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Responses of Five Locally Adapted Sweet Potato (*Ipomoea batatas* (L.) Lam) Cultivars to *in vitro* Plant Regeneration via Direct and Indirect Embryogenesis

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Abstract: The development of a reliable plant regeneration protocol in sweet potato forms the basis for sweet potato (*I. batatas*) genetic improvement. The success in production of the transgenic sweet potato is dependent on the reliability and efficiency of the regeneration protocol to produce somatic embryos capable of forming whole plants. The effect of direct and indirect embryogenesis on *in vitro* plant regeneration was studied and thereafter a suitable tissue culture protocol for 5 locally adapted Kenyan sweet potato cultivars *Mugande*, *SPK004*, *Kemb10*, *Japon tresmesino* and *Zapallo* established. Embryogenic calli was induced directly and indirectly from sweet potato leaf explants and auxiliary buds cultured on MS medium supplemented with 2,4-D (0, 0.5 and 1.0 mg L⁻¹). Abscisic acid was added to induce embryo maturation and when the hormone levels were reduced these embryos began to differentiate into shoots before whole plants were regenerated. For each treatment the number and days taken to form shoots, roots and plants that were regenerated were counted and used as a selection index of an efficient sweet potato regeneration protocol for the locally adaptable Kenyan cultivars. The test cultivar had a significant ($p \leq 0.05$) effect on both direct and indirect embryogenesis. The use of indirect embryogenesis was beneficial for the local Kenyan sweet potato cultivars as more calli formed hence ensuring higher plant regeneration and increased mass propagation of *in vitro* plants while direct embryogenesis took a shorter time to form shoots and roots but fewer plants were regenerated.

Key words: Sweet potato (*Ipomoea batatas*), plant regeneration, embryogenesis, somatic embryogenesis

INTRODUCTION

Sweet potato is ranked worldwide as third most important tuber crop after potatoes (*Solanum tuberosum*) and cassava (*Manihot esculentum*) (FAO, 2003). It is also an important subsistent crop after maize (Qaim, 1999). In Kenya, it consistently produces well below its potential because of yield-reducing viruses (Wambugu, 1995; Aritua *et al.*, 1998). The greatest challenge is with Sweet Potato Feathery Mottle Virus (SPFMV) because symptoms on infected plants are transient and farmers are unable to recognize them yet as much as 80% yield losses have been experienced (Wambugu, 1995; Odame *et al.*, 2002; Bioethics, 2004).

Genetic improvement by conventional breeding is limited due to biological complexities associated with the crop (Dhir *et al.*, 1998). This has led to more emphasis on the use of biotechnological tools such as gene transfers for genetic improvement of particular traits in sweet potato (Prakash, 1994; Sihachakr *et al.*, 1997). Research in Kenya has been directed to the development of sweet potato cultivars resistant to SPFMV; hence a transgenic

sweet potato resistant has been developed by genetic engineering using a virus Coat Protein (CP) gene (Odame *et al.*, 2002). The transformation procedures had earlier been shown to be genotype dependant and often difficult to regenerate (Lowe *et al.*, 1995). In addition the transformation procedures to produce transgenic sweet potatoes have been done in exotic cultivars such as Jewel, Beauregard and CPT 560 (Brink *et al.*, 2000), which have been unable to withstand the local environmental conditions as well as virus challenge in the field (Odame *et al.*, 2000).

A suitable tissue culture protocol that would be efficient and reliable in regenerating rapidly the local sweet potato cultivars adapted to local environmental conditions is essential to allow somatic embryos to be effectively induced and maintained in as wide range of cultivars as possible. The success in production of transformed material ultimately depends on the ability to regenerate whole plants from a totally undifferentiated cell mass (callus) by manipulation of the growth regulators under aseptic conditions (Yoshiaki, 2004).

Plant regeneration can occur directly from somatic embryos from the dividing cells or indirectly by the dividing cells reverting to undifferentiated cell mass (Merkle *et al.*, 1995). Direct embryogenesis has been reported to result in low frequency of chimeras and a high number of regenerates (Al-Mazrooei *et al.*, 1997) as with this type of embryogenesis pre-embryogenic cells are conditioned to produce embryos even before explant is cultured and need only an exogenous stimulant such as medium components supplemented by either an auxin or cytokinin to be able to express them (Merkle *et al.*, 1995). Indirect embryogenesis occurs through use of callus formation by manipulation of appropriate phytohormones, which programme embryogenic tissues to produce callus. Once induction process is initiated the callus cultures are transferred to auxin free media for further embryo development.

