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Research Note

Callus induction in *Hydnocarpus pentandra* (Buch. Ham.) Oken using shoot tips

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Abstract
Hydnocarpus pentandra (Buch. Ham.) Oken is a Vulnerable medicinal tree found in Western Ghats of India. It is highly exploited due to its medicinal properties and it is mainly used in leprosy treatment. An experiment was undertaken at Division of Plant Genetic Resources, ICAR-Indian Institute of Horticultural Sciences, Bengaluru during 2018-19. The experiment was laid down in Completely Randomized Design (CRD) with 16 treatments and three replications. Shoot tips from young seedlings were used as explant and treated in MS media with a different hormonal combination like Benzyl Amino Purine (BAP), Indole Acetic Acid (IAA), Kinetin and Thiaduzerol (TDZ). Among the treatments, highest shoot length (2.06 cm) and maximum number of shoots produced per explant (3.86) were found in the treatment combination of BAP (1.0 mg/l) and TDZ (0.5 mg/l) followed by the treatment combination of BAP (2.4 mg/l) and IAA (0.05 mg/l). Few treatments showed callus formation but no shoots were developed in these treatments.

Keywords
Vulnerable medicinal tree, Growth hormones, Explant, Callus induction

Hydnocarpus pentandra (Buch. Ham.) Oken is an important medicinal tree belonging to family Flacourtiaceae**.** The tree is endemic to the Western Ghats and very common in the south and central Sahyadri's. The tree grows up to 10 meters tall. Seeds are economic part and oil is extracted from the seeds. This oil is called chaulmugra oil and it is used in leprosy treatment. It is a biodiesel plant and also having anti-cancerous property (Shyam *et al*., 2013). Because of over-exploitation from the natural resources, its population decreased. So this tree is considered as a vulnerable plant in Red Data Book. In these days this tree is gaining importance due to its medicinal properties. But in this plant, seed germination is low and large amount of planting material production is difficult. No other vegetative method was developed in this plant due to woody its nature of the tree (Kumar *et al*., 2015 a, b). This species is dioecious in nature and having a long juvenile phase. So in order to solve these problems, an attempt was made to develop tissue culture techniques to produce a large number of quality planting materials.

Preparation of explant and surface sterilization: Shoot tips were collected from one-month-old seedlings at Division of Plant Genetic Resources, ICAR-Indian Institute of Horticultural Research, Bengaluru. The explants of 2.0 – 2.5 cm in length were dissected out from the seedlings and collected in distilled water. Collected explants were washed with tap water for five minutes to remove adhering dirt particles. The explants were disinfected with Tween 20 solution (0.1%) for 10 minutes and washed thoroughly with sterilized distilled water for 4 to 5 times. Then explants were brought to culture lab. Under the sterile conditions, the explants were treated with 0.1 per cent $HgCl_2$ solution for 5 to 6 minutes. The explants were then thoroughly washed (4-5 times of washings) with sterilized distilled water to remove traces of $HgCl_2$.

Preparation of media and inoculation:MS media was prepared by adding all its constituents and then the p^H of the medium was adjusted to 5.8 ± 0.2 using HCl or NaOH. Then the appropriate amount of growth regulators were added and agar was mixed at the time of boiling. About 15-20 ml of this media was filled in test tubes. The test tubes were plugged with polypropylene caps and autoclaved at 15 lb/in^2 pressure and 121^{0}C temperature for 15 to 20 minutes. After cooling of autoclaved media the sterilized explants were inoculated in the media. This process was carried out in the laminar chamber and all the cultures were maintained in standard culture conditions at a temperature of 26 ± 2^{0}C. The source of illumination consisted of four feet wide fluorescent tubes (40W) and incandescent bulb (25W) for 16 hours and darkness for eight hours. After the inoculation weekly observations were taken, the experiment was laid out in CRD by following the procedure outlined by Panse and Sukhatme (1967)

Table 1. Number of shoots initiated and length of induced shoots in explants of *Hydnocarpus pentandra* (Buch. Ham.) Oken at different combination of growth hormones in MS media.

Treatment	MS media + Growth regulator (mg/l)	Number of shoots produced per explant	Shoot length (cm)	Callus initiation
T_1	BAP (0.1)	0.0	0.0	--
T_2	BAP (0.1) + IAA (0.05)	0.0	0.0	++
T_3	BAP (0.1) + Kinetin (0.05)	0.0	0.0	--
T_4	BAP (0.2)	0.0	0.0	++
T_5	BAP (0.2) + IAA (0.05)	0.0	0.0	--
T_6	BAP (0.2) + Kinetin (0.05)	0.0	0.0	++
T_7	BAP (0.5)	0.0	0.0	--
T_8	BAP (0.5) + IAA (0.05)	0.0	0.0	++
T_9	BAP (0.5) + Kinetin (0.05)	0.0	0.0	++
T_{10}	BAP (1.2)	1.43	0.93	--
T_{11}	BAP (1.2) + IAA (0.05)	1.56	1.20	--
T_{12}	BAP (1.2) + Kinetin (0.05)	1.93	1.06	--
T_{13}	BAP (2.4)	2.43	1.46	--
T_{14}	BAP (2.4) + IAA (0.05)	2.62	1.63	--
T_{15}	BAP (2.4) + Kinetin (0.05)	2.60	1.53	--
T_{16}	BAP (1.0) + TDZ (0.5)	3.86	2.06	--
	Mean	1.03	0.61	
	S. Em.	0.094	0.054	
	CD @ 1%	0.35	0.19	

++ Callus produced, -- No callus production

In the present study, callus was initiated in five of the treatments (T_2, T_4, T_6, T_8 and T_9) and no callus was produced in a four of the treatments (T_1, T_3, T_5 and T_7). In other seven treatments no callus was initiated and direct organogenesis was found, i.e. Shoots were directly produced from explant without producing callus (**Table 1**). The maximum number of shoots produced per explant (3.86) and the highest shoot length (2.06 cm) were found in the treatment combination of BAP (1.0 mg/l) and TDZ (0.5 mg/l) followed by BAP (2.4 mg/l) and IAA (0.05 mg/l). Similarly the direct organogenesis was found in bael when treated with growth regulators like BAP, IAA, Kinetin and TDZ (Kumar and Seeni, 1998; Nayak *et al*. 2007). Similar results were obtained in *Cinnamomum camphora* when shoot tip and nodal segments were used as explant (Sharma and Vashishta, 2010a, 2010b, 2010c). Withal the nodal explants showed direct organogenesis in *Ficus religiosa* in treatment combination of BAP, TDZ and IAA (Siwach and Gill, 2011) and in *Lawsonia inermis* (Ram and Shekhawat, 2011) and in *Pongamia pinnata* (Sujatha and Hazra, 2007). It is due to the effect of BAP which is involved in the shoot proliferation. Similar results were obtained in *Oroxylum indicum* (Talari *et al*. 2016). Lower concentrations of TDZ are responsible for shoot initiation in woody plants (Lu, 1993).

Combination of BAP (1.0 mg/l) and TDZ (0.5 mg/l) gave maximum number of shoots per explant and highest shoot length. Higher concentration of BAP in combination with TDZ or IAA gave good results.

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