Auxin transport inhibitor 2,3,5 triiodobenzoic acid induced direct shoot morphogenesis in *Millingtonia hortensis* L.f.

Tissue culture studies have shown that *in vitro* response of explants is largely dependent on media composition, auxin-cytokinin ratio and the choice of explants. Pierik[1] defines the phenomenon wherein the position of explants in the mother plant influences its subsequent *in vitro* growth and development as topophysis. The plant hormone auxin is assumed to provide positional information for patterning during development. It is precise determination of auxins distributed across tissues and how it is sensed in space and time are uncertain. Interaction between several auxin response factors (ARFs), transcriptional activators and repressors in a complex network, control auxin-regulated gene expression[2]. Unwanted callus formation is an often encountered problem while establishing *in vitro* cultures of explants. Callus formation on the surface of the explants retards the incidence of direct shoot formation in *Millingtonia hortensis* L.f., a popular avenue tree.

The use of cytokinins in various concentrations is the usual approach for control of callus formation followed by shoot morphogenesis *in vitro*. Adventitious direct shoot generation has been reported with exogenous cytokinins application and amongst cytokinins, BAP has been used in a majority of cases[3]. TDZ has been reported to stimulate adventitious shoots in several plants of *Rosaceous* crops, including *Malus* species[4]. We report the use of TIBA concurrent with cytokinins BAP and TDZ for controlled callus formation resulting in successful direct shoot formation from second internodal explants of *M. hortensis.*

Young stems with elongated terminal internodes were collected from root suckers of an adult tree of *M. hortensis*. The plant material was washed under flowing tap water for 15 min and treated with a broad spectrum fungicide-Bavistin (Carbendazium, BASF) for 15 min. The stems were surface-sterilized with 0.1% w/v mercuric chloride and 0.1% w/v sodium lauryl sulphate for 8 min. The treated plant material was rinsed thoroughly thrice to ensure complete removal of mercuric chloride. Explants of 1.5 cm in length were prepared from the internodal regions and inoculated. One explant per tube was inoculated on solid MS media, 8/l agar supplemented with plant growth regulators BAP and TDZ. TIBA, in the concentration range of 1–5 mg/l was combined with BAP 1.5 mg/l and TDZ 0.5 mg/l. The pH of the media was adjusted to 5.6 preceding autoclaving at 121°C and 103.4 kPa for 20 min. The cultures were monitored and maintained at a 16-hour photoperiod with a photon-flux density of 10–15 uE m^{-2} s^{-1} provided by cool daylight fluorescent tubes for a period of 4 weeks. Data was collected from 30 replicates of each internodal explants. Statistical analysis was done with Student–Newman–Keuls multiple comparison tests using Graph Pad Software (San Diego, California, USA).

Amongst the three terminal internodes tested, only the second internode produced distinct bud initials of direct shoots in *M. hortensis*. Zeatin, kinetin and TIBA singly and in combination did not produce direct shoots in internodal explants of *M. hortensis*[5]. Endogenous hormonal content at specific zones in the explants is responsible for the *in vitro* tissue development[6]. The physiological gradient along the stem may determine the specific loci at which the meristemoids are initiated[7]. In *Populus* species, adventitious bud formation on internodal segments proximal to the shoot apex failed to produce shoot buds and the ability to form buds increased with increasing distance from the apex[8]. In Beech, the highest regeneration potential was obtained from apical internodes, while those distal to the apex were the least productive[9].

In the present study, BAP 1.5 mg/l and TDZ 0.5 mg/l produced direct shoots on the second internodal explants. Explants on MS media supplemented with BAP (1.5 mg/l) and TDZ (0.5 mg/l) singly produced 20.33% and 10.33% of direct shoots respectively (Figure 1). However, they rapidly turned into spots of callus

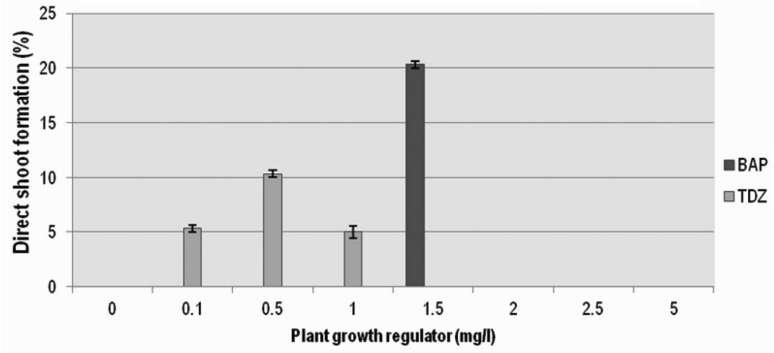

Figure 1. Graph showing direct shoot formation on second internodal explants cultured on MS media with 20 g/l sucrose, 8 g/l agar and varying concentrations of cytokinins BAP and TDZ singly at the end of 6 weeks.