In sweet potato, the synthetic auxin 2, 4-D is commonly used and has been reported to be the most effective for inducing embryogenic callus from tissue explants and regenerating plants (Al-Mazrooei *et al.*, 1997). However, the concentration used varies widely with genotype (Ammirato, 1984; Zheng *et al.*, 1996; Radhakrishnan *et al.*, 2001). The callus is then transferred to a 2, 4-D free medium supplemented with reduced forms of nitrogen in the form of NH_4^+ salts or amino acids. Such manipulations in plant tissue culture have been particularly successful in the case of Tobacco and Carrot tissue (Finer, 1994). Several studies exist on direct and indirect embryogenesis in sweet potato cultivars but none has been reported in the local adapted cultivars found in Kenya. The study aimed at evaluating the *in vitro* plant regeneration methods involving indirect and direct embryogenesis for their suitability in rapidly regenerating locally adaptable sweet potato cultivars.

MATERIALS AND METHODS

Study site: This study was carried out at the Biotechnology laboratory at the Kenya Agricultural Research Institute (K.A.R.I) Njoro, Nakuru District, Kenya under controlled temperature ranging from 27 to 29°C, 16/8 h photoperiod and light conditions of $700 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Plant material: The five sweet potato cultivars used were sourced from International Potato Centre (C.I.P) Nairobi, Kenya and included *SPK004*, *Kemb 10*, *Mugande*, *Japon tresmesimo* and *Zapallo* chosen based on their popularity, preferred taste and agronomic performance under local conditions.

Indirect embryogenesis medium: Medium for callus culture consisted of MS basal medium (Murashige and Skoog, 1962), supplemented with 100 mg L^{-1} Myo-inositol, 2 mg L^{-1} Thiamine-HCL, 40 g L^{-1} Sucrose, 10 m L^{-1} Vitamin stock solution (200 mg L^{-1} Calcium Pantothenate, 2 g L^{-1} Gibberelic acid, 10 g L^{-1} Ascorbic acid and 10 g L^{-1} Putrescine) and 3 g L^{-1} Phytagel. 2,4-D levels and BAP at a rate of 1 mL L^{-1} were filter sterilised and added to the medium. Leaves from most recently expanded *in vitro* plantlets were cut and placed with the adaxial side in contact with the medium. The cultures were transferred to a dark incubator for three weeks set at a temperature of 29°C after which lights were turned. After 3 to 4 weeks under light conditions the treatments that had formed callus were recorded and transferred to calli maturation and embryo initiation media comprised of half strength MS basal medium (Murashige and Skoog, 1962) supplemented with 2.5 mg L^{-1} Absisic acid. The embryogenic callus was transferred to a growth chamber set at a temperature of $27^\circ\text{C} \pm 0.5$ over a 16 h photoperiod and under a low light intensity of $700 \mu\text{mol m}^{-2} \text{s}^{-1}$ provided to regenerate plantlets.

Direct embryogenesis: Embryogenic tissues were initiated from auxiliary buds grown *in vitro* on sweet potato multiplication media comprised of 4.4 mg L^{-1} MS basal medium (Murashige and Skoog, 1962), 30 g L^{-1} Sucrose, supplemented with 0, 0.5 or 1.0 mg L^{-1} 2,4-D concentration, 100 mg L^{-1} Myo-inositol, 1 mg L^{-1} Thiamine HCL, 0.01 mg L^{-1} Nicotinic Acid, 0.01 mg L^{-1} Pyridoxine, 1 mL L^{-1} BAP, 2.235 g L^{-1} Potassium chloride (Newell *et al.*, 1995; Zheng *et al.*, 1996). Auxiliary buds from the *in vitro* plants were exercised from shoot tips from each sweet potato cultivar under a light microscope and placed on medium then incubated at a temperature of 27°C for a 16/8 h day: Night photoperiod for up to 6 weeks under low light. After 6 weeks the developing embryos were transferred using a scalpel to fresh media containing half strength MS basal media, 30 g L^{-1} Sucrose, 0.03 g L^{-1} Abscisic acid and 0.1 mL L^{-1} BAP, for two weeks in the dark to initiate embryo formation (Zheng *et al.*, 1996). Embryos were observed under a microscope and transferred to a light chamber set at 25°C for two weeks after which the developing embryos were transferred to hormone (2,4-D) free media to initiate shoots.

