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Standardization of callus induction in danthapala (*Wrightia tinctoria* R.Br.)

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Abstract
Wrightia tinctoria R.Br. (Family: Apocynaceae) commonly called "Indrajau" is distributed throughout the world and occurs abundantly in India. It is a deciduous tree with white fragrant flowers. The seeds and bark of this plant are used in Indian traditional medicine as anti-diarrheal, anti-dysenteric and also for the treatment of psoriasis. Callus induction is an important stage in micropropagation and was largely influenced by the medium, plant growth regulator, and explant.The present study was conducted at Onattukara Regional Agricultural Research Station, KAU, Kayamkulam to standardise the media for callus induction. The results revealed that callus induction from nodal segments started 20 days after inoculation and was high in MS medium containing BA 1 mg l^{-1} and 2,4-D 1.5 mg l^{-1}. The callusing from the leaf started within 25 days after inoculation. It shows best in MS medium containing 1 mg l^{-1} NAA and 2 mg l^{-1} 2,4-D.

Keywords: Wrightia tinctoria, callus, explant, nodal segment, leaf

1. Introduction
Wrightia tinctoria R. Br. belonging to the family Apocynaceae is commonly called as "Jaundice curative tree" in South India. It is a medium sized tree seen in most of the deciduous forests of India including Western Ghats. The juice of the tender leaves is used against jaundice. Crushed fresh leaves when filled in the cavity of decayed tooth relieve tooth ache. In Siddha system of medicine, it is used for psoriasis and other skin diseases. An oil prepared out of the fresh leaves of the plant has been assigned to analgesic, anti-inflammatory and antipyretic activities (Ghosh *et al*., 1985) [5], and to be effective in the treatment of psoriasis. Leaves indicated the presence of flavonoids, glycoflavones-iso-orientin and phenolic acids. Seed is well known for its medicinal effect and is being traditionally used for the treatment of various ailments such as anthelmintic, antidiarrheal, antidysenteric, astringent, febrifuge, tonic and in flatulence.

Lack of rapid natural regeneration and over-exploitation has reduced its population drastically. The use of tissue culture techniques for clonal propagation otherwise known as micro propagation has now been extended to a wide range of plants of economic importance. This includes trees, shrubs and herbaceous species. *In vitro* techniques can be either direct or callus mediated regeneration and are having advantages like high multiplication rates, potential for the production of disease free plants, plantlet production independent of seasons or time of the year and uniform plant quality. Micropropagation offers a rapid means of producing clonal planting stock for afforestation, woody biomass production and conservation of elite and rare germplasm (Bajaj, 1986) [2]. Cloning of mature tree is difficult because with ageing, the ability of shoots to root diminishes considerably, but success has been achieved in several systems where explants have been obtained from mature trees (Purohit and Kukda, 2004) [6]. Plant tissue culture techniques are also used as an alternative method for the production of specific metabolites. By standardising the callus induction protocol in *Wrightia tinctoria* R. Br. i*n vitro* production of secondary metabolites using cell cultures can also be initiated. As leaves are rich source of flavonoids, glycoflavones-iso-orientin and phenolic acids, the callus produced from leaves can be used as a source of these chemicals.

Callus is a group of dedifferentiated plant cells induced in media containing relatively high auxin concentrations or a combination of auxin and cytokinin under *in vitro* conditions. In medicinal plants, metabolites are present in leaves and establishing *in vitro* callus cultures from leaves and using them for the extraction of compounds would be an ideal alternative.

Callus cultures containing the bioactive substances are collected at a specific stage dried and the bioactive principle is extracted.

2. Materials and Methods

Experiment was conducted at plant tissue culture laboratory, Onattuka Regional Agricultural Research Station, Kayamkulam and experiments were laid out in Completely Randomized Design (CRD) with three replications.

2.1 Explant and sterilisation procedure

Nodal explants and leaves collected from mature trees maintained in the field of Onattukara Regional Agricultural Research Station, Kayamkulam were used as explants which was taken from the healthy and well maintained plants. Explants were washed thoroughly with sterilized distilled water having a few drops of Tween-20. Then the explants were surface sterilized with 0.1% $HgCl_2$ for 3 min. followed by rinsing three times with double distilled water inside the Laminar Air Flow chamber.

