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NOTES

IN VITRO PROPAGATION OF A TROPICAL BERRY, *PHYTOLACCA DODECANDRA*

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The soap berry plant, *Phytolacca dodecandra* L'Hér., belongs to the family Phytolaccaceae. Its distribution is reported in East, West, Central and Southern Africa, as well as parts of South America and Asia (Lemma 1984).

Berries of this plant have the potential of substituting expensive chemical molluscicides that are used in controlling the snail intermediate host of bilharziasis (Lemma 1984). *Phytolacca dodecandra* can be propagated from either seed or cuttings, though sexual propagation poses the problem of genetic variability in the offspring. In contrast to conventional vegetative propagation, through tissue culture, both disease-free and genetically uniform plants can be produced more rapidly.

Cytokinins such as benzyladenine (BA) have been used in micropropagation in order to improve the formation of multiple shoots (Edson *et al.* 1994, Yeo & Reed 1995, Molinar *et al.* 1996). A number of cytokinins are used in multiple shoot formation during *in vitro* propagation and their effectiveness is dependent on the plant genotype (Uddin *et al.* 1988). Of all the cytokinins that are currently in use, BA has been commonly applied because it is cheaper and reasonably effective on many plants (Lindgren & McCown 1992, Edson *et al.* 1994, Yeo & Reed 1995). Tigst and Hughes (1990) were able to generate up to three and five shoots of *Phytolacca dodecandra* on shoot tips and nodal explants respectively cultured on MS supplemented with 0.44 µM BA. The *in vitro* culture of *Phytolacca dodecandra* is relatively new, hence literature on the subject is scanty. The objective of this study was therefore to establish the response of *Phytolacca dodecandra* shoot tips to *in vitro* propagation.

Shoot tips, about 2 cm long, were excised from a clone planted outside the laboratory in 1982. Explants were rinsed under running tap water for 1 h followed by a 10 s immersion in 70% (v/v) ethanol and in 40% (v/v) aqueous sodium hypochlorite containing two drops of a detergent per 100 ml as a surfactant, followed by three successive 5-min rinses in sterile deionised water under the laminar flow hood.

The basal medium consisted of half-strength Murashige and Skoog (MS) inorganic salts, MS vitamins (Murashinge & Skoog 1962), 20 g l^{-1} sucrose and 0.8% Difco Bacto agar. The basal medium was supplemented with various concentrations of BA (0, 2.5, 5.0, 10.0, 20.0 or 40.0 µM) plus 0.5% activated charcoal (AC). Each treatment was replicated 20 times. The pH of the medium was adjusted to 5.0 before autoclaving at 121 °C for 20 min. The medium was dispensed in 10 ml aliquots into 25 × 150 mm borosilicate culture tubes capped with polypropylene closures (Bellco Kaputs, Vineland, N.J.). Cultures were grown at 27± 2 °C for a 16-h photoperiod under cool-white fluorescent lamps.

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Plantlets were rooted on half-strength MS medium without any auxin and were transferred to *ex vitro* conditions as potted plants after a total period of 10 weeks in an *in vitro* system. Acclimatisation was achieved in the screen-house by transferring plantlets straight into the soil for two weeks. Establishment under potted conditions in ordinary loamy soil was accomplished within a period of 6 months.

Data were analysed after ten weeks in an *in vitro* system by first evaluating shoot height, number of shoots, number of leaves and number of roots per explant. The experimental layout was a completely randomised design (CRD) and was repeated twice. Before the analysis was performed, data were transformed to $\log_e (x + 10)$ in order to make it nearly normal. The one way analysis of variance was performed using the MSUstat.

After 10 weeks, a significant mean *in vitro* height on 40 µM BA was achieved ($p = 0.01$). All other treatments had insignificant means at the same probability level (Table 1). Although BA may sometimes cause an increase in shoot height, this was not the case in all treatments other than 40 µM BA.

Similarly, the mean number of leaves $stem^{-1}$ (Table 1) over the same period was highest at 40 µM BA ($p= 0.01$).