Data collection: To select the most suitable plant regeneration method for the local sweet potato cultivars the influence of both direct and indirect embryogenesis on the mean number of days taken to form shoots and

roots, number of leaves and roots formed and the overall number of plants regenerated *in vitro* was determined for each cultivar.

Statistics: The experimental design was a factorial treatment arranged in a completely random design with the factor cultivar at five levels, regeneration protocols at two levels and three levels of 2,4-D, replicated three times. Treatments were randomly assigned after which data collected was subjected to Statistical Analysis systems (SAS) and where F-test was significant at 5% level means were separated by LSD (SAS Institute, 2001).

RESULTS

Days taken for shoots to form: Significant ($p \leq 0.05$) interaction between sweet potato cultivars and regeneration method for mean number of days taken to form shoots were observed but there was no significant ($p \leq 0.05$) three-way interaction between cultivar, 2,4-D concentration and regeneration method. In this study, using indirect embryogenesis by callus induction increased the mean number of days taken for shoots to develop compared to direct somatic embryogenesis (Fig. 1). Nevertheless, plant regeneration under both methods took a significantly ($p \leq 0.05$) shorter time to form shoots with the local sweet potato cultivars when 0 or 0.5 mg L⁻¹ 2,4-D levels were used. With the transfer of embryogenic callus to culture media free of 2,4-D but supplemented with 2 mL L⁻¹ ABA embryos began to form clearly on the surfaces of calli (Fig. 3a). These embryos turned green against the surface of calli (Fig. 3b). The regenerated shoots from the test cultivars had similar phenotypic characteristics as mature plants (Fig. 3c and d). As the shoots developed a thickening wall began to gradually form around the shoot (Fig. 3e) which was followed by progressive elongation of the shoot. Further shoot development resulted in the gradual regeneration of leaves (Fig. 3f).

Days taken for roots to form: Generally, the shortest time taken to form roots was attained at 0.5 mg L⁻¹ 2,4-D with direct somatic embryogenesis and in most cultivars this time increased by almost six days when the medium was without 2,4-D. The types of roots produced were adventitious and appeared at the base of the developing shoots. The cultivar *Zapallo* took the shortest time to form roots while *Mugande* took the longest. In initiation of plants indirectly from callus, the cultivar *Kemb10* took the longest period of time to form and *SPK004* took the shortest period of time (Fig. 2).

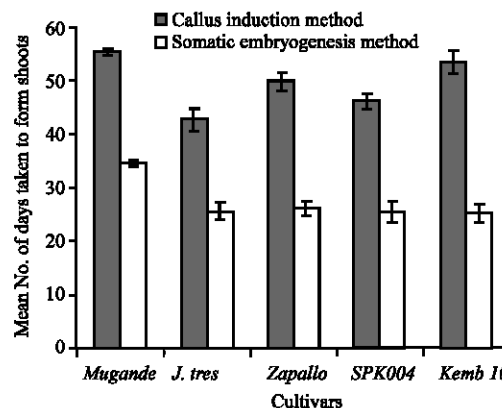


Fig. 1: Mean number of days taken to form shoots from somatic embryogenesis and callus induction methods in five sweet potato cultivars tested

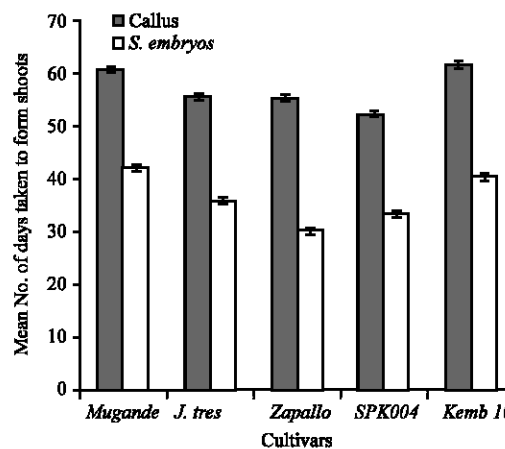


Fig. 2: Interaction effect of cultivar and regeneration method on mean number of days taken to form roots in sweet potato

