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IN VITRO RESCUE OF ZYGOTIC EMBRYOS FOR THE ENHANCEMENT OF HYBRID PLANTAIN (*MUSA*, AAB GROUP) PRODUCTION.

Harry[1], G. I., Ebong[1], U. U. and Vuylsteke[2], D. R.

ABSTRACT

[1]*Dept. of Crop Science, Faculty of Agriculture, University of Uyo, Uyo.*
[2]*International Institute of Tropical Agriculture, Onne, Rivers State. E-mail: hdrgamaliel@yahoo.com*

Two sets of experiments were carried out to determine the seed production capacity of the French plantain cv. 'Bobby Tannap' and to determine the best age to obtain the optimum germination of plantain hybrid embryos. Results obtained showed that there was a decrease in the number of ovules per hand. The number of ovules per hand decreased from hand 1 to hand 8. The finger length of plantains decreased from the first hand to the eighth hand. The total number of seeds extracted was highest from the 60-65 days old bunches (1123 seeds). While the lowest number of seeds (794 seeds) were extracted from the 50-55 days old bunches. Germination percent (%) decreased as the age of embryos increased. The germination percent was highest (10%) for the youngest embryos (50-55 DPP) and lowest (2.5%) for the embryos that were 70-75 days old. The seed success rate decreased as the embryos grew older. The plant recovery rate was highest for the younger (50-55 DPP) embryos (79%) and lowest for the older embryos 70-75 DPP (20%). Embryo production was not affected by year. However, significant differences (P>0.5) were found between the months of the year with the highest number of embryos extracted in the month of February (320 embryos) as against 25 embryos extracted in the month of July. Monthly variation in seed set was significant. Seed set was high in the early part of the year with a peak in February (450 seeds) and lowest in September (30 seeds) per bunch.

Key words: Black sigatoka, Bobby Tannap, Calcutta 4, Parthenocarpic.

INTRODUCTION

Plantains (*Musa* spp. AAB group) have played important roles in the history of human civilization are economically important crops in tropical Africa, America and Asia (Robinson, 1996). The green fingers are reported to contain 29% starch on a fresh fruit basis (Mateo, 1993, INIBAP,1988). Plantains have traditionally been considered easy to cultivate in low input production system while offering consistent high yields (Valmayar *et al*, 1990). Over the last 10-15 years, international concern for plantains has increased through two developments: a growing appreciation of their role in nutrition and household food security over a significant part of the globe, and the threat posed by the introduction to Africa of black sigatoka disease, sometimes referred to as black leaf streak (Mateo, 1993). The black sigatoka disease caused by the *ascomycetes* fungal pathogen (*Mycosphaerella fijiensis*, Morelet) is generally considered to be the most serious threat to plantain production in the sub-Saharan Africa (IITA, 1992). It attacks the leaves, causing severe and extensive necrosis and thereby reducing yields by 30-50% (Stover, 1983).

Black sigatoka leaf spot disease can be controlled with fungicides but these are too expensive for most small-holders and subsistent farmers. Moreover, the toxicity of the chemicals constitutes a health hazard since plantains are generally cultivated around the homestead. Commonly cultivated plantains are parthenocarpic allotriploids with low female fertility (Tomekpe, *et al*., 1998). These attributes together with a restricted level of variability in the accessible *Musa* gene pool have led to the recalcitrance of this crop to orthodox breeding techniques. Natural seed set is rare due to the absence of male fertile clones. However, even artificial pollination with viable pollen results in extremely low seed set as compared to the number of ovules in each inflorescence. (Stozky *et al*., 1962). These impediments to plantain breeding have prompted interest in the use of in vitro techniques which have the potential to increase the efficiency of recovering hybrid plantains. The aim of this study was therefore to overcome two basic limitations to plantain improvement viz: low seed germinability and post germination developmental failure.

