MICROPROPAGATION OF *STRYCHNOS HENINGSII* FOR SUSTAINABLE CONSERVATION

R. K. Ngenoh[1] P. K. Njenga[2], V. W. Ngumi[3], J. Onguso[4], J. Kahia[5]
[1,4]Institute of Biotechnology Research, Jomo Kenyatta University of Agriculture and Technology, Nairobi
[2,3] Department of botany, Jomo Kenyatta University of Agriculture and Technology, Nairobi
[5]Department of Plant Physiology, Coffee Research Foundation, Kenya

Abstract
Strychnos henningsii is an important medicinal herb of the family *Logoniaceae* common name Muteta (Kikuyu and Kamba), Muchimbi (Meru). It is an endangered plant that is facing threat of extinction owing to indiscriminate and unsustainable harvesting in the wild. Conventional breeding is difficult in this plant, and *in-vitro* multiplication is important to conservation and propagation. Tissue culture techniques are useful for *ex-situ* conservation of rare endemic or threatened plant species. An efficient protocol for rapid production of micro shoots was established. Multiple shoots were obtained using nodal segments with two auxiliary buds as explants on Ms medium supplemented with various concentrations of Cytokinins (Kinetin, Benzyl Amino Purine) and/or GA_3, at concentrations ranging from 0.5 to 10 mg/l. High frequency bud break 74.67% and multiple shoot formation were induced from nodal segments cultured on MS medium supplemented with 2.0mg/l BAP. Faster bud break coupled with enhanced frequency of shoot development 87.53% and internode elongation were dependent on the synergistic effect of GA3 (0.2 mg/l).

Key words: Cytokinins, *Ex-situ*, medicinal plant, multiple shoots, *In-vitro*, *Strychnos henningsii*

1.0 Introduction

Strychnos henningsii (Gilg), Logoniaceae common name: Muteta (kikuyu/kamba), Muchimbi (Meru). The genus has a small erect, much-branched tree of medicinal important and a native of Angola, Kenya, Mozambique, South Africa, Swaziland, Tanzania and Uganda. It is a herb with dark green, flossy foliage. "Muteta" is reported to be widely used in East Africa; among the Kikuyu, Masaai and Kamba, roots, stem and barks are boiled and used in the preparation of milk soups and fatty-meat (Palgrave, 1988; Beentle, 1994 and Maundu *et al*., 2005). The fruits are used for flavouring beer among the Mbeere. It also has significant medicinal uses.

In Africa traditional medicine *S. heningsii* has been used for various treatments including; rheumatism, syphilis, gastrointestinal disorders (purgative) and snakebites (Titus *et al*., 1991). The bark is a mouth antiseptic and applied on to wounds in cattle and horses to hasten healing (Palgrave, 1998; Noad and Birnie 1989). *S. heningsii* has potential in development of antinociceptive (anti-inflammatory and analgesic drugs) and antispasmodic drugs. Some of its application can be partially explained by the presence of retune-like alkaloids (Titus *et al.,* 1991).

This plant species has now become endangered due to depletion in the natural habitat that supports vegetation. With rapid modernization and demand for space, vegetation cover especially the forests has continued to deplete. The species also has a slow growth rate, seed production is erratic and seed germination is poor because seeds exhibit orthodox storage behavior.

"Muteta" have been documented to be among 30 species identified for conservation due to wide-scale use. The gradual decline in the population of this species demands launching of conservation efforts so as to ensure continuous and ample supply by establishing a balanced cycle of harvest and renewal (Odum, 1971). Such conservation efforts would ensure continuous and ample supply of this valuable material which is in great demand by the pharmaceutical industry. Only a small percentage of medicinal plants, used in the industry are cultivated. Most of them are collected from the wild, very often in a destructive and unsustainable manner. Keeping the above facts in mind, namely the gradual decline in this endangered species, the present study was undertaken to develop a suitable protocol for its rapid multiplication.

2.0 Materials and Methods

2.1 Disinfection of the explants

Nodal explants were obtained from seedlings growing in the green house (Figure 1). The explants were thoroughly washed for 30 minutes under running tap water, followed by trimming of leaves. The explants were then rinsed before transferring them to the clean bench. Inside the clean bench, the explants were disinfected by soaking in 70% ethanol for 60 seconds, followed by 25 minutes in 30% (v/v) commercial bleach (Jik) before rinsing four times with distilled water.