Number of leaves formed per plant: Regeneration by indirect embryogenesis resulted in significantly ($p \leq 0.05$) higher overall mean number of leaves per regenerated plant than through direct embryogenesis. With indirect embryogenesis by callus induction the highest mean number of leaves was obtained at 0 mg L⁻¹ 2,4-D with the cultivar *Zapallo* followed by *SPK004* when the medium was supplemented with 0.5 mg L⁻¹ 2,4-D, cultivar *SPK004* recorded the highest number of leaves with callus induction method while direct somatic embryogenesis *Mugande* gave the highest number of leaves (Fig. 4). Using 1.0 mg L⁻¹ 2,4-D reduced the number of leaves across all treatments. The lowest mean number of leaves was recorded at 1.0 mg L⁻¹ 2,4-D with cultivar *J. tresmesino* using both indirect and direct embryogenesis, followed by *Kemb 10*.

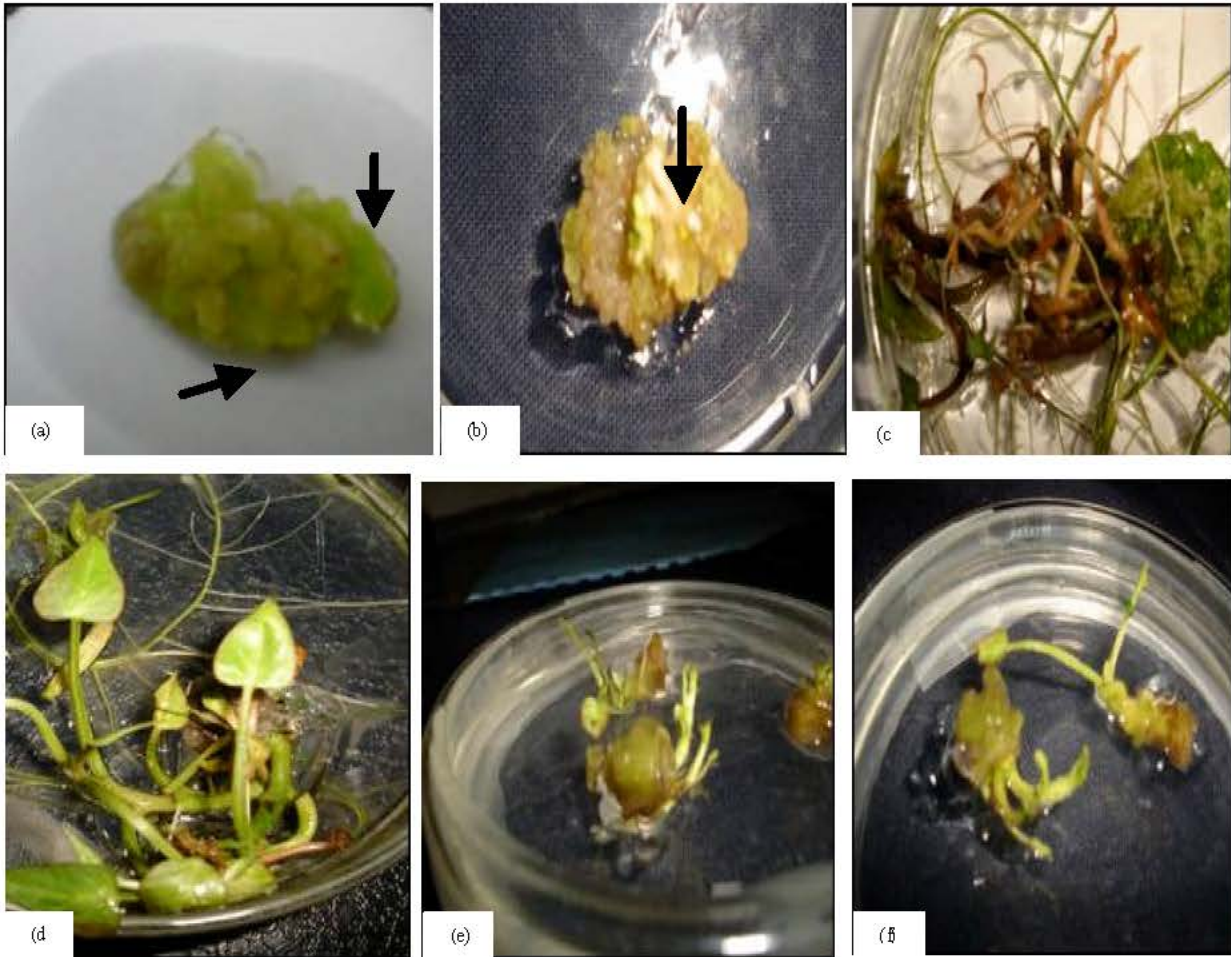


Fig. 3: a) Embryos begin to form on the surfaces of leaf explants from sweet potato cv. *SPK004* cultured on media supplemented with 2 mL L^{-1} ABA without 2,4-D, b) Embryos begin to form at the centre of embryogenic calli of leaf explants from sweet potato cultivar *Zapallo* cultured on media supplemented with 2 mL L^{-1} ABA without 2,4-D, c) Shoots regenerated from the cultivar *Zapallo* showing purple stem colouration and narrow leaves characteristics of the mature plantlet, d) Shoots regenerated from the cultivar *Mugande* showing green stem colouration and broad leaves characteristics of the mature plantlet, e) Somatic embryos from the sweet potato cultivar *Kemb 10*, five weeks culture in $\frac{1}{2}$ MS medium and 1 mL L^{-1} BAP, began to develop shoots and f) Shoots from the sweet potato cultivar *Kemb 10* increase in length and leaves begin to develop seven weeks after culture in $\frac{1}{2}$ MS medium and 1 mL L^{-1} BAP