MATERIALS AND METHODS

This study was conducted at the Tissue culture laboratory and fields of the International institute of Tropical Agriculture, high rainfall station, Onne, Rivers State, Nigeria. Crosses were made between the French plantain cv. 'Bobby Tannap' which is a local cultivar from Cameroun as the female parent and the wild AA clone 'Calcutta 4' which is a wild seeded non-edible diploid banana (*Musa acuminata* spp. *Burmaniccoides*) from Burma (De langhe

and Devreux,1960) as the male parent due to it's black sigatoka (BSR) resistance. Emerging inflorescences were bagged to avoid contamination with alien pollen. Pollinations were then carried out at anthesis between 7.30 and 10.30 am for each of the experiments. The flowers were hand pollinated.

Experiment 1.The experiments were carried out in two parts. The first set of experiments were regarded as preliminary – 1) Evaluation of the seed efficiency of the female parent 'Bobby Tannap' The objective of this experiment was to determine the seed production capacity of the French plantain cv. 'Bobby Tannap'. Immature bunches of the French plantain cv. 'Bobby Tannap" were harvested as soon as all the hands had opened and taken to the laboratory. The plantain 'hands' were detached from the main stalk of the plantain bunch and the fingers (fruits) were also detached from the hands by the use of ordinary kitchen knives. The middle finger in each hand was selected for hands that had odd numbers, while hands with even numbers had the finger nearest to the middle selected and used for the experiment. The two ends of the fruit were cut off before the fruit was sliced open longitudinally. The fruit length was measured with the aid of a ruler, before the ovules were counted. The ovules were recognized as the tiny black dots placed in double rows and running the entire length of the plantain fruit.

The treatments being compared were the middle finger of hands 1,2,3,4,5,6,7 and 8. The control was the middle finger of the 1st hand. The experiment was repeated seven times. The experimental design was completely randomized design. The number of ovules in each hand were counted .The length of each fruit was measured in centimeters using a measuring tape. The number of fruits per hand was counted and the total number of hands was recorded.

Experiment 2: The second sets of experiments were carried out to determine the best age at which embryos could be 'rescued'. Pollinated bunches were harvested from the field plots at 50-55 days post- pollination, 60-65 DPP, 70-75 DPP and 80-85 DPP. Vuylsteke and Swennen (1992) had reported that the plantain cultivar 'Bobby Tannap' attains full maturity at 13 weeks. The age of bunches was calculated as the number of days from the first day of pollination to the days of harvest of bunches. The number of fingers per hand of each bunch was recorded. Bunches were then placed in a ripening room and ripened with acetylene. After 4 days of ripening, the fruits were squashed in a locally fabricated hand press and the seeds extracted. Hard and black seeds were selected for the experiments, while the soft and empty seeds were discarded as bad seeds. The hard seeds were those containing the whitish powdery endosperm and embryo while the soft seeds were the seeds lacking the endosperm and embryo (empty seeds).

Selected seeds were washed in 70% ethanol for 1 minute and disinfected for 15 minutes in 1% silver nitrate (AgNo3). One drop of teepol (detergent) was added. Disinfection was followed by rinsing in sterile distilled water 3 times. The seeds were viewed with a stereomicroscope and cracked longitudinally with the aid of alcohol sterilized scalpels and the embryos extracted. Excised embryos of different ages were placed one each in test-tubes containing the Murashige and Skoog (1962) media and incubated in a growth chamber. The chamber had a mean temperature of 26^{o}C. The temperature in the chamber was cooled and controlled by an air-conditioning unit. Cool-white fluorescent tubes of 40w provided artificial light. A photoperiod of between 12-16hrs light was maintained by the use of time switches.

The treatments were embryo ages: 50-55 DPP, 60-65 DPP, 70-75 DPP and 80-85 DPP. The design of the experiment was a completely randomized design. The embryos that were 80-85DPP were used as the control since it has been established that plantains mature at that time (Vuylsteke and Swennen, 1992) and the embryos also matured by then. The number of germinated embryos was counted and the percent germination was taken. Other observation recorded were the number of embryos of a particular age. The number of seeds containing endosperm and embryos and seeds lacking endosperm and embryos from each treatment were recorded. The seeds were graded, on a scale of 1-5 using the presence of embryos and endosperm as criteria. The experiment was repeated 17 times and each repetition was regarded as a replicate. The chi-square test was used to analyse the data and X^2 homogeneity test was used for the mean separation.