2.2 Shoot Multiplication

The basal medium was MS medium Ms medium supplemented with various concentrations of Cytokinins (Kinetin, Benzyl Amino Purine) and/or GA_3, at concentrations ranging from 0.5 to 10 mg/l.

The pH of all the media was adjusted to 5.8 with 1 mol/L HCL or NaOH prior to gelling with 0.8% (*w/V*) agar, dispensed (10 ml) into culture tubes (25mm x 150mm) and sterilized by autoclaving at 120 ^{0}C for 20 min. After sterilization, the explants were inoculated (Fig, 2) on MS medium. The cultures were maintained in the culture room under a regime of 16 h photoperiod (intensity - 40-50 $\mu E\ m^2$ /min/sec) at 25 ^{0}C. For each treatment, 8 replicates were used and each experiment was repeated three times.

Figure 1: One of the seedlings growing in the greenhouse

3.0 Results and Disscussion

To our knowledge, there are no published reports on the multiplication of *Strychnos henningsii* through micropropagation techniques. Micropropagation of medicinal plants is considered to be one of the most promising ways for multiplying a selected variety true to its type. Such individuals are genetically similar showing the same agronomic characteristics. Bud breakage and development of shoots from nodal explants was a function of cytokinin activity. The morphogenic responses of nodal explants cultured in various concentrations of cytokinins are shown in Table 1. There was no sign of bud break even after 30 days on MS basal medium without any growth regulator supplement. Of the two cytokinins tested, BAP was more effective than Kinetin in inducing bud break as well as multiple shoot formation. There was a linear correlation between the increase in concentration of BAP up to the optimum level (2.0 mg/l) and percentage shoot development. The number of shoots per explant also increased with increments in BAP concentrations up to the optimum levels (2.0mg/l). MS medium containing 2.0 mg/l BAP induced bud break in ca. 74.67% of the nodal explants.

The explants cultured in the medium showed their first response by an initial enlargement of the existing axillary bud following bud break within 10–15 days. From each developing bud, a single shoot emerge after 15 days. The percentage bud break and induction decline with the increase in BAP concentration beyond the optimal level (2.0 mg/l, Table 1). Above 2.0mg/l, BAP caused a suppression of sprouting. A combination of optimal concentration of BAP (2.0mg/l) and GA_3 (0.2mg/l) in the culture medium not only induced a faster bud break (within 7 days) but also enhanced the frequency of bud break. In this treatment, 87.53% of cultured explants produced multiple shoots and the number of shoots per explant was 4.13 ± 0.14 (Table 1).

The synergistic effect was also noticed in a combination of Kinetin (3.0mg/l) and GA_3 (0.2mg/l), but with respect to percentage shoot development, shoot number and shoot length it was inferior to BAP - GA_3 coupling. In the present study, the stimulatory effects of a singular supplement of BAP on bud break and multiple shoot formation in *S. henningsii* was similar to that reported earlier in other medicinal plant species including *Chlorophytum borivilianum* (Purohit *et al.,* 1994) and *Ocimum spp.* (Pattnaik and Chand, 1996 and Sahoo *et al.,* 1997).Our observations on the suppression of sprouting at higher BAP concentrations were in accordance with those of Pattnaik and Chand (1996) in *O. americanum* and *O. sanctum*. In another species of *Ocimum viz*. *O. basilicum*, although the nodal segments required the presence of BAP at 1.0 mg/l at the initial stage of bud break their further growth and proliferation demanded transfer to a medium containing BAP at a relatively very low concentration (0.25 mg/l) (Sahoo *et al* 1997).

In the present study, coupling of BAP (2.0mg/l) and GA_3 (0.2mg/l) had a noticeable synergistic influence on multiple shoot formation but in *Plumbago indiga* (Nitsch and Nitsch, 1967) and *Duboisia myoporoides* (Kukreja and Mathur, 1985), GA_3 has been shown to suppress shoot bud differentiation. Thus the role of GA_3 in shoot induction and development in medicinal plants species remains controversial.

Table 1: Morphogenic Response of Nodal Explants of S. henningsii to Different Concentrations of Cytokinins (KIN, BAP) and/or GA_3.