Number of roots formed per plant: The highest number of roots was obtained with cv. *SPK004* at 1.0 mg L^{-1} 2,4-D using indirect embryogenesis followed by *Zapallo* at 0.5 mg L^{-1} 2,4-D. With direct somatic embryogenesis the highest number of roots was with *Kemb 10* at 0.5 mg L^{-1} 2,4-D level. With the somatic embryogenesis method, increasing the 2,4-D levels from 0 to 0.5 mg L^{-1} 2,4-D led to an increase in the mean number of roots formed per plant but a decline at 1.0 mg L^{-1} 2,4-D in all cultivars with the exception of *Mugande*. However, the cvs. *SPK 004* and *J. tresmesino* revealed no significant differences ($p \leq 0.05$) in the methods used to form roots (Fig. 5).

Number of plants regenerated: The quality, quantity and frequency of embryogenic calli induction influenced the mean number of plants that were to regenerate. The number of plants that were regenerated was highest with the indirect callus induction method and when no 2,4-D levels were added followed by when 0.5 mg L^{-1} 2,4-D were supplemented in the culture medium. The cultivars *Mugande* and *SPK004* responded best with the indirect embryogenesis while *J. tresmesino*, *Kemb 10* and *Zapallo* produced more number of plants with direct embryogenesis. The lowest mean plants regenerated were recorded with *Kemb 10* with indirect embryogenesis

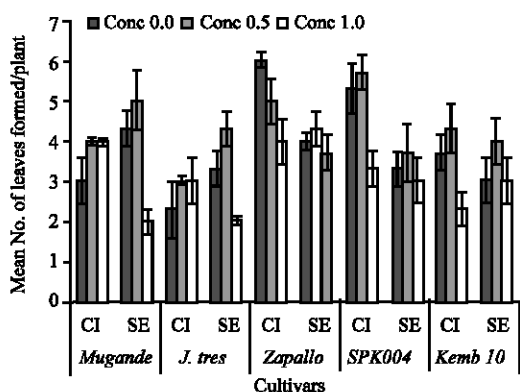


Fig. 4: Interactive effect between the sweet potato cultivars, regeneration method and 2,4-D concentration on the mean number of leaves formed per plant

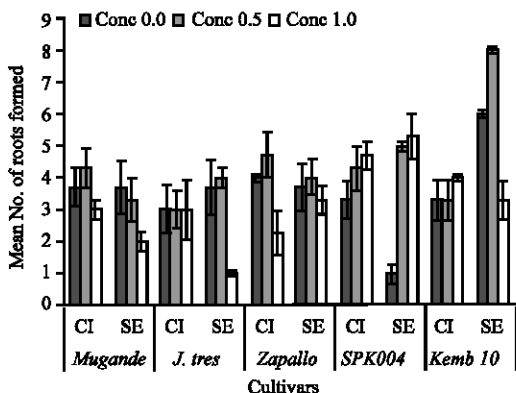


Fig. 5: Interactive effect between the sweet potato cultivars, regeneration method and 2,4-D concentration on the mean number of roots formed per plant

which produced 2 plants while with direct somatic embryogenesis *Mugande* had the lowest mean number plants regenerated.

