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CALLUS INDUCTION AND MULTIPLE SHOOT REGENERATION FROM SHOOT TIP CULTURES OF *TURNERA SUBULATA*

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Calli were induced from shoot tip cultures on MS medium using 3 conc. of phytohormones NAA and BAP with 1.5% sucrose in diffused light as primary cultures In 4 weeks organogenetic centres were induced in callus with rooting, shooting and rooting + shooting depending upon the concentrations of phytohormones used. Subcultures on MS medium with IAA, NAA, KN and BAP with 3% sucrose at intense light conditions resultud in multiple shoots and multiple shoots with roots in 3 weeks. Effect of sucrose concentration, light quality, hormonal concentrations on callus are discussed.

Keywords : Phytohormones; Multiple shoot; *Turnera subulata*.

Regeneration of plants *via* sho ot tip culture is common in plant tissue culture studies (Barghi, 1988; Bentz *et al.*, 1988). Several factors like medium, hormone manipulation and selection of incoculum plays pivotal role inducing organogenesis. Shoot tip is a giant explant with ready made pre-shoot organisation and requires only growth factors for shoot elongation and rooting (Murashige, 1974; Thorpe and Biondi, 1981). Evaluation and preservation of disease free stocks and to resist the steri-litant effect the shoot tips are ideal source with enhanced viability (Bajaj, 1983).

Turnera subulata, a bushy perennial ornamental exhibits dimorphic incompatibility and is rich in caffiene content (Rao *et al.*, 1988a) needs *in vitro* work combining horticultural, medicinal and genetical investigations. This communication deals with a method for rapid propagation of Turneras for commercial exploitation.

Shoot tips of field grown plants were washed 3–5 times in running tap water and detergents. They were also washed in ethanol (6–10 sec) and $HgCl_2$ of 0.1% (1–2 min.) with frequent washings in intervals. Since

the epidermal hairs of shoot tip hold traces of sterilitant, additional washings were given. The explants were inoculated on MS basal medium after cutting its distal end. The medium was gelled with 0.8% agar at pH 5.6, autoclaved for 15 min. at 15 lb^2 pressure. The basal medium was fortified with 3 concentrations of NAA and BAP as primary culture source with 1.5% sucrose (Table 1). Cultures were incubated at diffused light conditions at 25±2°C. The secondary cultures contained IAA, NAA, BAP, KN at different conc. and 3% sucrose and maintained 14–16 hrs. photoperiod and observations were noted after 3 weeks.

Calli were induced in all 3 concentrations of NAA and BAP as a set of primary cultures. Induced callus was soft, friable and white light green in two weeks. In 3–4 weeks the induction of organogenetic centres in calli were observed. Formation of the mature structures, *viz.*, rooting, shooting, rooting+ shooting depends on the phytohormones and their concentrations used. After 4 weeks the primary cultures need to be subcultured into fresh medium because reduced sucrose (1.5%) in medium promotes early anthocyanin pigment induction but diffused light delays its induction in *Turnera subulata* (Rao *et al.*, 1988c). The primary cultures were subcultured on 4 different media fortified with IAA, NAA, KN, BAP along with control with 3% sucrose (Table 1). For the sake of convenience the results are presented as under :

(i) MS + NAA 0.125 mg/l + BAP 0.065 mg/l (primary culture).

The subcultured calli of above traetment showed more multiple shoots with roots (MSR) in 3 weeks (Table 1). Here the cultures were with profuse rooting because of more NAA than BAP in primary culture medium which induces rapid root organogenesis. Among the subcultures MS + IAA 0.5 mg/l + NAA 0.25 mg/l + BAP 0.25 mg/l showed good response than other combinations and control for rapid multiple shoots with roots with less callogenesis (C)

(ii) MS + NAA 0.065 mg/l + BAP 0.125 mg/l (primary culture).

