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In vitro Axillary Mass Multiplication of *Trichodesma indicum*

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ABSTRACT: In vitro mass propagation has been regarded as a significant technique for mass propagation of plantlets in a short period and utilization of cell suspension cultures for metabolite generation. It has helped in biodiversity conservation of many endemic plants since it provides a way for the utilization of plant germplasm without destroying natural habitats. *Trichodesma indicum* is one of the most significant medicinal herbs and desired for many formulations in the medical sector. Its overexploitation from natural habitats may be a threat to its diversity. The authors have proposed an optimized protocol for plantlet generation with greater viability potential and could be used to mass propagate plants in a short period. It was evident from the study that variable combinations of plant growth regulators and sucrose were able to alter the multiplication potential of axillary buds and it could be enhanced with the proper combination.

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INTRODUCTION

Herbal medicines have been used as good plant origin products for the treatment of various diseases and disorders [1, 2]. Not only the Indian traditional medicinal system as Ayurveda but Yunani literature also emphasized using plants as sources in multiple therapies. *Trichodesma indicum* is a significant herb used in the Indian system of medicine to cure for fever and disorders related to eye and ear. The anti-inflammatory, anticancer potential and treatment of joint disorders using the extract of this plant are very popular [3, 4]. It is found in western Shivalik hills and adjacent plains in scattered patches. It is also reported in other parts of the country in dry wasteland areas. It is an erect, branched annual herb with hairs springing from tubercles. The leaves are stalkless opposite, lance-shaped, 2-8cm long, pointed at the tip and heartshaped at the tip [5, 6]. The flowers occur singly in the axils of the leaves. Flower colour is usually violet, light blue or purple. The calyx is green, hairy with pointed sepals. The fruit is ellipsoid and is enclosed by the calyx. The plant is reported to have antimicrobial [7], Antidiabetic [8]; insecticidal and herbicidal activities [9].

Tissue culture is now being commonly used for not only mass propagation of plants with medicinal potentials but they also provide suitable plant models for studies related to stress plant physiology due to strictly controlled growth conditions [10-13]. Authors have standardized and optimized mass propagation protocols with various combinations of plant growth regulators for the generation of *in vitro* plantlets for its commercial uses.

MATERIALS AND METHODS

Young plants of *Trichodesma* were collected from plains nearby Shivalik hills, western Uttar Pradesh, India and nodal explants with axillary buds were taken from young & healthy plants for culture initiation. Explants were surface sterilized using 3-4 drops of Tween-20 for 3-4 min. followed by surface disinfection with 0.2% $HgCl_2$ (w/v) for 3 minutes [13-17]. The nodal segments were then inoculated under aseptic conditions on Murashige and Skoog's (MS) medium [10, 11]. The medium was supplemented with usual salts and vitamins and 3% sucrose (w/v; Hi- Media), 100 mg/litre myo-inositol (E. Merck) and 0.8% agar (w/v; Hi- Media).

Media were supplemented with various concentrations of BAP (6-benzylamino purine) alone and in combinations with NAA (α- naphthalene acetic acid) and IAA (Indole-3- Acetic acid). The pH of the media was adjusted to 5.8. The cultures were kept at 25 ± 2^0C under illumination with white fluorescent tubes (50 µM $m^{-2}s^{-1}$) at 82% relative humidity. They were maintained under light for 14 h. followed by 10 h. dark period.

Each treatment was used in replicates to avoid manual errors and to get repeated accuracy in results.

Sprouting of axillary buds was seen on nodal segments after 15-25 days of culture (Fig. 1 a). These buds, with part of the growing nodal segments, were subcultured on media supplemented with BAP (1.30-4.40 µM) + IAA (1.40-2.30 µM) or NAA (0.44-1.33µM) for further shoot multiplication. Nodal explants (0.7-0.9 cm) from the axenic shoots were recultured on agar solidified medium containing different concentrations of BAP, NAA and IAA. Roots were then induced in shoots that attained the height of 1.5 cm± 0.5 cm by transferring to MS medium supplemented with different combinations of IAA, and NAA. The roots were initiated in the rooting medium as well as in basal medium. The basal medium was unable to produce roots.