RESULTS AND DISCUSSION

Number of ovules per finger

There was a significant decrease in the number of ovules per finger from hand 1 to hand 8 (Fig.1). The rate of decrease was not the same for all the hands. There was no significant difference in the number of ovules between 1st,2nd, and the 3rd hands as they all contained about 300 ovules each. The number of ovules in the 4th and 5th hands were almost equal (about 230 ovules each), while the number of ovules in the 8th hand was about 50% (150 ovules) of the number of ovules in the 1st hand. There was a decrease in the number of ovules per finger, from the older and more mature 1st, 2nd and 3rd hands to the more distal hand 4, 5, 6, 7 and 8th hands. The 7th and 8th hands were the younger and most recently opened hands. It would appear that fertilization took place in the older fingers of the older hands after artificial pollination was carried out in the older hands than in the younger hands. Moreover, post- fertilization developmental failure as noted by Stozky et al (1962) is responsible for the decreasing number of ovules in the distal fingers. Hence, the number of ovules found in the fingers of older hands

was more than that of younger hands. Stozky et al (1962) made a general observation that artificial pollination with competent pollen resulted in extremely low ovules and seed set but did not indicate the trends in the age of the fingers or hands.

Total number of ovules per hand

The number of ovules per hand significantly decreased from hand 1 to hand 8. The rate of decrease was not the same for all the hands (Fig. 2). The highest number of ovules was in hand 2.The total number of ovules in hands 1,2,3,4, and 5 were not significantly different. The smallest number of ovules was found in hand 8. The total number of ovules in the hands followed the same trend as in the fingers. However, it was difficult to find a plausible explanation for the higher number of ovules in the second hand than the 1st hand, except probably to assume that the artificial pollination was more successful in the second hand than in the first.

Finger length of immature hybrid plantain

The finger length of the hybrid plantain decreased from the first to the eight hand (Fig.3) There was no significant difference in the finger length between the 1,2,3, and 3rd hands. The 7th hand had the shortest finger length, while the 5th and the 6th hands had equal finger lengths. The average finger length decreased from the 1st hand to the 8th hand. This could be due to the fact that the fingers in the first hand formed and filled out while photosynthetic surface of the leaves of plantain were still active. The decrease in finger length of the distal fingers could be as a result of the fact that they were formed after the photosynthetic surface has been destroyed by the black sigatoka disease. Resulting in less food manufactured and stored.

Number of ovules per centimeter of finger length

The number of ovules per finger length did not follow any definite trend (Fig. 4). The number of ovules per finger length was lowest in the 1st hand. The 4th and 5th hands had the highest number of ovules per finger length. Monthly variation in the number of embryos extracted from the Bobby Tannap x Calcutta 4 cross. Embryo production was not affected by year (Fig. 5). However, significant differences were found between months of a year. The total number of embryos of all ages (50-55DPP, 60-65DPP, 70-75DPP, 80-85DPP) produced in a month were pooled together. Figure 5 illustrates the pattern of monthly variation of embryo extracted for the two years. The number of embryos extracted was very high in the early part of the year (with a peak in February), but declined to a very low level in May and reached a second high point in November – December. The same pattern held true for the two years under study.

The results obtained indicated that season influenced the number of embryos extracted. This agrees with the findings of Vuylsteke et al (1993). The number of embryos extracted correlated with monthly weather variation as measured by rainfall, temperature and relative humidity. This pattern was repeated yearly with only slight variation. Furthermore, the highest number of embryos extracted was in February and the second peak was in the November-December periods. The January-February and November-December periods are very dry periods with low rainfall and high temperatures which is characteristic of the dry season. This suggests that pollination made during dry season is favourable to the production of embryos.