The cultures showed less callogenesis (C) rooting (R) because of more BAP than NAA in primary cultures which induced multiple shoots (MS) even when higher auxin concentration was used in subcultured medium. This probably explains that the pre-organogensis in primary culture is more effective than morphogenesis in subcultures (Table 1).

The subcultures showed increased efficiency of multiple shoots with roots (MSR) in MS + IAA 0.5 mg/l + NAA 0.25mg/l + BAP 0.25 mg/l.

Table 1. High Frequency of Multiple Shoot Regeneration in Shoot Tip Callus Cultures of *Turnera subulata.*

	C	R	S
1) MS+ NAA 0.125 + BAP 0.065 (primary cultures)			
Subculture			
1) MS basal medium	—	++	—
2) MS+IAA 0.5 + NAA 0.25+KN 0.25	+	+++	+
3) MS+IAA 0.5 + NAA 0.5 + BAP 0.5	++	+++	++
4) MS+IAA 0.5 + NAA 0.25 + BAP 0 25	+	+++	+++ (MSR)
ii) MS + NAA 0.065 + BAP 0.125 (primary culture)			
Subculture			
1) MS basal medium	+	—	+++
2) MS+IAA 0.5 + NAA 0.25 + KN 0.25	++	+	+
3) MS+IAA 0.5 + NAA 0.5 + BAP 0.5	+	+	+++ (MS)
4) MS+IAA 0.5 + NAA 0.25 + BAP 0 25	—	+	+++ (MS)
iii) MS + NAA 0.065 + BAP 0.065 (primary culture)			
Subculture			
1) MS + IAA basal medium	++	—	++
2) MS + IAA 0.5 + NAA 0.25 — KN 0.25	+	++	+
3) MS + IAA 0.5 + NAA 0.5 + BAP 0.5	+	++	++ (MS)
4) MS + IAA 0.5 + NAA 0.25 + BAP 0.25	—	++	++ (MSR)

MS : Murashige skoogs basal medium
\+ : Sign indicate the extent of response
C : Callogenesis
R : Rhizogenesis
S : Shoot bud induction
(MS) : Multiple shoots
(MSR) : Multiple shoots with roots

Note : All conc. of phytohormones are in mg/L.

This resulted in less callogenesis (C) and optimum organogenesis for rooting and shooting in subculture (Table 1).

The average number of shoots per inoculum is more in second treatment (20-30 shoots) than control and other treatments (1–12 shoots). Highest number of shoots was noticed in MS + IAA 0.5 mg/l + NAA 0.5 mg/l + BAP 0.5 mg/l (MS) while large number of largest shoots in MS + IAA 0 5 mg/l + NAA 0.25 mg/l + BAP 0.25 mg/l (MS) in second treatment (Table 1). The difference in response between large and total shoot number may be attributed to increased production of small shoots at higher rate. Higher conc. of total hormones induces calli among subcultures with reduced morphogenesis.

NAA in the medium helps in root elongation and IAA supported the action of NAA to counter the action of BAP which is known to stimulate shoot bud induction even in less concentration (Rao *et al.*, 1988b). NAA alone in high concentrations promotes exuberant callogenesis reducing thereby the regenerative potential of the callus. Kinetin appears to be a good substitute for BAP to control multiple shoot formation and indirectly for increased rooting efficiency.

The common practice of rooting of shoot tips is not required in the present system. Moreover, the phenol production from the cut ends of the wound tissue supresses rooting which is common in plants like *Coffea arabica* (Monaco *et al.*, 1977). To over come inhibition of rooting, induction of organogenetic centres and their subsequent regeneration was found to be convenient and equally easy.

These findings are comparable to *Yucca glauca* (Bentz *et al.*, 1988) and the only difference is the induction of preorganogenesis during subculture. Such low hormonal pre-treatment among primary cultures appears to be a good system to conserve germ plasm of disease free plants and to reduce the cost, time and space. The present findings suggest *Turnera subulata* to be an ideal experimental system for further investigation.

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