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# Performance of micropropagation-induced off-type of East African highland banana (*Musa* AAA - East Africa)

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## 1 SUMMARY

**Tissue culture derived off-type plants with both good and poor field performance have been reported in banana and researchers have viewed the variants as a new source of genetic variability for crop improvement. In vitro micropropagation of East African highland banana (Musa - AAA East Africa) cv.' Uganda' resulted in high incidence of off-type plants. This study was conducted to evaluate the performance of the in vitro derived off-type banana in comparison with the in vitro micropropagation (MP) derived normal banana and conventionally propagated (CP) banana with no tissue culture history in its ancestry as controls. The evaluation of the off-type was carried out in 2005/2007 at Sokoine University of Agriculture based on number of days to plant flowering and fruit maturation, yield, fruit quality and shelf life. Results showed that the off-type banana produced significantly ($P < 0.05$) higher yield with bigger bunches and fruits of 52.2 t/ha, 21.1 kg per bunch and 125.3 g per fruit compared with 40.7 t/ha, 16.5 kg and 109.5 g of the MP derived normal banana and 45.7 t/ha, 18.5 kg and 118.3 g of the CP derived banana, respectively. The off-type fruits were significantly ($P < 0.05$) firmer with higher dry matter content of 12.4 kg/cm$^2$ and 33.7 %. The firmness and dry matter content of the MP derived normal banana were 8.5 kg/cm$^2$ and 20.0 %, and those of the CP derived banana were 8.9 kg/cm2 and 21.1 %, respectively. The off-type fruits had significantly ($P < 0.05$) longer shelf life of 17 days compared with 7.2 and 7.0 days of the MP and CP derived normal banana, respectively. However, the off-type banana was constrained by a significantly ($P < 0.05$) delayed flowering by one month and maturation by two months compared with the true-to-type banana. The observed agronomically desirable characters of the off-type banana underscore the potential of tissue culture technology as an alternative strategy for creation of genetic variability and improvement of East African highland banana.**

## 2 INTRODUCTION

Cultivated banana is propagated vegetatively using field-collected suckers (Vuylsteke, 1989). This method leads to inadequate sucker production for one banana stool can hardly produce 8 - 10 suckers per year (Macias, 2001). Moreover, field-collected suckers can easily facilitate the transmission of pests from old to new banana plantations (Maerere *et al*., 2003). *In vitro* micropropagation is widely applied for mass production of pest-free banana suckers (Banerjee and De Langhe, 1985; Talengera *et al*., 1994). Tissue culture processes such as explant preparation and growth regulators used to induce regenerative competence are stressful to plant cells (Benson, 2000; Cassells and Curry, 2001). The tissue cultured induced stress can alter the plant genetic regulation and the defect caused by such stress is referred to as somaclonal variation or tissue culture-induced variation (Kaeppler *et al*., 2000; Sahijram *et al*., 2003). Somaclonal variation is unfavourable to the clonal fidelity of *in vitro* regenerated planting materials (Lee, 2003).

A wide range of somaclonal variation has been reported and some researchers have viewed the variation as a new tool for increasing genetic variability for crop improvement (Trujillo and Garcia, 1996; Vuylsteke, 2001; Nwauzoma *et al*., 2002). For instance, *in vitro* derived off-type of

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true horn plantain had more fertile flowers and out-yielded the true-to-type plantain (Vuylsteke, 2001). Conversely, Smith *et al.* (1999) reported on an *in vitro* derived off-type of AAB banana cv. 'Lady Finger' with slow plant growth, and reduced bunch and fruit size. Tissue culture-derived off-type with slow plant growth, late flowering and low yield has also been reported in African plantain cv. 'Agbagba' (Vuylsteke, 2001).

## 3 MATERIALS AND METHODS

**3.1. *Description of study area and plant materials*:** East African highland banana (*Musa* – AAA East Africa) cv. 'Uganda' with no micropropagation history in its ancestry was *in vitro* micropropagated at Sokoine University of Agriculture (SUA) according to Maerere *et al.* (2003). The *in vitro* suckers were planted in May 2003 at SUA for evaluation of field performance in comparison with conventional suckers. Sokoine University of Agriculture lies at an altitude of 525 m above sea level and experiences annual rainfall and temperatures of 700 - 900 mm and 16 – 34 °C, respectively. Four off-type plant stools were accidentally detected in the field in April 2004 based on visual observation. The off-type plants were multiplied *in vivo* to increase the number of suckers for their subsequent performance evaluation.

