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Research Article

MICROPROPAGATION OF *WRIGHTIA TINCTORIA*R.BR., – A TRADITIONAL MEDICINAL PLANT

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ABSTRACT

A standard protocol was established for mass propagation of the valuable medicinal plant*Wrightia tinctoria*R.Br., **(**Apocynaceae)through *in vitro* culture using nodal explants. The highest percentage (80%) of shoot induction was observed from nodal explants cultured on MS medium augmented with BAP (1.5mg/l), NAA (0.4 mg/l). The *in vitro* raised shootlets were transferred for rooting and the best result was achieved in MS medium supplemented with IBA (0.8mg/l). The plantlets were subsequently hardened to green house conditions.

Keywords :-Wrightia tinctoria, Internodal explants, Cytotoxicity.

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INTRODUCTION

Medicinal plants are gaining great importance right now because they contain large number of pharmaceutical compounds which are highly valuable to the human welfare. One of the important medicinal plants namely *Wrightia tinctoria*R.Br., contains rich anticancer activity that shows maximum cytotoxicity against skin cancer as well as substantial cytotoxicity towards cervical and lung cancer cell lines[1]. The juice of the tender leaves used efficaciously in jaundice. Crushed fresh leaves when filled in the cavity of decayed tooth relieve toothache; In Siddha system of medicine, it is used for psoriasis and other skin diseases [2,3]. It has got a very important place in traditional healing and thus it has been widely recognized as a medicinal plant[4]. An oil 777 prepared out of the fresh leaves of the plant has been assigned to analgesic, anti-inflammatory and antipyretic activities[5] and to be effective in the treatment of psoriasis[6]. The chemical constituents of this mature plant contain co-occurrence of β-amyrin, ursolic acid and olianolic acid along with β- sitosterol. Immature seed pods contain cycloartenone, β-amyrin, β-sitosterol, cycloeucalenol and a new terpenewrightial. A new sterol 14 α–methylzymosterol in addition to four rare plant sterols, desmosterol, cholesterol, 24-methylene-25-methylcholesterol and 24-dehydropollinastanol have also been isolated from seeds [7]. *De novo* regeneration *in vitro* has been reported for several medicinal, aromatic, economically important plants [8, 9]. The present study was aimed to develop a large scale multiplication protocol for *Wrightia tinctoria* using the nodal as well as internodal explants.

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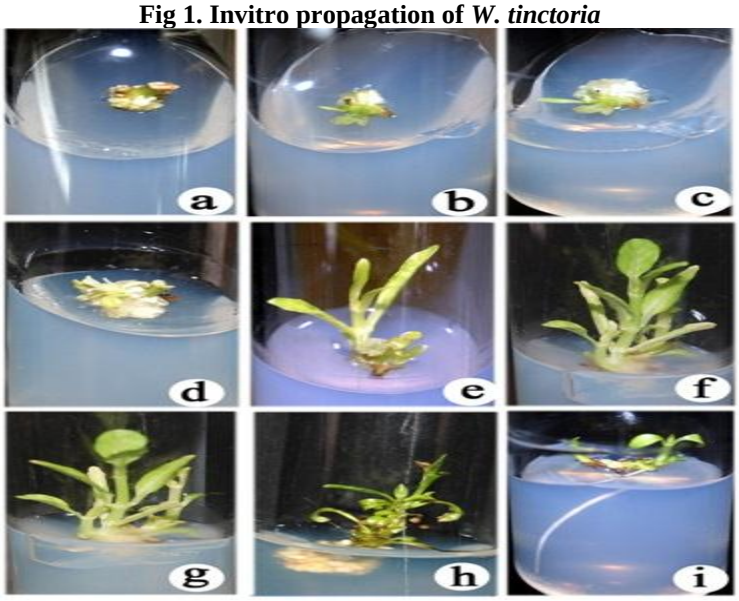
MATERIALS AND METHODS

The explants for this study was collected from the mother plant *Wrightia tinctoria* R.Br., that is internodal explants were collected from the healthy plants, thoroughly washed with running tap water for 10 min. followed by Bavistin (2%), tween 20 growing in the Herbal garden of Centre for Biodiversity and Biotechnology (CBB),St. Xavier's College (Autonomous), Palayamkottai. The nodal and treatments for 10-15 minutes to remove the superficial dust particles as well as fungal and bacterial spores. Then the explants were surface sterilized with 0.1% $HgCl_2$ for 3 min. followed by rinsing them three times with double distilled water inside the Laminar Air Flow chamber. Nodal segments of about 1.0 cm were prepared aseptically and were implanted vertically on MS medium fortified with 3% sucrose, 0.6%(w/v) agar and different concentrations and combinations of BAP (0.5–3.0 mg/l), and NAA (0.2 - 1.0 mg/l) for calli and shoot induction. The pH of the medium was adjusted to 5.8 with 0.1N NaOH before autoclaving at 121^{o}C for 20 min. The cultures were incubated at a constant temperature of 25 ± 2^{o}C with 12 hour photo period (2000 Lux). Proliferated shoots initiated from nodal segments were sub cultured for further multiple shoot induction. Regenerated multiple shoots were cut and individual shoots were transferred into MS medium containing different concentrations of IBA (0.2-2.0 mg/l) for root induction. For hardening, the *in vitro* raised plantlets with roots were removed from culture vials, washed thoroughly with double distilled water and planted in small polycups filled with sterile garden soil and sand (3: 1), covered by un-perforated poly bags and hardened for four weeks in a mist chamber before transfer to the field.

