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Reduction in genome size and DNA methylation alters plant and fruit development in tissue culture induced off-type banana (*Musa* spp.)

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1 SUMMARY

Shoot tip *in vitro* micropropagation of banana (*Musa* AAA East Africa) cv.' Uganda' resulted in off-type plants with an altered plant and fruit development. This study was carried out to determine epigenetic mechanisms underlying the altered plant and fruit development of the *in vitro* derived off-type banana. The off-type banana was compared with *in vitro* micropropagation (MP) derived normal banana and conventionally propagated (CP) banana plants with no tissue culture history in their ancestry as controls. Plant development was estimated based on plant height, girth and number of days from planting to flowering. Fruit development was measured as the number of days from flowering to fruit maturation and senescence. Mechanisms underlying the altered plant and fruit development were determined based on global cytosine DNA methylation and 2C nuclear DNA content. Leaf cytosine DNA methylation and 2C nuclear DNA content were determined using reversed phase HPLC and flow cytometer, respectively. Results showed that the off-type banana was significantly ($P < 0.05$) taller and delayed to flower compared with the MP and CP derived banana. Similarly, the fruits of the off-type banana had significantly ($P< 0.05$) longer maturation, ripening and senescence periods than those of the true-totype fruits. The off-type derived plants had lower ($P < 0.05$) leaf global cytosine DNA methylation and 2C nuclear DNA amount compared with the MP and CP derived plants. These findings suggest that the altered plant and fruit development of the of-type banana are possibly under the control of reduced cytosine DNA methylation and nuclear DNA content. Further studies are required to identify specific genes which affect plant and fruit development upon undergoing demethylation.

1 INTRODUCTION

During banana shoot tip *in vitro* micropropagation, shoots arise from either epidermal and parenchyma cells (i.e. adventitious shoots) or intercalary meristem of leaf bases (i.e. axillary shoots) (Israel *et al*., 1991; Zaffari *et al*., 2000; Filippi *et al*., 2001). Differentiated plant cells like parenchyma must undergo dedifferentiation to acquire regenerative competence (Cassells and Curry, 2001). The use of growth regulators to induce regenerative competence involves reprogramming of cell cycles (Harding *et al*., 1996) and this may result in a genetic instability with an altered plant growth and yield (Vuylsteke, 2001; Nwauzoma *et al*., 2002; Roux, 2004). An *in vitro* derived off-type of AAB banana cv. 'Lady Finger' with a slow plant growth, and a reduced bunch and fruit size has been reported (Smith *et al*., 1999). Moreover, a tissue culture-derived off-type with a slow plant

growth, late flowering and low yield has been reported in African plantain cv. 'Agbagba' (Vuylsteke, 2001).

Somaclonal variation has been reported to be under the control of DNA methylation (Finnegan *et al*., 1993; Harding *et al*., 1996). DNA methylation can reversibly affect chromatin conformation, thereby influencing gene expression by altering the ability of RNA polymerase and transcriptional proteins to bind to gene or promoter sequences (Reik and Murrell, 2000; Slater *et al*., 2003). A high amount of indole-3-acetic acid and 2, 4-dichlorophenxyacetic acid in the growth media increased DNA methylation in a callus-derived carrot (Arnholdt-Schmitt *et al*., 1991). Similarly, a treatment of wheat seeds with 6-benzylaminopurine increased DNA methylation in germinating embryos and seedlings (Vlasova *et al*., 1995). A high DNA hypermethylation results in a gene repression, silencing, imprinting, culture ageing and loss of culture regenerability (Kazmierczak *et al*., 1998). A loss in DNA methylation has frequently been reported in tissue culture derived plants. An elevated concentration of kinetin in a growth media decreased DNA methylation in a callus-derived carrot (Arnholdt-Schmitt *et al*., 1991). A reduction in DNA methylation increases gene transcription, *in vitro* culture regenerability, plant juvenility and tallness (Finnegan *et al*., 1993; Harding *et al*., 1996).