Table 1. Direct shoots on second internodal explants cultured on MS medium with 20 g/l sucrose, 8 g/l agar and varying concentrations of TIBA in combination with BAP and TDZ singly at the end of 6 weeks

	Explants with direct shoot formation (%)	
TIBA (mg/l)	BAP (1.5 mg/l)	TDZ (0.5 mg/l)
0.0	20.33 ± 0.33 a[1]	10.33 ± 0.33 a[1]
1.0	34.67 ± 0.33 a	21.33 ± 0.00 b
2.0	46.00 ± 0.00 a	11.00 ± 0.58 a
3.0	63.33 ± 3.33 c	0.00 ± 0.00 c
4.0	72.00 ± 0.00 d	0.00 ± 0.00 c
5.0	10.00 ± 0.00 b	0.00 ± 0.00 c

[1]Values followed by the same letter in each column do not differ significantly from one another at $P > 0.05$ according to Student–Newman–Keuls multiple comparisions test.

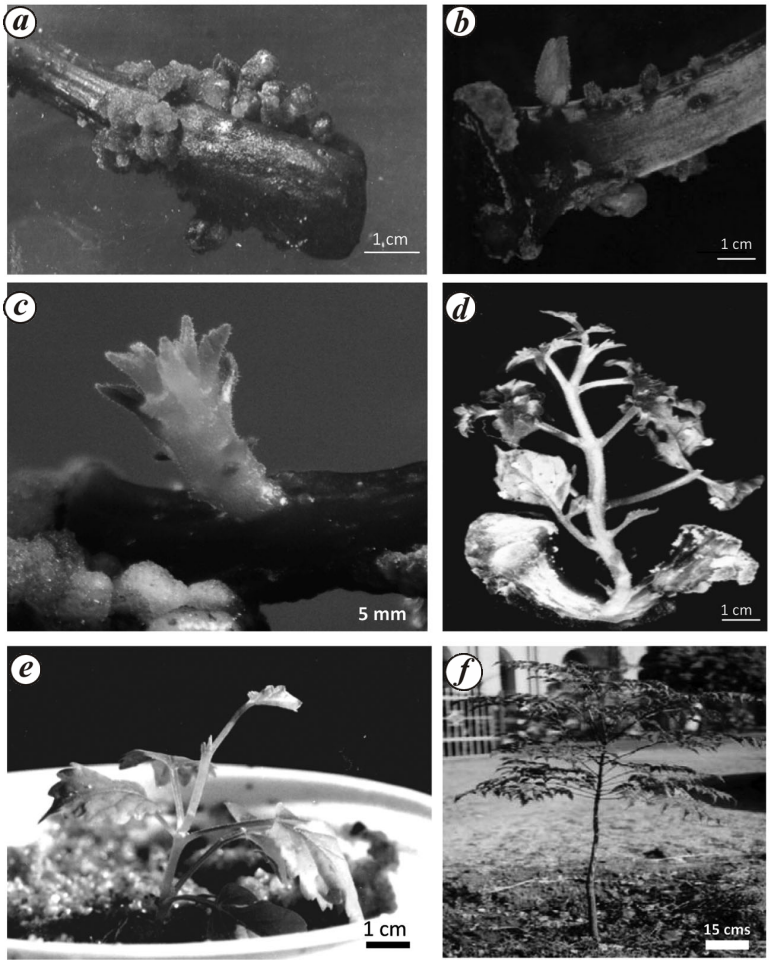

Figure 2. ***a***, Direct shoot formation on second internodal explants of *Millingtonia hortensis* cultured on MS media with BAP 1.5 mg/l without TIBA. ***b***, Direct shoots induced with reduced callus formation on second internodal explants with TIBA 4 mg/l and BAP 1.5 mg/l. ***c***, Early stages of elongation of direct shoot formed. ***d***, Elongated shoot. ***e***, Hardening of the rooted plant in sand: soil mixture. ***f***, Hardened *in vitro* plant after six months in the field.

on the internodal region (Figure 2 *a*). While BAP showed a very specific response to 1.5 mg/l concentration, TDZ responded in a gradient range between 0.1 and 1.0 mg/l. Direct shoot initials were not observed in concentrations beyond the mentioned range.

Incorporation of TIBA, an anti-auxin to the shoot initiation medium resulted in decreased callus formation in the internodal region along with significant increase in the number of explants with direct shoot initials (Table 1). Second internodal stem cultures (72%) produced direct shoots when cultured in combination with BAP 1.5 mg/l with TIBA 4.0 mg/l (Figure 2 *b*).

Auxin depletion from leaf primordia contributes to organ patterning[10]. The role of auxin inhibition on apical dominance[11] and direct somatic embryogenesis[12] has been reported. In *Arabidopsis*, auxin inhibitors are known to regulate phyllotaxy[13]. This is the first report of auxin depletion induced cytokinin aided direct shoot meristem establishment on internodal explants in *M. hortensis*. TIBA induced enhanced shoot formation at 4 mg/l concentration in combination with the cytokinin BAP (1.5 mg/l). Direct shoot formation (Figure 2 *c*) is a tissue-dependent (second internode) and auxin-cytokinin gradient dosage-dependent event indicating the role of topophysis in internodal explants cultures of *M. hortensis*.

The shoots produced by this technique were elongated (Figure 2 *d*) and rooted. The rooted plants were hardened (Figure 2 *e*) on sand : soil (1 : 1) mixture and successfully transferred to the field. Rooting and hardening were carried out according to methods standardized for rooting multiple shoots from nodal meristems[14]. The plant had grown up to 55 cm in six months (Figure 2 *f*). This improved method of direct shoot formation offers an alternative to micropropagation method of nodal culture propagation in *M. hortensis*.

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