Monthly embryo and seed success rate (%)

The two years data on embryo culture of plantain seeds obtained after triploid x diploid cross was analysed. Seed success (percentage of seedlings germinated over total seed produced) and embryo success, percentage of seedling germinated over total seeds containing embryos were not affected by the year effect ($P>0.05$). However, a significant monthly variation was observed (Figs.6 and 7) for both seeds success ($P>0.05$) and embryo success ($P<0.05$). The interaction between year x month was significant ($P>0.05$) only for seed success.

Monthly variation in seed set in the plantain hybrid

Seed production was not affected by year. However, significant differences were found between months of a year. The total number of seeds produced in a month was pooled together irrespective of age. Figure 8 illustrates the pattern of monthly variation of seed production (average over 2 years). Seed set was very high in the early part of the year (peak in February) then declined to a very low level in May and reached a second peak in November – December. The results obtained indicate that season influenced seed sets (seed production). Seed production in the Bobby Tannap x Calcutta 4 cross was analysed over a period of two years. The seed set pattern was correlated with monthly weather variation as measured by rainfall, evaporation, air temperature at Onne. Significant correlations were found between maximum relative humidity and seed set for Bobby Tannap X Calcutta 4 whereas the variation in seed set was negatively associated with changes in total rainfall and maximum relative humidity and positively correlated with average relative humidity

Some parameters of the seed and embryos of hybrid plantain progenies

The highest number of seeds (1123) (Table 1) was extracted from the 60-65 days old bunches while the lowest number (794) were extracted from the 50-55 days old bunches. There was no significant difference in the number of seeds extracted from the older bunches of 70-75 and 80-85 days. The percentage of embryos excised from the given number of extracted seeds was highest for the 70-75 days (49%) and only 38% of the seeds from the 50-55 days old embryos produced embryos. There was no significant difference between the number of embryos extracted from the 80-85 and 60-65 days old seeds. On the average, thirteen seeds per bunch were extracted from

the control, 80-85 days old embryos and seventeen seeds which was the lowest number was extracted from the 70-75 days old bunches. Eighteen seeds were extracted from each bunch of the 60-65 and 50-55 days old bunches.
However, the highest number of progenies (plantlets) were recovered from the immature embryos of 50-55 days old and the least number of progenies were recovered fro the 70-75 days old embryos. 14 plantlets which is almost 50% the number of plantlets recovered from the youngest embryo age were recovered from the 80-85 days old embryos which was the control.

Effect of embryo age on germination of embryos

(a) Germination percent of embryos

The germination percent decreased as the embryo age increased. The rate of decrease was not the same for all the ages (Fig. 9). The germination percent was highest for the youngest (50-55 DPP) embryos. However, the germination rate was lowest for 70-75 days old embryos, there was no significant difference between the germination rate of embryos of 60-65, and 80-85 days old embryos which was the control.

(b) The seed success rate

The seed success rate (Fig.10) decreased as the embryos grew older. The rate of decrease was not the same for all the ages. The seed success rate was highest for the youngest age (50-55 DPP) and lowest for the 70-75 days old embryos. However, there was no significant difference between the seed success rate for the 60-65 and 80-85 days old embryos.

(C) Plant Recovery rate (%)

The plant recovery rate (Fig.11) decreased as the embryo age increased. The rate of plant recovery was highest for the youngest age (50-55 DPP) and lowest for the 70-75 days old embryos. There was no significant difference between the plant recovery rate for the 60-65 and 80-85 days old embryos.

The result that the younger the embryos, the higher the germination rate (%) agrees with the finding of Mathias et al, (1990) on *Cuphea paucipetala.*

Since a large percentage of the older seeds (41% of the 80-85 days old seeds, 70-75 day – 32% and 60-65 days – 40%) contained no endosperm, it means that the development of the endosperm stops after 50-55days. Even where the seeds lacking endosperm contained embryos as most of them did, the embryo would be rendered unviable as result of starvation due to the absence of endosperm, which is the source of nutrients. This obviously contributed to the low germination rate of the older embryos of 60-65 days, which had no endosperm or had endosperm but had seeds rendered unviable due to the absence of endosperm.