Growth Regulators(mg/l)	Shoot Development (%)	Shoot Number/Explant	Shoot Length (cm)
0.0	-	-	-
KIN			
0.5	-	-	-
1.0	24.67 ± 1.36	1.33 ± 1.76	1.47 ± 0.11
2.0	37.47 ± 1.22	3.67 ± 1.32	3.27 ± 0.16
3.0	62.50 ± 1.74	2.33 ± 1.64	2.33 ± 0.17
5.0	12.50 ± 1.46	1.33 ± 1.40	1.13 ± 0.09
BAP			
0.5	12.40 ± 1.70	1.33 ± 1.45	1.33 ± 0.16
1.0	50.33 ± 0.24	1.33 ± 0.74	1.53 ± 1.00
2.0	74.67 ± 1.38	3.67 ± 0.40	3.27 ± 0.45
5.0	37.60 ± 1.94	2.33 ± 0.24	2.40 ± 0.74
10.0	25.00 ± 1.13	1.67 ± 0.13	2.20 ± 0.46
KIN + GA_3			
3.0 + 0.1	62.50 ± 0.06	3.33 ± 0.20	2.77 ± 0.78
3.0 + .02	74.67 ± 0.34	2.67 ± 0.48	3.33 ± 0.22
3.0 + 0.3	49.67 ± 0.42	2.33 ± 0.32	2.83 ± 0.43
3.0 + 0.4	49.37 ± 0.58	2.33 ± 0.12	2.63 ± 0.35
3.0 + 0.5	37.57 ± 0.27	2.00 ± 0.00	2.33 ± 0.45
BAP + GA_3			
2.0 + 0.1	49.67 ± 0.33	3.31 ± 0.45	2.83 ± 0.04
2.0 + 0.2	87.53 ± 0.03	3.67 ± 0.33	4.13 ± 0.14
2.0 + 0.3	62.47 ± 0.09	3.33 ± 0.26	3.13 ± 0.03
2.0 + 0.4	62.07 ± 0.24	2.33 ± 0.38	2.77 ± 0.38
2.0 + 0.5	37.53 ± 0.12	1.33 ± 0.41	2.33 ± 0.21

Data presented as the mean value ± standard error after 45 days of culture from three independent experiments each with 8 replicates.

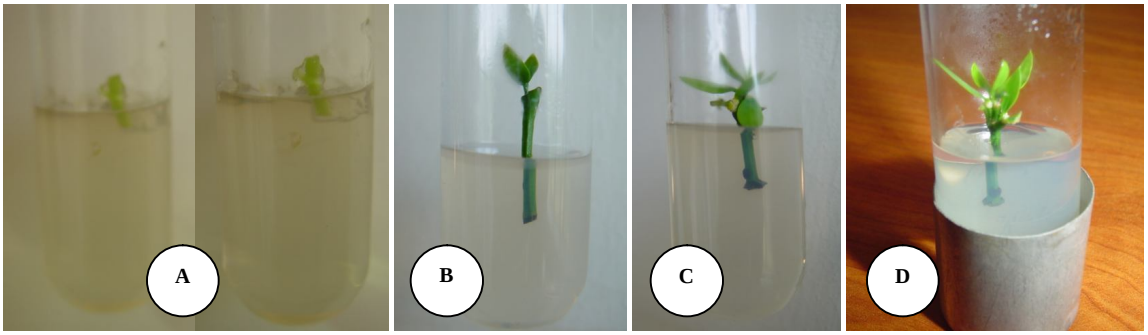

Figure 2. A-D. A: Freshly inoculated nodal explants on MS Medium Supplemented with 2.0 mg/l of BAP and 0.2 mg/l of GA_3. B. Early stage of development of micro shoots. C. Formation of multiple shoots. D. Well development of multiple shoots