Fig 1. Invitro propagation of *W. tinctoria*



a-h) At MS + BAP (1.5mg/l) + NAA (0.4 mg/l) for shoot multiplication;
i) At MS + IBA (0.8mg/l) for root induction

Table 1. Effect of PGR on multiple shoots induction from nodal explants of *Wrightia tinctoria*

Plant growth regulators (mg/l)		No.of explants inoculated	% of response	No. of shoots/ explants ± SD	Intensity of Callus Development
BAP	NAA				
0.5	0.0	20	20%	1.42 ± 0.8	-
1.0	0.2	20	60%	4.62 ± 0.7	+
1.5	0.4	20	80%	7.62 ± 1.6	++
2.0	0.6	20	60%	5.21 ± 0.5	++
2.5	0.8	20	66%	3.76 ± 0.3	++++*
3.0	1.0	20	55%	-	+++
0.5	0.0	20	40%	1.14 ± 0.9	-
1.0	0.0	20	55%	2.42 ± 0.4	+
1.5	0.0	20	70%	4.67 ± 0.4	++
2.0	0.0	20	60%	4.09 ± 1.2	++

2.5	0.0	20	52%	3.06 ± 0.3	+++
3.0	0.0	20	50%	-	++++*

- Nil; +- Poor; ++- Moderate; +++- profuse; ++++*- Callus with shoots.

Table 2. Effect of IBA on multiple root regeneration from *in vitro* shoots of *W. tinctoria*

MS medium with IBA (mg/l)	Percentage of response	Maximum No. of Roots	Intensity of Callus Development
0.2	Nil	Nil	+
0.4	10%	1.20 ±0.09	+
0.6	20%	2. 21±0.10	+
0.8	60%	4.78±0.23	+
1.0	50%	2.55±0.15	+
1.2	30%	2.32±0.11	++
1.5	18%	1 11±0.15	++
2.0	Nil	Nil	++++

RESULTS AND DISCUSSION

Among the various concentrations of growth regulators such as BAP (0.5–3.0 mg/l), and NAA (0.2 - 1.0 mg/l) used for callus induction and shoot initiation, maximum percentage of white friable Calli basal calli was obtained on MS medium supplemented with BAP (2.5mg/l) and NAA (0.8mg/l) and maximum percentage (80%) of shoot initiation was obtained on MS medium with BAP (1.5mg/l) + NAA (0.4 mg/l) and the maximum number of shoots per nodal explants was observed as 7.62 ± 1.6. (Table 1; Fig.1.d & f). The results revealed that, BAP alone in the medium is not sufficient to induce multiple shoot initiation indicating the necessity of using combinations of auxins with that of cytokines. Similar work was done in *Aralia elata* and *Phellodendron amurense* nodal segments cultured on MS medium supplemented with 2.0mg/l BAP and 0.5mg/l NAA respectively[10]. However, multiple shoots induced from nodal explants of a 30-year-old tree of *Wrightia tinctoria* on MS medium supplemented with 2.0 mg/l of BAP only which affirms that only BAP could induce multiple shoots[11]. Whereas, multiple shoots obtained from cotyledons and shoot tips of wood apple (*Aeglemarmelos*) on MS medium augmented with BAP (2.0mg/l) and NAA (0.2mg/l) within fourteen days of inoculation[12]. This report portrays the similar combination of plant growth regulators used for the present study. In the same way, in *Alstroe meria* when the explants were inoculated on medium supplemented with BAP (1.5 mg/l) and NAA (0.2 mg/l) which is very close to the results obtained in the present study [13].

The *in vitro* raised shoots were transferred to MS medium augmented with IBA(0.2-2.0 mg/l) and maximum of 60% of rooting was obtained within 12 days on MS medium supplemented with IBA (0.8mg/l) and the maximum number of roots were 4.78±0.23 per shoot (Table 2; Fig 1). However, IBA (2.0 mg/l) alone induced basal callus instead of roots. The root induction of *Paederia foetida* were cultured the shootlets on MS medium supplemented with IBA (0.1 mg/l) and they were able to induce 95% of rooting which is very close to the present study undertaken[14]. The maximum (96.7%) percentage of roots induction was obtained in the ½ MS medium supplemented with NAA (5mg/l) were get good results[15]. In *Guizotia scabra* and *G. abyssinca* contain the multiple roots obtained from *in vitro* shoots were cultured onMS medium supplemented with NAA (0.2 mg/l)[16]. All these results show that NAA and IBA have been used either individually or in combination to induce rooting from the *in vitro* raised shouts. In short we can say that the results of this present study are in agreement with earlier reports.

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CONFLICT OF INTEREST

No interest

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