A plant genome size or 2C nuclear DNA content is highly plastic in a response to environmental conditions. An increase in a genome size is caused by an entire chromosome duplication, a multiplication of retrotransposable elements and DNA repeat sequences (Price and Johnson, 1996; Bennetzen, 2002). Naphthalene acetic acid and 2,4-dichlorophenoxyacetic acid used to induce rooting have been reported as polyploidy inducers (D'Amato, 1997) whereas 6-benzylaminopurine at 15 mg/L resulted in a formation of a tetraploid somaclone 'CIEN BTA-03' from a triploid banana cv. 'Williams' (Trujillo and Garcia, 1996). Conversely, a reduction in a genome size is frequently caused by a deletion of mobile genetic elements and repeat DNA sequences, and an impaired DNA replication (Deumling and Clermont, 1989; Petrov, 2001). Benzylaminopurine in a growth media enhanced a reduction of a copy number of DNA repetitive sequences in a carrot callus (Arnholdt-Schmitt *et al*., 1991; Pluhar *et al*., 2004). Short photoperiodic conditions also reduced the amount of DNA in a sunflower (Price and Johnson, 1996). The loss in nuclear DNA amount has been linked to aplant rejuvenation (Arnholdt-Schmitt *et al*., 1991) and a loss of plant adaptation to winter (Deumling and Clermont, 1989).

A shoot tip *in vitro* propagated off-type banana (*Musa* AAA East Africa) cv. 'Uganda' exhibited altered plant and fruit developmental cycles. Mechanisms underlying the altered plant and fruit development in this off-type banana are hardly known. The objective of this study was to determine the underlying epigenetic causes of the altered plant and fruit development in the tissue culture derived off-type banana cv. 'Uganda' based on 2C nuclear DNA content and global cytosine DNA methylation.

3 MATERIALS AND METHODS

3.1 Description of study area and plant materials: East African highland banana 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 the field at SUA in May 2003 and four off-type plant stools were accidentally detected in the field based on visual observation. The off-type visually differed from the true-to-type plants by altered plant size, flowering and fruit maturation time. The off-type plants were multiplied *in vivo* in the field to increase the number of suckers.

3.2 Experimental design: The setup of the experiment was a randomised complete block design with three treatments. These treatments were the off-type banana, *in vitro* micropropagated (MP) normal banana and conventionally propagated (CP)

banana with no tissue culture history in its ancestry. A treatment was replicated three times and each replicate consisted of 10 plants. Sword suckers were collected from field-grown of the off-type, MP and CP plants and transplanted in a new plot at a spacing of 3 x 4 m in 2006. The crop received an appropriate management including weeding, irrigation and desuckering to maintain three plants per stool. The evaluation of plant and fruit development was carried out from May 2006 to August 2007.

3.3 Plant and fruit development: Ten plants per replicate were selected for a measurement of pseudostem height and girth. The pseudostem height was measured from the root collar to the level of the inflorescence emergence using an extendable ruler whereas the pseudostem girth was measured at 100 cm above the root collar using a field veneer calliper (Msogoya *et al.*, 2006). The flowering time was determined using 10 plants per replicate as the number of days from the date of planting to the emergence of inflorescence (Swennen and De Langhe, 1985). The fruit maturation stage was determined as the number of days from the day of plant flowering to the date when 50 % of the plants had harvestable bunches (Samson, 1986). The fruit ripening was estimated using a colour chart (RHS Colour Chart) as the number of days from the date of harvesting to the day when 50 % of the fruits in a hand had yellow peel. The fruit shelf life was recorded as the number of days from the date of harvesting to the day when 50 % of fruits in a hand were considered unmarketable.

3.4 Epigenetic mechanisms underlying altered plant and fruit developmental cycles: Mechanisms underlying the altered plant and fruit development were determined based on 2C nuclear DNA content and cytosine DNA methylation. The 2C nuclear DNA content and global cytosine DNA methylation was determined from cigar leaves of the off-type, MP and CP derived banana plants.

3.4.1 Nucleus DNA content: Fresh cigar leaf tissues of banana samples and those of the calibration standard were macerated using 'bead beating' method according to Roberts (2007). Parsley (*Petroselinum crispum* Mill.) cv. Nyman with the leaf 2C nuclear DNA content of 4.46 pg was used as a calibration standard (Yokoya *et al.*, 2000). The macerated leaf tissue was filtered through 30 µm nylon mesh and the filtrate was treated with RNase at 150 µg/mL, stained using propidium iodide and incubated at 25 °C for 20 minutes (Hanson *et al.*, 2005). The staining solution consisted of 0.06 mg/mL propidium iodide, 56.8 mg/mL disodium hydrogenphosphate, 3.6 mg/mL sodium sulphate and 4.9 mg/mL trisodium citrate. The flow cytometric analysis was carried out using CAIII flow cytometer (Partec GmbH, Munster, Germany) according to Yokoya *et al.* (2000). The effectiveness of the sample preparatory procedure was assessed using coefficients of variation of the peaks in the histograms, which was as low as 2.05 and 2.76 % for the calibration standard and banana samples, respectively. The 2C nuclear DNA amount of the banana sample was calculated according to Yokoya *et al.* (2000) as follows:

$$DNA_b = DNA_p * \frac{G_b}{G_p}$$

Where DNA_b: 2C nuclear DNA content of banana (test plant), DNA_p: 2C nuclear DNA content of parsley (calibration standard), G_b: Fluorescence intensity peak of banana and G_p: Fluorescence intensity peak of parsley.