DISCUSSION

The study showed that the levels of 2,4-D concentration affected the number of plants that were regenerated hence sufficient 2,4-D levels had to be provided when using the two regeneration methods to promote somatic embryo formation and subsequent plant regeneration. In this study the plant regeneration tendencies varied significantly with the regeneration methods used and genotype as reported by Chee *et al.* (1990) and Radhakrishnan *et al.* (2001). Each of the local sweet potato cultivar varied widely in their response to

tissue culture embryogenesis and plant regeneration. Higher concentrations of 2,4-D resulted in lower plant shooting, rooting and leaf incidences and a reduced mean number of plants regenerated per cultivar. In this study, the number of days taken for shoots to develop decrease when plant regeneration was through direct somatic embryogenesis as compared to regeneration by indirect somatic embryogenesis. This could be that regeneration by somatic embryogenesis, involved embryogenic cells already conditioned to produce embryos therefore enhancing organogenesis as reported by Merkle *et al.* (1995). These cells were subjected to less genetic variability while in regeneration by indirect somatic embryogenesis through callus, the embryogenic cell mass had to be initiated and only a small percent contributed to formation of calli as reported by Chee *et al.* (1990), hence leading to an increase in the mean number of days taken to form shoots. Likewise, the high production of leaves with callus induction method could be that somatic embryos arose from a large mass of disorganized and undifferentiated cells unlike in somatic embryogenesis where the cells were already organized to develop into leaf or shoots (Merkle *et al.*, 1995). When the disorganized cells differentiated into organized cells there may have differentiated into more shoots, which subsequently increased the mean number of leaves that formed per plant. Studies by Nguyen *et al.* (1997) indicated that increasing the 2,4-D levels led to a significant increase in the number of days taken to form shoots. Great variability in genotype depended on the different sensitivities to the endogenous levels of 2,4-D levels. If the level of auxin and cytokinins were in correct amounts, shoots and roots developed rapidly also reported by Chrispeels and Sadaava (2003). Rolando *et al.* (1999) observed that shoot and root regeneration took a longer time at higher 2,4-D levels and although root emission and sporadic shoot formation were obtained, these occurred at low frequency. In this study, a reduction in 2,4-D levels could be that low 2,4-D levels supported production of secondary embryogenic calli that led to organogenesis as compared to high levels which in this study led to cultivars forming non-embryogenic calli therefore hindering shoot formation. 2,4-D played an important role in regenerating the local sweet potato cultivars by influencing the rate of cell division hence affecting the success of morphogenesis of tissues and subsequent increased development of regenerated plants. It was important to select a protocol that had a higher plant conversion in order to increase the likelihood of getting higher transformed plants similar phenotypic characteristics as mature plants.