Table 1. Effect of benzyladenine on *in vitro* growth of *Phytolacca dodecandra* shoot tips after 10 weeks in culture*

Conc. of BA (µM)	Mean shoot height (cm)	Mean no. of leaves $stem^{-1}$	Mean root number $explant^{-1}$
0.0	3.52 ± 0.01^{a}	2.5 ± 0.03^{a}	2.59 ± 0.05^{b}
2.5	3.17 ± 0.10^{a}	2.4 ± 0.03^{a}	2.30 ± 0.05^{a}
5.0	3.26 ± 0.11^{a}	2.4 ± 0.03^{a}	2.37 ± 0.05^{ab}
10.0	3.48 ± 0.09^{a}	2.5 ± 0.03^{a}	2.39 ± 0.05^{ab}
20.0	3.44 ± 0.10^{a}	2.5 ± 0.03^{a}	2.39 ± 0.05^{ab}
40.0	3.95 ± 0.11^{b}	2.7 ± 0.03^{b}	2.58 ± 0.05^{b}

*Means separation in columns by Newman-Keuls multiple comparison test (Lund 1986), $p = 0.01$.

The effect of BA on *in vitro* rooting of *Phytolacca dodecandra* did not give a well-defined pattern. However, in all treatments, root formation was possible without the exogenous application of an auxin (Table 1). In this study there was no apparent inhibition of rooting by BA.

Plantlets could be transferred to field conditions with 81% survival after six months (Figure 1).

The exogenous application of cytokinins like BA has been used in enhancing multiple shoot proliferation during *in vitro* propagation (Yeo & Reed 1995). The effects of BA tend to increase as the concentration increases. However, increased levels of cytokinins cause a progressive inhibition of the rooting system. In fact, even in shoots, higher BA levels lead to a strong inhibition of shoot elongation. Increased BA concentration can lead to an increased number of stunted shoots with little or no capacity for shoot elongation. On the other hand, some plants need the appropriate type and balance of both auxins and cytokinins in order to induce massive shoot proliferation (Uddin *et al.* 1988). The difference between these results and those of Tigst and Hughes (1990) on shoot formation may be attributed to genotypic differences in the experimental material that was used in the two cases (Uddin *et al.* 1988). However, more work needs to be done on shoot proliferation.

In conclusion, it has been established that *Phytolacca dodecandra* is amenable to *in vitro* propagation and that *in vitro* rooting is possible without exogenous auxins. This work further indicates that tissue culture has potential application in the propagation of *Phytolacca dodecandra*.

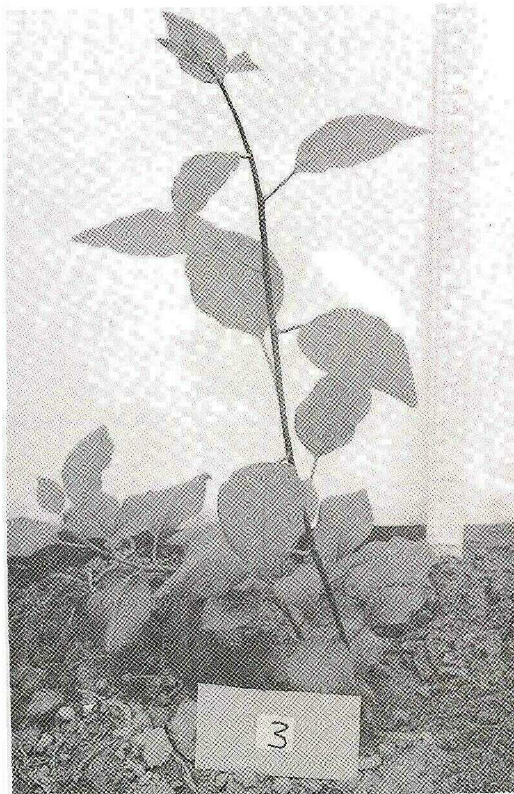

Figure 1. *Ex vitro Phytolacca dodecandra* at 6 months in the field

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