3.4.2 DNA methylation: Nucleic acids for the determination of DNA methylation were extracted from cigar leaves using CTAB-based procedure with modifications according to Ramage *et al.* (2004). The nucleic acids were digested into nucleotides and nucleosides using nuclease P1 (Sigma N-8630) and bacterial alkaline phosphatase (Sigma P-4252) (Chakrabarty *et al.*, 2003; Johnston *et al.*, 2005), respectively. Nucleoside chromogram was generated by a reversed phase HPLC and the percentage global cytosine DNA methylation (*m*DNA) was calculated according to Johnston *et al.* (2005) as follows:

$$mDNA = 100 * \frac{[mdC]}{[dC + mdC]}$$

Where *mdC*: Methylated DNA cytosine (µM) and *dC*: Non-methylated DNA cytosine (µM).

3.5 Data analysis: Percentage data were first arcsin-transformed before undertaking data analysis. Data analysis was performed using 'SPSS 15.0 computer statistical programme (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 at a probability of 5% (Zar, 1997).

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4 RESULTS

4.1 Plant development: The off-type banana plants were significantly ($P < 0.05$) bigger. They had a taller pseudostem height and height to circumference ratio of 329 cm and 6.0 compared with 226 cm and 5.3 of the MP derived banana and 236 cm and 4.6 of the CP derived banana, respectively (Table 1). The off-type banana plants significantly ($P < 0.05$) delayed to flower at 293.0 days from the date of planting compared with 263.6 and 262.2 days of the MP and CP derived banana plants.

Table 1: Plant size and number of days to flowering of *in vitro* derived off-type banana cv. 'Uganda'

Banana type	Plant height (cm)	Plant circumference (cm)	Plant height to circumference ratio	Number of days to plant flowering
Off-type	$329^{b} \pm 11$	$55^{b} \pm 20$	$6.0^{c} \pm 0.3$	$293.0^{b} \pm 6.0$
MP banana	$226^{a} \pm 15$	$42^{a} \pm 00$	$5.3^{b} \pm 0.1$	$263.6^{a} \pm 6.0$
CP banana	$236^{a} \pm 20$	$51^{b} \pm 10$	$4.6^{a} \pm 0.1$	$262.2^{a} \pm 8.0$

Means bearing the same superscript letter within the column are insignificantly ($P < 0.05$) different according to Tukey-HSD test. ± SE: standard error of the mean.

4.2 Fruit development: The fruits of the off-type banana significantly ($P < 0.05$) matured later at 124 days from the date of flowering whereas those of the MP and CP derived banana matured at 90.9 and 87.8 days, respectively (Table 2). Similarly, the fruits of the off-type banana had longer ($P < 0.05$) ripening period of 7.0 days from the date of harvesting compared with 4.0 and 4.5 days of the MP and CP derived banana, respectively. The off-type fruits also had slower senescence (long shelf life) of 17 days from the date of harvesting while those of the MP and CP derived banana had shelf life of 7.2 and 7.0 days, respectively.

Table 2: Fruit maturation, ripening and senescence of *in vitro* derived off-type banana cv. 'Uganda'

Banana type	Number of days from flowering to fruit maturation	Number of days from harvesting to fruit ripening	Fruit shelf life (days)
Off-type	$124.0^{b} \pm 3.0$	$7.0^{b} \pm 2.0$	$17.0^{b} \pm 2.1$
MP banana	$90.9^{a} \pm 6.0$	$4.0^{a} \pm 1.8$	$7.2^{a} \pm 1.8$
CP banana	$87.8^{a} \pm 5.0$	$4.5^{a} \pm 2.1$	$7.0^{a} \pm 2.1$

Means bearing the same superscript letter within the column are insignificantly ($P < 0.05$) different according to Tukey-HSD test. ± SE: standard error of the mean.

