeds resulting from the artificial hand pollination. Swennen and Vuylsteke working at IITA have identified 12 females fertile French plantains although they concluded that fertility varies from variety to variety in unpredicted manner. From the table 1, USTPx/01/01 and USTPx/01/02 are fertile, USTPx/01/03, USTPx/01/05, USTPx/01/06 and KM5 are slightly fertile and USTPx/01/07 is sterile. Low seed set in triploids are attributed to embryo mortality, deranged embryo - endosperm relations, irregular growth of pollen tube in styles of female flower and great variations in potency existing between pollens of different cultivars. Pollens of the different cultivars were collected which were used for interspecific crosses to ascertain fertility and cross compatible between and among the different species. Cultivated edible *Musa* species produce fruits through vegetative parthenocarpy, diploids like USTPx/01/01, USTPx/01/02 and some tetraploids are prone to produce viable seeds due to even chromosomal number. The seeds obtained were counted and subjected to floatation test to sort out the viable seeds from the non-viable ones. It was also discovered that USTPx/01/07 does not produce visible pollen grains which is pistil morphological characteristics. Pistil morphological characteristics are related to female fertility of a cultivar. Style length, ovary length, number of ovules and the diameter of the stigma are significantly affects the female fertility of a cultivar, exhibiting a negative correlation. Cultivars with long styles and ovaries have less chance of setting seeds after hand pollination. However, these cultivars do not produce pollen, but only bare stamens called staminoids. This infers male sterility and lack of pollen results in the absence of seeds in their fruits. Due to the difficulty of genetics and the sterility of the crop, the development of new varieties through hybridization, mutation or transformation was not very successful in the 20th century (Heslop-Harrison and Schwarzacher, 2007).

Nevertheless, some plants produce seeds after hand pollination which became an indispensable incidence to be used in breeding programs for the production of varieties with important traits (Ortiz and Vuylsteke, 1995).

Hybridization

Hybridization as a method is the mating of different species of plants with dissimilar genotype which is aimed at producing hybrids that may or may not genectically different from paternal and maternal parents in a cross. Pollination was done at anthesis of both female and male flowers with pollen from fertile flowers being brushed against the stigma of the female flower. This is an essential process for the fertilization of the flowers and for the development of seeds. The most effective time of pollination according Simmonds, 1952 is between 7.00 a.m and 10.30 a.m. Pollen grains are structures that house the male gametophyte generation of seed plants and are the vehicles through which the microsporangium is carried to the female gamete. The pollen grain has a very complex structure that may be reflective of specific species' functional adaptations. The ability of fertilization is considered to indicate the ability of the pollen grain to perform its function of delivering the sperm nuclei to the embryo sac and finally fertilization after compatible pollination. Hence the seed set is dependent on pollen viability (Oselebe *et al*., 2014). Nevertheless, some plants produce seeds after hand pollination which became an indispensable incidence to be used in breeding programs for the production of varieties with important traits (Ortiz and Vuylsteke, 1995). Hand pollination shown in fig 2

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Fig 2 Hand pollination

Large inflorescence size, visual conspicuousness, floral odor and copious nectar production in Musaceae have been identified as classic examples of adaptations for cross-pollination. In conformity with Simmonds, 1952, both male and female flowers of the different USTPx plantain hybrid series are nectiferous and male flowers produce sticky pollens which are dispersed by a variety of natural pollinators. In the female flowers of plants, nectaries are limited to the upper part of the ovary above the locules. In male flowers with aborted ovaries, the nectaries entirely occupy the aborted ovaries.

It was discovered that in diploids such as USTPx/01/01, the pollen grains deposited on the stigma developed pollen tubes faster than in triploids such as USTPx/01/02. Four hours after pollination, pollen grains were not visible on the stigma anymore, which indicates that germination was complete. In triploids, pollen germinated on the stigma, but apparently it didn't penetrate into the style, which suggests the lack of some sort of stimulus to direct the pollen tube, or the presence of a physical barrier.

According to Simmonds, (1952) both *M. balbisiana* and *M. acuminata* are considerably differentiated. In these two species, pollination among closely related clones can produce significantly higher number of seeds than self-pollinated plants. In-line with this, pure-line crosses were made in the seven (7) *Musa* accessions and few seeds were obtained from such crosses while significantly higher number of seeds were obtained from self-compatible interspecific crosses.

Plate 1: Plantain seeds from the cross between USTPx/01/01and USTPx/01/02 cultivars

Stigma receptivity

Specific cell-cell (intercellular) interactive events (interactions) are important components in pollination and fertilization processes. In a successful pollination, shortly after pollen capture, the interaction between a pollen grain and the stigma surface leads to hydration and germination of the pollen grain (Heslop-Harrison, 1975). As a result, pollen grains were collected and possible crosses were made in the morning hour which is the most receptive period of the stigma and to circumvent the eventual dehydration and death of pollen grains due to high environmental temperature.

CONCLUSION

Conventional plant breeding approach of *musa* accessions is time consuming yet it is essential in most developing nations which is geared towards promoting food security for the ever increasing population through the production of high quantity and quality produce. The success of breeding plantain and banana should be measured by the extent to which new *Musa* cultivars are used profitably and sustainably by farmers. Access to bred germplasm of plantain and banana is therefore exceeding vital, especially in a crop with a few active breeding programs but with a great demand from farmers worldwide.

Both male and female sterility prevail predominantly in *Musa* clones, it is essential to screen the cultivars for male and female fertility before selecting them as potential seed parent in any hybridization programme. This is done by crossing female flowers of the selected cultivars with pollen grains of a fertile diploid male. The relatively high female fertility of plantains is a key component of the rapid breeding advances at IITA (Vuylsteke *et al*.,1997)

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