**3.2. *Experimental design*:** The setup of the experiment was a randomised complete block design with three replications. The treatments were the off-type banana and *in vitro* micropropagated (MP) normal banana and conventionally propagated (CP) banana with no tissue culture history in its ancestry. Each replication consisted of plot of 10 plants.

**3.3 *Crop establishment and management*:** Planting holes had dimension of 100 x 100 x 100 cm and were spaced at 300 cm between plants and 400 cm between rows. The holes were filled up with 20 litres of farmyard manure mixed with top soil. Suckers of the off-type, MP and CP derived banana were planted in the holes in May 2005. After the establishment, the crop received appropriate management such as irrigation during the dry spell, weeding, desuckering to maintain three suckers per stool, farmyard manure application, removal of old and diseased leaves. The performance evaluation of the crop was carried out up May 2007. Mature banana bunches were harvested and taken to the Plant Postharvest Laboratory. In the laboratory, the average minimum and maximum temperatures ranged from 25 to 31 °C and relatively humidity from 60 to 70 %.

*In vitro* derived East African banana cv. 'Uganda' exhibited high incidence of off-type plants in the field. Desirable tissue culture-induced variation could be useful for the improvement of cv. 'Uganda'. The objective of this study was to evaluate the performance of the *in vitro* derived off-type banana cv. 'Uganda' based on number of days to plant flowering and fruit maturation, yield, fruit quality and fruit shelf life.

**3.4 *Data collection and analysis:*** Data on number of days to flowering, fruit maturation and bunch weight were collected using 20 plants per replication according to Swennen and De Langhe (1985). Fruit maturation or harvest stage was determined visually based on loss of fruit cross-section angularity (Samson, 1986). The numbers of hands per bunch, fruits per hand and fruit weight were recorded according to Swennen and De Langhe (1985). Data on the number of fruits per hand and fruit weight were taken from the proximal second hand of the bunch because this hand exhibits low variability (Ortiz, 1997).

Twenty fruits per replication were used to determine fruit shelf life, dry matter content, fruit pulp firmness and total soluble solids (Brix). The shelf life of mature green fruits was scored as the number of days from the date of bunch harvest to the date when 50 % of the fruits changed their colour to yellow. On the other hand, the shelf fruit life of yellow ripe fruits was recorded as the number of days from harvesting to the date when 50 % of fruits were considered unmarketable for dessert consumption. Fruit pulp firmness, dry matter and soluble solid content were measured using fruits in the proximal second hands of the bunch. Fruit pulp firmness was determined using penetrometer (David Bishop Instruments) and fruit dry matter content was measured according to Ferris (1993a). Total soluble solids of fruit pulp were measured using digital refractometer (A. Krüss Optronic DR 5000). Fruit maturation level was calculated as a ratio of total soluble solid content to dry matter content multiplied by 100.

Data analysis was performed using 'SPSS 15.0 computer software (SPSS, 2006). The data were subjected to analysis of variance ($P < 0.05$) and multiple means comparison was performed based on Tukey honest significant difference (Tukey-HSD) test ($P < 0.05$) (Zar, 1997).

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## 4 RESULTS AND DISCUSSION.

**4.1 *Plant developmental cycle:*** The off-type banana had significantly ($P < 0.05$) longer developmental cycle with delayed plant flowering and fruit maturation than the MP and CP derived banana plants (Table 1). *In vitro* derived off-types with increased apparent juvenility and slow plant growth have been reported in African plantains, potato and grape vines (Cassells *et al*., 1991; Harding *et al*., 1996; Nwauzoma *et al*., 2002). This apparent juvenility has been associated with an alteration in plant developmental programme as a result of induction of *in vitro* regenerative competence (Harding *et al*., 1996). It is hypothesised that the alteration in plant developmental programme is under the control of cytosine DNA methylation (Kaeppler *et al*., 2000; Cassels and Curry, 2001). Moreover, the fruits of the off-type banana delayed to mature possibly due to either slow starch accumulation or increased yield. Vuylsteke (2001) reported delayed fruit maturation in tissue culture derived African plantain off-type with bigger bunches.

**4.2 *Yield and yield components:*** The off-type banana significantly ($P < 0.05$) produced higher yield with bigger bunches and fruits compared to the MP and CP derived normal banana (Table 1). The high yield of the off-type banana was possibly due to increased carbohydrate accumulation, bunch and fruit weight. High yielding *in vitro* derived banana off-types have been reported in true horn plantain (Vuylsteke, 2001). Bunch and fruit weights in polyploidy banana are under the control of epistatic gene interactions (Ortiz and Vuysteke, 1993).