4.3. Nuclear DNA content and cytosine DNA methylation: The *in vitro* derived off-type plants had significantly ($P < 0.05$) smaller leaf 2C nuclear DNA content of 1.72 pg compared with 1.81 and 1.82 pg of the MP and CP derived banana plants, respectively (Table 3). Furthermore, the off-type banana had lower ($P < 0.05$) leaf global cytosine DNA methylation of 11.3 % compared with 17.4 and 22.5 % of the MP and CP derived banana, respectively (Table 3). The *in vitro* derived MP derived banana had also significantly lower leaf cytosine DNA methylation than the CP derived banana.

Table 3: Nuclear DNA content and cytosine DNA methylation in tissue culture derived off-type banana cv. 'Uganda'.

Banana type	Leaf 2C nuclear DNA content (pg) (± SE)	Cytosine DNA methylation (%) (± SE)
Off-type banana	$1.72^{a} \pm 0.02$	$11.3^{a} \pm 0.5$
MP banana	$1.81^{b} \pm 0.01$	$17.4^{b} \pm 0.3$
CP banana	$1.82^{b} \pm 0.01$	$22.5^{c} \pm 1.3$

Means bearing the same superscript letter within the column are insignificantly $P < 0.05$) different according to Tukey-HSD test.

5 DISCUSSION AND CONCLUSION

The off-type banana was taller and flowered later than the true-to-type plants of either *in vitro* or conventional derived normal banana plants. The increased plant height and number of days to flowering of the off-type plants were probably due to the high juvenility. *In vitro* derived off-types with an increased juvenility have been reported in African plantains and potato (Cassells *et al*., 1991; Nwauzoma *et al*., 2002). The tallness and juvenility among *in vitro* derived regenerants has been associated with an alteration in a plant cell developmental programme during tissue culture (Harding *et al*., 1996). The increased plant height and delayed flowering of the off-type banana in this study were possibly under a control of DNA hypomethylation and a loss in nuclear DNA content. A loss in DNA methylation and nuclear DNA content has been linked to an increase in a plant height and juvenility in a callus-derived carrot (Arnholdt-Schmitt *et al*., 1991; Finnegan *et al*., 1993; Harding *et al*., 1996; Cassells and Curry, 2001).

The fruits of the off-type banana delayed to mature possibly due to the higher bunch weight and carbohydrate content in the fruit as earlier reported in the same off-type banana (Msogoya *et al*., 2011). According to Harada *et al.* (2005), fruit development involves an increase in size caused by a cell multiplication at early stage and an enlargement (i.e. due to accumulation of soluble solids and water) at a later stage. The fruits of the off-type banana had also slower fruit ripening and senescence (i.e. longer shelf life). These defects were possibly associated with an alternation of genes involved in a fruit ripening. Several genes involved with changes in a fruit colour, aroma, texture, sugars, acids and an activation of ethylene synthesis do express differentially and their cumulative effect brings about fruit ripening and senescence (Alexander and Grierson, 2002; Pech *et al*., 2008). In banana fruit ripening, many genes belonging to stresses or defences are epigenetically expressed in addition to genes related to ethylene biosynthesis, cell wall hydrolysis, metabolite transport and transcription or translation machinery (Kesari *et al*., 2007). The epigenetic expression of genes belong to stresses is associated with a loss in DNA methylation (Galaud *et al*., 1993; Burn *et al*., 1993). The slow ripening and senescence of the off-type banana fruits encountered in this study were probably under the control of cytosine DNA hypomethylation. Cytosine DNA hypomethylation does silence several fruit ripening genes, especially those related to stresses, causing a delay in a fruit ripening (Manning *et al*., 2006; Seymour *et al*., 2008).

It is concluded that the increased plant height and delayed plant flowering, fruit ripening and senescence in the off-type banana cv. 'Uganda' are epigenetically under the control of genes which are altered as a consequence of a reduction in nuclear DNA content and DNA methylation. This is a first report in which losses of cytosine DNA methylation and 2C nuclear DNA content are associated with alterations in plant and fruit development in the tissue culture derived East African highland banana cv. 'Uganda'. Further studies are required to identify specific genes which affect plant and fruit development upon methylation at cytosine base.

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7 REFERENCES

Alexander L. and Grierson D: 2002. Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. *Journal of Experimental Botany* 53: 2039 - 2055.

Arnholdt-Schmitt B., Holzapfel B., Schillinger A. and Neumann KH: 1991. Variable methylation and differential replication of genomic DNA in culture of carrot root explants during growth induction as influenced by hormonal treatments. *TAG Theoretical and Applied Genetics* 82. www.springerlink. com/content.