Nine-week old plantlets were transferred to pots containing sterilized soil and sand mix (1:1), covered with polythene bags with perforations, for 10 days and the pots were kept below 25±2ºC, for acclimatization. These were then transferred to green house, after removing polythene covers, for hardening [16-19].

RESULTS AND DISCUSSION

Best induction of multiple shoot formation from leaf explants occurred on medium containing BAP (3.40 µM) and IAA (2.80 µM). Amongst different combinations of plant growth substances used, maximum shoot regeneration per explants was found to take place with BAP (3.4 µM) + NAA (2.40 µM) & BAP (3.3 µM) + NAA (2.80 µM) (**Figure 1a and b**) with about 22 & 24 shoots respectively in 50 days. A high dose of BAP (>5 µM) and NAA (>4 µM) resulted in inhibition of shoot multiplication and induction rate/s (**Table 1**).

Root initiation was tried with combinations of BAP, IAA and NAA, but the best root growth was promoted by BAP (0.50 µM) used with NAA (1.75 µM, **Figure 1c**). Using GA3 (0.30-0.45 µM) along with BAP and NAA was observed to be most suitable for shoot elongation and maturation (**Figure 1d**). It was recorded from the experiments that differences in sugar concentration (2.4-4% w/v) also affected the shoot multiplication from axillary buds (**Figure 3**). It was also evident from the studies that very high BAP concentration affected the nodal multiplication ratio and higher concentrations of BAP were observed to be inhibitory rather than being stimulatory in nature (**Figure 2**). It was observed in the present investigations that multiple plant regeneration from nodal explants of *Trichodesma indicum* could be induced on MS medium with PGRs. The plant multiplication rate was dependent on appropriate combinations of plant growth substances (PGSs). The acclimatized plantlets with moderate humidity in green house (70%-80%) and temperature 28± 5^0C were best for the generation of viable plantlets. % viability was recorded to be more than 87% that could be regarded as one of the best viability percentage (**Table 2**). The current work provides preliminary information and methodology for the rapid propagation of this valuable plant from axillary bud explants that might help in the improvement of conservation methods.

Table 1. Effect of Plant Growth Substances on shoot multiplication from cultured explants in *Trichodesma indicum* (Values are means ± SE of five replicates per treatment)

S. No.	Plant Growth Substances (conc. in µM)	25 Days		50 Days	
		No. of shoots	Average length of shoots (mm)	No. of roots	Average length of roots (mm)
1.	Control	0	-	0	-
2.	BAP (2.35)	5	1.7 ±0.3	7	11 ±0.9
3.	BAP(2.40)+NAA (2.10)	10	1.8 ±0.4	12	12 ±0.85
4.	BAP (1.65)+ IAA (1.80)	12	2.8.0 ±0.8	16	14 ±0.8
5.	BAP (3.40)+ NAA (2.80)	14	3.2 ±1.2	22	17±1.2
6.	BAP (3.40)+ NAA (2.40) + GA_3 (0.40)	15	4.7 ±0.95	24	25 ±1.4
7.	BAP (4.50)+ NAA (3.00)	5	1.3 ±0.2	9	22 ±1.2

*Only those combinations are included in the table that exhibited optimum results.

Table 2: Survival of plantlets under *ex vitro* conditions (Values are means ± SE of five replicates repeated thrice)

Group No.	No. of plantlets produced & transferred to pots	No. of surviving plants after 60 days of plantation	Survival percent (%)
1	35	29	82.85
2	40	34	85.00
3	37	32	86.5
4	45	41	91.1
5	36	33	91.7
Average survival % 87.43			

Figure 1 *In vitro* mass propagation of *Trichodesma indicum* axillary bud proliferation (a & b), Root development (c) and maturation of a viable shoot (d).