**Table 1**: Plant developmental cycle, yield and yield components of the *in vitro* induced off-type banana

| Variable | Off-type banana | MP banana | CP banana |
|---|---|---|---|
| Number of days to flowering | $293.0^{b} \pm 6.0$ | $263.6^{a} \pm 10.0$ | $262.2^{a} \pm 8.0$ |
| Number of days to maturation | $417.0^{b} \pm 3.0$ | $354.5^{a} \pm 6.0$ | $350.0^{a} \pm 5.0$ |
| Fruit yield (t/ha) | $52.2^{b} \pm 10.0$ | $40.7^{a} \pm 8.0$ | $45.7^{a} \pm 6.6$ |
| Bunch weight (kg) | $21.1^{b} \pm 5.6$ | $16.5^{a} \pm 6.1$ | $18.5^{a} \pm 4.9$ |
| Number of hands per bunch | $9.5^{a} \pm 0.2$ | $7.6^{a} \pm 0.2$ | $7.4^{a} \pm 0.2$ |
| Number of fruits per hand | $20.4^{a} \pm 0.5$ | $18.5^{a} \pm 0.1$ | $19.9^{a} \pm 0.2$ |
| Fruit fresh weight (g) | $125.3^{b} \pm 4.6$ | $109.5^{a} \pm 5.9$ | $118.3^{a} \pm 6.7$ |

Mean bearing the same superscript letter within the row are not significantly ($P < 0.05$) different according to Tukey-HSD test. ± SE: standard error of the mean.

**4.3 *Fruit physico-chemical properties and shelf life:*** The fruits of the off-type banana were significantly ($P < 0.05$) firmer with higher dry matter content and had longer shelf life (Table 2). Bugaud *et al*. (2006) reported a positive correlation between fruit firmness and dry matter content. The dry matter content of the off-type plants of 33.7 % was comparable with that of the plantains of 36 – 37 %, but higher than that of the AAA dessert banana of 23 – 29 % (Ferris, 1993a). The high fruit firmness and dry matter content increase fruit resistance to mechanical damage and shelf life (Ferris, 1993b; Vuylsteke, 2001).

**Table 2**: Physico-biochemical properties and shelf life of the *in vitro* derived off-type banana fruits

| Variable | Off-type banana | MP banana | CP. Banana |
|---|---|---|---|
| Mature fruit shelf life (days) | $7.0^{a} \pm 2.0$ | $4.0^{a} \pm 1.8$ | $4.5^{a} \pm 2.1$ |
| Ripe fruit shelf life (days) | $17.0^{b} \pm 2.1$ | $7.2^{a} \pm 1.8$ | $7.0^{a} \pm 2.1$ |
| Mature fruit pulp firmness ($kg/cm^2$) | $12.4^{b} \pm 0.2$ | $8.5^{a} \pm 0.2$ | $8.9^{a} \pm 0.2$ |
| Mature fruit dry matter content (%) | $33.7^{b} \pm 0.1$ | $20.0^{a} \pm 0.2$ | $21.1^{a} \pm 0.2$ |
| Mature fruit soluble solids (%) | $3.6^{a} \pm 0.2$ | $4.0^{a} \pm 0.1$ | $4.2^{a} \pm 0.1$ |
| Fruit maturation level (%) | $10.6^{a} \pm 0.6$ | $20.5^{c} \pm 0.4$ | $20.2^{c} \pm 0.4$ |

Mean bearing the same superscript letter within the row are not significantly ($P < 0.05$) different according to Tukey-HSD test. ± SE: standard error of the mean.

The long shelf life of the off-type fruits is an important attribute taking into consideration that most dessert banana fruits have shelf life of hardly seven days with high postharvest loss of up to 50 % (Aked *et al*., 2000). Being climacteric, the ripening of banana fruits is delayed by treatments with 1-methylcyclopropene and gibberellin (A4A7) (Jiang *et al*., 1999; Sisler and Serek, 2000). Alternatively, ripening of banana fruits is slowed down by packing them in polyethylene bags containing ethylene-absorbing vermiculite blocks (Sisler and Serek, 2000).

## 5 CONCLUSION

The *in vitro* derived off-type banana cv. 'Uganda' produces high yield with bigger bunches and fruits, and longer fruit shelf life. However, the delayed plant flowering and fruit maturation are the major agronomic constraints of the off-type banana. The findings on agronomically desirable characters of the off-type banana underscore the potential of tissue culture technology as an alternative strategy for creation of genetic variability and improvement of East African highland banana. Further studies are required to evaluate the acceptance of the off-type as dessert banana by farmers, traders and consumer.

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