Bennetzen JL. 2002. Mechanisms and rates of genome expansion and contraction in flowering plants. *Genetica* 115: 29 – 36.

Burn JE., Bagnall DJ., Metzger J.D., Dennis ES. and Peakock WJ. (1993). DNA methylation, vernalisation, and the initiation of flowering. *Proceedings of the*

National Academy of Sciences (*USA*) 90: 287 – 291.

Cassells AC. and Curry RF: 2001. Oxidative stress and physiological, epigenetic and genetic variability in plant tissue culture: implications for mocropropagators and genetic engineers. *Plant Cell, Tissue and Organ Culture* 64: 145 – 157.

Cassells AC., Deadman ML. Brown CA. and Griffin E: 1991. Field resistance to late blight (*Phytophthora infestans* Mont. De Baary) in potato (*Solanum tuberosum* L.) somaclones associated with instability and pleiotropic effects. *Euphytica* 56: 75 – 80.

Chakrabarty D., Yu KW. and Peak KY: 2003. Detection of DNA methylation changes during somatic embryogenesis of Siberian ginseng (*Eleuterococcus senticosus*). *Plant Science* 165: 61 – 68.

D'Amato F: 1997. Applied and fundamental aspects of plant cell, tissue and organ culture, in Reinertard, J.M. and Y.P.S. Banajj (eds.) Cytogenetics of differentiation in tissue and cell culture, New York: Springer Verlag, 150 – 180.

Deumling B. and Clermont L: 1989. Changes in DNA content and chromosome size during cell culture and plant regeneration of *Scilla siberica*: selective chromatin diminution in response to environmental conditions. *Chromosoma* (Berl) 97: 439 – 448.

Filippi SB., Appezzato-da-Gloria B. and Rodriquez MPA: 2001. Histological changes in banana explants, cv. Nanicao (*Musa* spp. Group AAA) submitted to different auxins for induction of somatic embryogenesis. *Revista Brasileira Botanica* 24: 595 - 602.

Finnegan E., Brettell RIS. and Denis ES: 1993. The role of DNA methylation in the regulation of plant expression, in Jost, J.P. and H.P. Saluz (eds.) DNA methylation: Molecular Biology and Biological Significance, Basel: Birkhauser, 218 – 261.

Galaud JP., Gaspar T. and Boyer N: 1993. Effects of anti-DNA methylation drugs on growth, level of methylated DNA, peroxidase activity and ethylene production of *Bryonia dioca* internodes. *Physiologia Plantarum* 87: 528 – 534.

Hanson L., Boyd A., Johnson MAT. And Bennett MD: 2005. First nuclear DNA C-values for 18 eudicot families. *Annals of Botany* 96: 1315 - 1320.

Harada T., Kurabayashi W., Yamai M., Wakasa Y., Satoh T: 2005. Involvement of cell proliferation and cell enlargement in increasing the fruit size of *Malus* species. *Scientia Horticulturae* 105:447–456

Harding K., Benson EE. Kaliope A. and Roubelakis-Angelakis AK: 1996. Methylated DNA changes associated with the initiation and maintenance of *Vitis vinifera in vitro* shoot and callus cultures: A possible mechanism for age-related changes. *Vitis* 35: 79 – 85.

Israel Y., Reuven O. and Lahav E: 1991. Qualitative aspects of somaclonal variation in banana propagated by *in vitro* techniques. *Scientia Horticulturae* 48: 71 – 88.

Johnston JW., Harding K., Bremner DH., Souch G., Green J., Lynch PT., Grout BWW. and Benson EE: 2005. HPLC Analysis of DNA Methylation in Plants. *Plant Physiology and Biochemistry* 43: 844 – 853.

Kazmierczak J: 1998. Effect of DNA methylation on potential transcriptional activity in different tissues and organs of *Vicia faba* spp. minor. *Folia Histochemistry and Cytobiology* 36: 45 – 49.

Kesari R., Trivedi PK. and Nath P: 2007. Ethylene-induced ripening in banana evokes expression of defense and stress related genes in fruit tissue. *Postharvest Biology and Technology* 46 (2):136-143.

Maerere AP., Kusolwa PM., Msogoya TJ. and Nsemwa TLH: 2003. Comparison of effective *in vitro* regeneration and multiplication potential of local and introduced banana, in Proceedings of the second collaborative research workshop on food security, Morogoro, Tanzania, 28 - 30th May 2002, 169 – 174.

Manning K., Tor M., Poole M., Hong Y., Thompson AJ., King GJ., Giovannoni JJ., Seymour GB: 2006. A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. *Nature Genetics* 38: 948 - 952.