However, the immature embryos that were rescued before 55 days had a higher germination rate (10%) because they were rescued before the disintegration and subsequent abortion of the embryos. According to Raghavan (1986) embryo failure is associated with retarded growth of the endosperm and a deficiency in the development of the conducting elements within the seed. The consequent abnormal distribution of nutrients in the seed leads to failure of the embryo to complete full development. This is based on the frequent observation that triploid by diploid crosses in the AAB *Musa* plantain group having many embryoless seeds have normal endosperm while those containing an embryo, the endosperm invariably degenerates. It could be as a result of the difficulty encountered in the meiotic division or gamete formation when triploid by diploid crosses is involved.

CONCLUSIONS

This study has shown that the younger the embryos, the higher their viability and the older the embryos the lower their viability. The results confirm this fact since the 50-55 days old embryos produced a germination rate of 10%, 60-65 days old embryos had a germination rate of 4%, 70-75 DPP recorded a germination rate of 2.5% and 80-85 DPP had a germination rate of 4%. From the results obtained, it indicates that embryos extracted from immature bunches 50-55 days were more viable than embryos extracted 80-85 days after pollination. It is therefore recommended that plantain bunches for embryo culture should be harvested when the bunches are 50-55 days old. This has proven to be the best embryo rescue age after this age, the embryo looses viability. Furthermore, rescuing embryos at this age has the added advantage of conserving the resources that would have been spent on maintaining the plantains in the field for the extra 30-35 man-days.

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Table 1: Some yield characteristics of the four ages of the hybrid plantain progenies

Embryo Age	No. of bunches	Total no. of seeds	% embryos excised	No. of seeds per bunch	Total no. of progenies recovered
80-85 DPP	45a	858a	40.6b	19a	14c
70-75 DPP	45a	828a	49.0a	17c	10d
60-65 DPP	45a	1123a	42.2b	18b	19b
50-55 DPP	45a	794a	38.0c	18b	30a

Within columns, means followed by the same letter are not different.