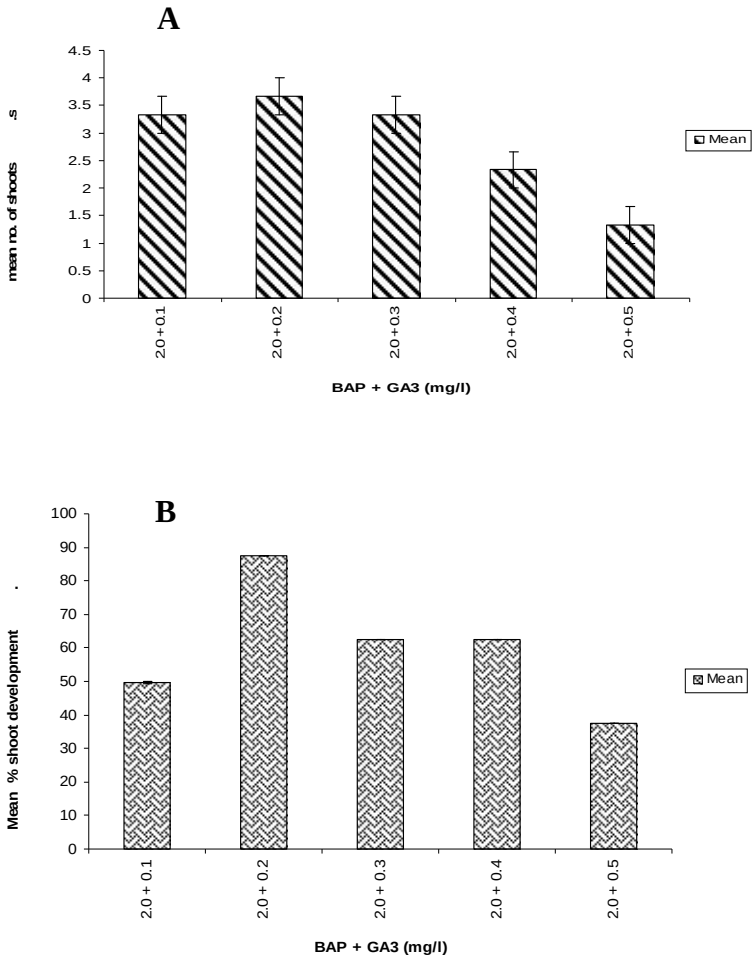

Figure 3 (A and B): The effects of five different concentrations of cytomix between BAP (2.0mg/l) and GA_3 (0.2mg/l) on (A) mean % shoot development and (B) mean number of shoots per explant

In conclusion, the present investigation has resulted in shooting protocol, which could be used in developing a rooting protocol for *ex-situ* conservation and true to type mass propagation of this plant with immense pharmaceutical relevance.

Future prospects

Micropropagation should play an important role in the conservation of medicinal plants, in the rapid multiplication and reintroduction to nature of endangered species, in the assessment and monitoring of biodiversity, as a source of new tools for conservation and in the search for new gene product of therapeutic use. Species of medicinal and aromatic plants at risk need to be multiplied with minimum loss of time and reintroduced for establishment in their natural habitats. In vitro protocol for multiplication of endangered species could be very useful for those taxa whose propagation through conventional means was difficult.

Acknowledgment

Many thanks go to the Principal Investigator of this research project especially for providing financial support for the Research through RPE, JKUAT. My deep appreciation also goes to the research, production and extension (RPE) division of Jomo Kenyatta University for Agriculture and Technology for funding the research.

References

Kukreja, A. K. and Mathur, A. K. (1985). Tissue culture studies in *Dubosia myoporoides. Planta Medica*, **2**: pp 93–96.

Maundu, P. and Teugas, T. (2005). Useful Trees and Shrubs of Kenya Technical handbook No. 35. Nairobi, Kenya: World Agroforestry centre-Eastern and Central Africa regional Programme (ICRAF-ECA).pp 400.

Nitsch, C. and Nitsch, J. P. (1967). The induction of flowering *in vitro* in stem segments of *Plumbago indica*. 1.The production of vegetative buds. *Planta*, **72**: pp 355-370.

Noad, T. C. and Birnie, A. (1989). Tree of Kenya. Pg 135.

Odum, E. P. (1971). Fundamentals of Ecology, W.B. Sacenders Company, USA, pp. 74-75.

Palgrave, K. C. (1988). Trees of South Africa 5th Ed, Struik publishers, Cape Town Pg 765-766.

Pattnaik, S. K. and Chand, P. K. (1996). *In vitro* propagation of the medicinal herb *Ocimum americanum* L. Syn. *O. canumsims* (hoary basil) and *Ocimum sanctum* (holy basil). *Plant Cell Reports*, **15**: pp 846–850.

Purohit, S. D., Dave, A. and Kukda, G. (1994). Micropropagation of Safed musli (*Chlorophytum borivilianum*), a rare medicinal herb. *Plant Cell, Tissue and Organ Culture*, **39**: pp 93–96.

Sahoo, Y., Pattnaik, S. K., Chand, P. K. (1997). In vitro clonal propagation of an aromatic medicinal herb *Ocimum basilicum* L (Sweet basil) by axillary shoots proliferation. In Vitro Cellular and Developmental. *Biology –Plant*, **33**: pp 293-296.

Titus, M., Damas, J., Quetin-Leclercq, J. and Angenot, L. (1991). From ethnobotanical uses of *Strychnos henningsii* to anti-inflammatory, analgesics and antispasmodics. *Journal of Ethnopharmacol*. **34** (2-3): 261-7.