Msogoya TJ., Maerere AP. and Grout B: 2006. Field performance of micropropagated East African highland banana (M*usa* AAA East)

in the eastern zone of Tanzania. *Journal of Agronomy* 5 (3): 471 – 474.

Msogoya TJ., Grout BW. and Maerere AP: 2011. Performance of micropropagation-induced off-type of East African highland banana (Musa AAA - East Africa). *Journal of Animal and Plant Sciences* 10 (3): 1334-1338.

Nwauzoma AB., Tenkouano A., Grouch JH., Pillay M., Vuylsteke D. and Kalio LAD: 2002. Yield and disease resistance of plantain (*Musa* spp. AAB group) somaclones in Nigeria. *Euphytica* 123: 323 – 331.

Pech JC., Bouzayen M., Latch A: 2008. Climacteric fruit ripening: ethylene-dependent and independent regulation of ripening pathways in melon fruit. *Plant Science* 175: 114 - 120.

Petrov DA: 2001. Evolution of genome size: new approaches to old problem. *Trends in Genetics* 17: 23 – 28.

Pluhar SA., Erickson L. and Pauls KP: 2004. Effects of tissue culture on a highly repetitive DNA sequences (E180 satellite) in *Medicago sativa*. *Plant Cell, Tissue Culture and Organ Culture* 67: 195 – 199.

Price JH. and Johnson J.S: 1996. Influence of light on DNA content of *Helianthus annuus* L. *Proceedings of the national Academy of Sciences* (USA) 93: 11264 – 11267.

Ramage CM., Borda AM, Hamill SD. and Smith MK: 2004. A simplified PCR test for early detection of dwarf off-types in micropropagated Cavendish bananas (*Musa* spp. AAA). *Scientia Horticulturae* 103: 145 – 151.

Reik W. and Murrell A: 2000. Silence across the border. *Nature* 405: 408 – 409.

Roberts A: 2007. The use of bead beating to prepare suspensions of nuclei for flow cytometry from fresh and herbarium leaves, petals and pollen. *Journal of Cytometry* 71: 1039 – 1044.

Roux NS: 2004. Mutation induction in *Musa* – Review, in Banana improvement: Cellular, molecular, biology and induced mutation (S. M. Jain and R. Swennen (eds). IPGR /FAO/INIBAP, Science publishers, Enfield (NH), USA, Plymouth, UK. 23 – 32.

Samson JA: 1986. Tropical Fruits (2nd Edition). Longman Scientific and Technical, New York.

Seymour G., Poole M., Manning K., King JG: 2008. Genetics and epigenetics of fruit development and ripening. *Current Opinion in Plant Biology* 11: 58 - 63.

Slater A., Scott N. and Folwer M: 2003. Plant Biotechnology: Gene manipulation in plants. New York: Oxford University Press.

Smith MK., Hamill SD., Doogan VJ. and Daniells JW: 1999. Characterisation and early detection of an off-type from micropropagated 'Lady Finger' banana. *Australian Journal of Experimental Agriculture* 39: 1017 – 1023.

SPSS^R (2006. Statistical package for the social sciences (SPSS) (Version 15.0). SPSS Inc., Chicago.

Swennen R. and De Langhe E : 1985. Growth parameters of yield of plantains (*Musa* AAB cv. Agbagba). *Annals of Botany* 56: 197 – 204.

Trujillo I. and Garcia E: 1996. Strategies for obtaining somaclonal variants resistant to yellow sigatoka (Mycosphaerella musicola). *Infomusa* 5: 12 – 13.

Vlasova TI., Demidenko ZN., Kirnos MD. and Vanyushin BF: 1995. *In vitro* DNA methylation by wheat nuclear cytosine DNA methyltransferase: effect of phytohormones. *Gene* 157: 279 – 281.

Vuyslsteke D: 2001. Strategies for utilisation of genetic variation in plantain improvement. Published PhD Thesis, Katholieke Universiteit Leuven.

Yokoya K., Roberts AV. and Mottley J: 2000. Nuclear DNA amount in roses. *Annals of Botany* 85: 557- 561.

Zaffari GR., Kerbauy GB., Kraus JE. and Romano EC: 2000. Hormonal and histological studies related to *in vitro* banana bud proliferation. *Plant Cell, Tissue and Organ Culture* 63: 187 – 192.

Zar JH: 1997. Biostatistical Analysis (3rd Edition). Prentice-Hall International Inc., Upper Saddle River.