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REFERENCES

- Al-Mazrooei, S., M.H. Bhatti, G.C. Henshaw, N.J. Taylor and D. Blakeslew, 1997. Optimisation of somatic embryogenesis in fourteen cultivars of sweet potato (*Ipomoea batatas* (L.) LAM). Plant Cell Rep., 16: 710-714.
- Ammirato, P.V., 1984. Control of Expression of Morphogenesis in Embryogenic Culture. In: Handbook of Plant Tissue Culture and Agricultural Applications. Evans, D.A., W.R. Sharp and P.V. Ammirato (Eds.), Macmillan Publishers, New York, pp: 23-40.
- Aritua, V., E. Adipala, E.E. Carey and R.W. Gibson, 1998. Incidences of sweet potato virus disease: Virus resistance of sweet potato grown in Uganda. Ann. Applied Biol., 132: 399-411.
- Bioethics, Nuffield Council, 2004. The Use of GM Crops in Developing Countries. Case study 5: Improved resistance to viruses in sweet potato.
- Brink, J.A., B. Prior and E.J. Da Silva, 2000. Developing Biotechnology around the world. Nat. Biotechnol., 17: 434-436.
- Chee, R.P., J.R. Schultheis and D.J. Cantilfee, 1990. Plant recovery from Sweet potato somatic embryos. Hortic. Sci. J., 25: 795-797.
- Chrispeels, M.J. and D.E. Sadaava, 2003. Plant Genes and Crop Biotechnology. Jones and Barlett Publishers. Inc. USA., pp: 561.
- Dhir, S.K., J. Oglesby and A.S. Bhagsari, 1998. Plant regeneration via somatic embryogenesis and transient gene expression in sweet potato protoplasts. Plant Cell Rep., 17: 665-669.
- Finer, J.J., 1994. Plant regeneration via embryogenic suspension cultures. Plant Cell Rep., 8: 586.
- Food Agricultural Organization, 2003. Bulletin of Statistic FAO Secretariat Rome, Italy, 4: 46-47.
- Lowe, K.M., W.D.O. Hamilton and C.A. Newell, 1995. Genetic transformations in *Ipomoea batatas* (L.) LAM. Biotechnol. Agric. For., 29: 308-320.
- Merkle, S.A., W.A. Parrot and B.S. Flinn, 1995. Morphogenic Aspects Of Somatic Embryogenesis. In *in vitro* Embryogenesis in Plants. Thorpe, T.A. (Ed.), The Netherlands: Kluwer Academic Publishers, pp: 155-203.
- Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol. Plant., 15: 473-497.
- Newell, C.A., J.A. Lowe, A. Merryweather, L.M. Rooke and W.D.O. Hamilton, 1995. Transformation of Sweet potato (*Ipomoea batatas* (L.) LAM) with *Agrobacterium tumefaciens*. Plant Sci., 107: 215-227.
- Nguyen, T.T., E. Santagelo and A. Sommino, 1997. Effect of genotype, explant size and medium composition on somatic embryogenesis and plant regeneration in Sweet potato. Plant Cell Rep., 51: 63-67.
- Odame, H., P.K. Mbote and D. Wafula, 2000. Report on the case of transgenic sweet potato in Kenya. In: Kenya Agricultural Research Institute (KARI), Annual Report.
- Odame, H., P.K. Mbote and D. Wafula, 2002. The Role of Innovation in Policy and Institutional Change: Influence of modern biotechnology on institutional and policy change in Kenya. IELRC. Environment Team, IDS, Sussex, 2002.
- Prakash, C.S., 1994. Sweet Potato Biotechnology: Progress and Potential. Biotechnology and Development Monitor, 18: 1819-1822.
- Qaim, M., 1999. The Economic Effects of Genetically Modified Orphan Commodities: Projections for Sweet Potato in Kenya ISAAA Brief No. 13 (Ithaca, NY: ISAAA).
- Radhakrishnan, T., T.G.K. Murthy, K. Chandran and A. Banyopadhyay, 2001. Somatic embryogenesis in *Arachis hypogaea*: Revisited. Aust. J. Bot., 49: 753-759.
- Rolando, G.G., D.S. Sanchez, J.M. Campos, E.P. Vazquez, Z.Z. Guerra and A.L. Quesada, 1999. Plant regeneration from leaf and stem of Sweet potato (*Ipomoea batatas* (L.) Lam.) cultivars. Biotecnologia Aplicada, 16: 11-14.
- SAS Institute, 2001. Statistical Analysis System (SAS) user's guide, Release 8.1 1999-2000. North Carolina, U.S.A.
- Sihachakr, D., R. Houcour, A.J.M. Cavalcante, L. Umboh, D. Nzoghe, A. Servaes and G. Ducreux, 1997. Plant regeneration of Sweet potato (*Ipomoea batatas* L.). Euphytica, 96: 143-152.
- Wambugu, F., 1995. Control of african sweet potato virus diseases through biotechnology and technology transfer. ISNAR.
- Yoshiaki, Y., 2004. Tissue culture and Morphogenesis: The adventive embryo for formation and plant regeneration. Botanical Database of Morning Glories Kokei, Tokyo, pp: 1-6.
- Zheng, Q., P.D. Desai, S. Chaumpatua and C. Prakash, 1996. Rapid and repetitive plant regeneration in sweet potato using Somatic embryogenesis. Plant Cell Rep., 15: 381-385.