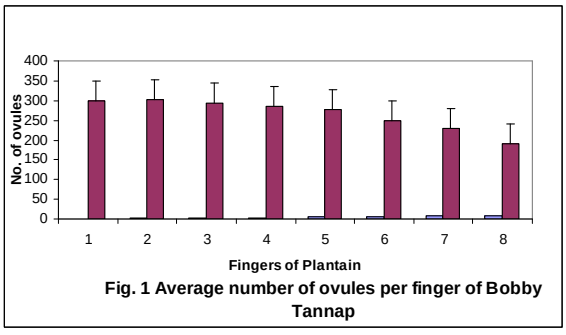


Fig. 1 Average number of ovules per finger of Bobby Tannap

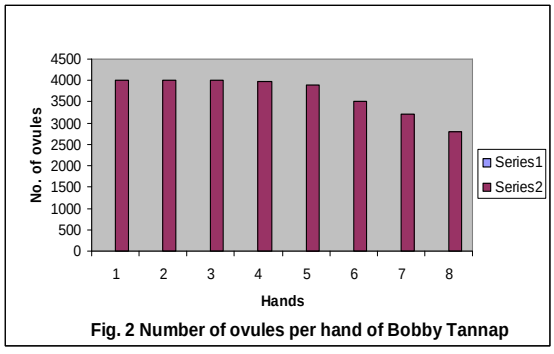


Fig. 2 Number of ovules per hand of Bobby Tannap

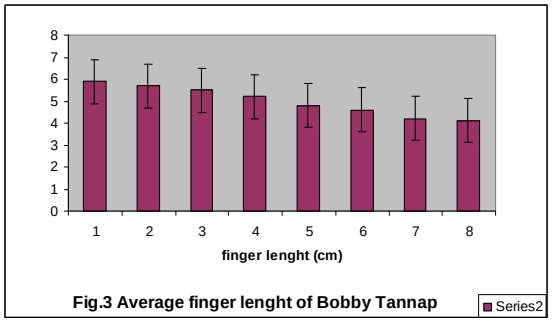


Fig.3 Average finger lenght of Bobby Tannap

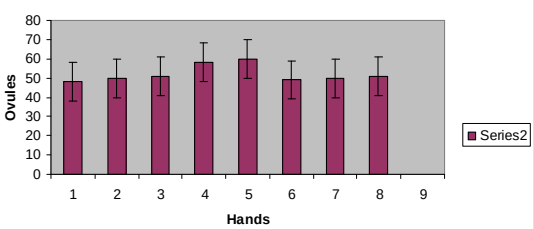


Fig. 4 Number of ovules per centimetre of finger of bobby Tannap

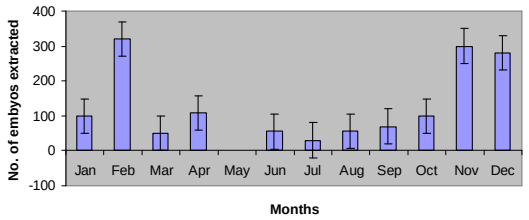
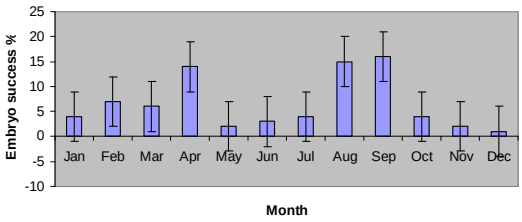


Fig.5 Monthly variation in the number of embyos extracted



Fig, 6 Monthly embryo germination rate of in vitro res. hybrid embryo

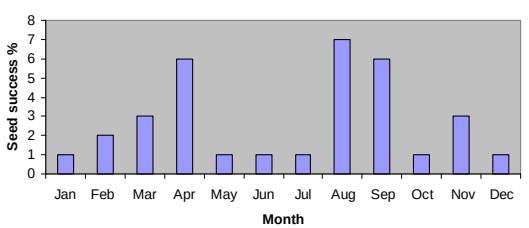


Fig. 7 monthly seed success of the in vitro res. hybrid (average for 2yrs.

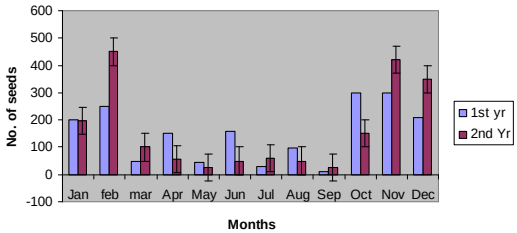


Fig.8 Monthly variation inseed set over 2 years

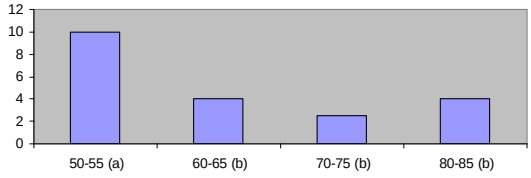


Fig. 9 Effect of embryo age on germination. Ages followed by diff. lett. were signif. diff. at 0.01 probability level(x2 test for homogeneity freq. distribution

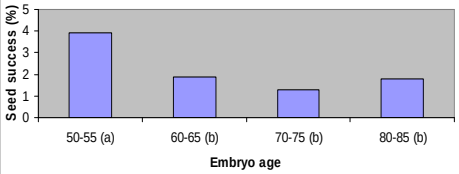


Fig. 10 Seed success rate of hybrid plantain embryos. Ages followed by diff. lett.in bracket were signif. diff. at 0.01 probability level (X2 test for homogeneity freq. distribution

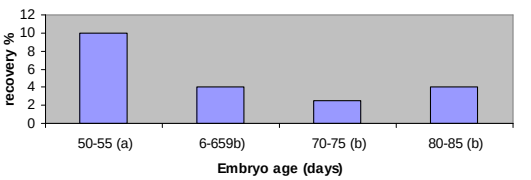


Fig.11 Plant recovert rate of hybrid plantain embryos. Ages followed by diff. lett. in bracket were signif. diff. at 0.01 probability (x2 test for homogeneity freq. distribution