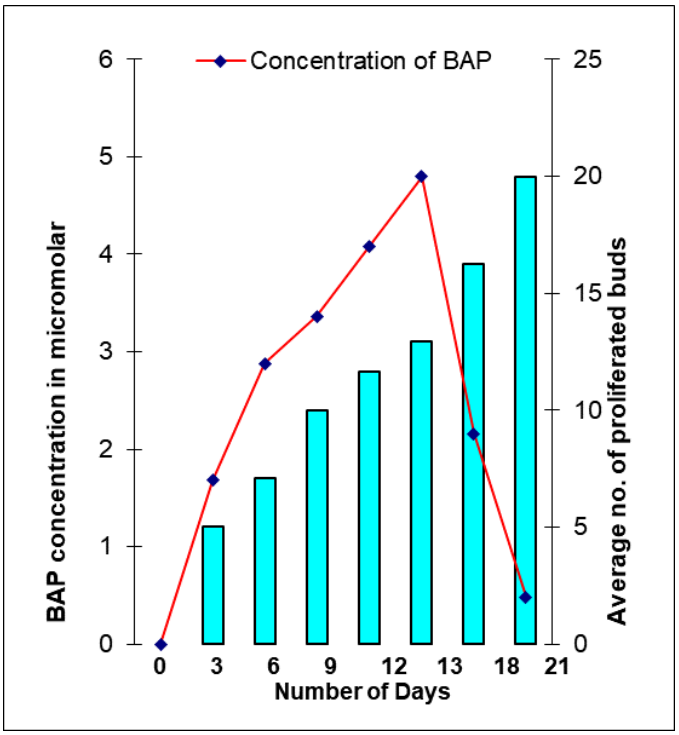

Figure 2 Effect of BAP on nodal multiplication and its relation with the multiplication ratio.

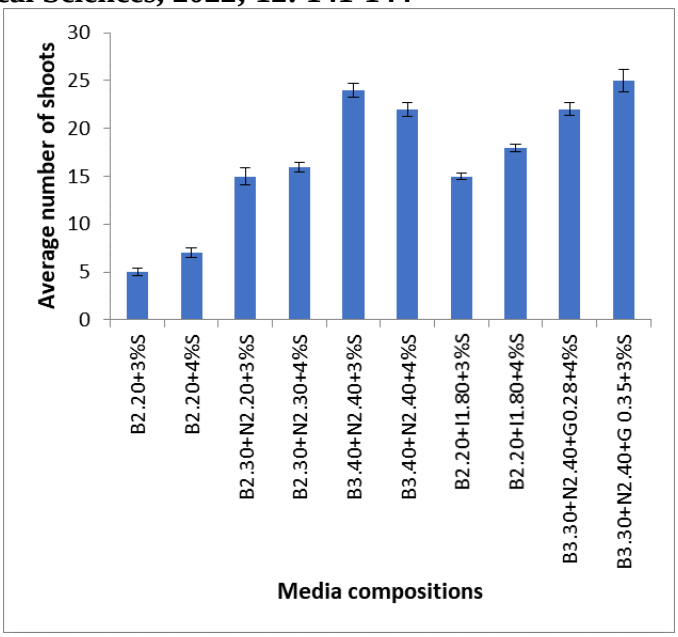

Figure 3 Alteration in shoot multiplication with combinations of PGRs and sucrose concentrations.

Trichodesma indicum is high-value medicinal plants and its mass propagation strategy can be used further in suspension cultures to obtain suitable secondary metabolites for commercial uses in antioxidant, antimicrobial, anticancer and other activities [17]. It is widely used in many traditional medicines prescribed under different systems of medicine. The current study provides supplementary data for not only mass propagation at a large scale but also can help in getting model plantlets under optimized conditions for stress physiology experiments.

CONCLUSION

The mass propagation of endemic and endangered plants is significant with respect to the biodiversity conservation strategies. Authors have made an attempt to mass propagate *Trichodesma* plantlets in shorter period. It can help researchers to utilize the data for suspension cultures and metabolite production *in vitro*.

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DATA AVAILABILITY

Not declared

ETHICS STATEMENT

The authors have taken all the necessary permissions as per ethical guidelines wherever applicable. The authors will be responsible for all the technical content mentioned in the manuscript. Journal and Publisher will not be responsible for any copyright infringement and plagiarism issue.

CONFLICTS OF INTEREST

The authors have no known conflict of interest.

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AUTHORS' CONTRIBUTION

All the authors were contributed for manuscript preparation, conducting of the experiments, data collection, interpretation and analysis of the result.

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