2.2 Culture conditions and callus induction

Explants were inoculated on various culture media like full and half strength MS with varying concentrations of hormones. The cultures were kept under controlled conditions of temperature (28±2°C), light (4000 lux) for 16 hrs/day provided by fluorescent tubes and 60-70% relative humidity (RH). For each experiment, a minimum of 10 replicates were taken and repeated thrice. Observations were recorded every 3 weeks.

2.3 Results and Discussion

In the present study callus induction of nodal segments and leaf discs of *W. tinctoria* was done by using MS medium as the basal medium which is supplemented with different combinations of auxin and cytokinin. Auxins used were 2,4-D, NAA, IBA and cytokinin used was BA. The results are presented in Table1 and Table 2 respectively.

In the micropropagation of danthapala high callus induction from nodal segments was observed in MS medium containing BA 1mg l^{-1} and 2,4-D 1.5mg l^{-1}. The callusing from leaf started within 25 days after inoculation. It showed best in MS medium containing 1mg l^{-1} NAA and 2mg l^{-1} 2,4-D. The leaf callus obtained was compact in texture and cream in colour. Callus induction was also observed in other media combinations like MS + 1mg l^{-1} NAA + 1.5mg l^{-1} 2,4-D, MS + 1mg l^{-1} BA + 1mg l^{-1} 2,4-D, MS + 0.5mg l^{-1} IAA + 1.5mg l^{-1} 2,4-D, MS + 1mg l^{-1} BA + 0.5mg l^{-1} 2,4-D, MS + 0.5mg l^{-1} NAA + 2.5mg l^{-1} 2,4-D, MS + 0. mg l^{-1} NAA + 0.5mg l^{-1} 2,4-D, MS + 1mg l^{-1} IBA + 2mg l^{-1} 2,4-D, MS + 1mg l^{-1} NAA + 2.5mg l^{-1} 2,4-D. The results revealed that, auxins or cytokinins alone in the medium is not sufficient to induce callusing which indicates the necessity of using combinations of hormones. Arulanandam *et al.* (2017) [1] also reported that maximum percentage of white friable calli in *W. tinctoria* was obtained on MS medium supplemented with BAP (2.0mg l^{-1}) and NAA (0.8mg l^{-1}). All combinations of plant growth regulators were conducive to callus induction from leaf and nodal segments, where the ratio of explants with callus induction was very high in media with high concentration of 2,4-D. Compact and white callus had high regeneration capacity.

Plant tissue culture represents an important technique in basic science and commercial application. In all major families of terrestrial plants, wounded tissue is recovered by non-differentiated callus cells. These callus cells can be cultured *in vitro* for biotechnological applications. Almost any part of the plant can be used to generate callus cultures. Explants taken from plant tissues slowly grow *in vitro* into a cell mass that ranges from amorphous and colorless to pale-brown, if they are obtained under sterile conditions avoiding microbial infection and cultured on solid gel medium supplemented with growth hormones. By passaging the cells regularly, callus cultures can be indefinitely maintained *in vitro*. Callus cells are similar to non-differentiated meristemic cells; they reveal only small vacuoles and lack chloroplasts for photosynthesis, among other features. Callus cultures can re-differentiate into entire plants, if maintained under appropriate growth media that differ from standard culture media. They can be distinguished between cultures that grow in a rather compact form, and those that are friable. Friable callus cultures can be used to generate single-cell cultures that are maintained in slowly shaken liquid medium.

Callus cultures may be used for the sustainable and large-scale production of secondary metabolites in pharmaceuticals, cosmetics, food, and related industries. Callus cultures from medicinal plants produce bioactive phytochemicals that can be used to treat a wide variety of diseases; furthermore, the produced chemical substances do not seem to be limited to certain chemical classes, but have a wide chemical variety. As phytochemicals can be directly extracted from calli without sacrificing the entire plant, the callus technology may help to protect rare and endangered plant species, and sufficient amounts of secondary metabolites can be produced. Callus cultures can also be converted to single-cell suspension cultures growing in flasks on shakers or in biofermentors in order to produce the desired secondary metabolites. This allows growth under controlled conditions without the influence of varying environmental factors, seasonal variation microbial diseases, pests, and geographical constraints. Hence, secondary metabolites with constantly high quality can be produced. As callus and suspension cultures harbor the full genetic information of whole plants, they possess the totipotency for the biosynthesis of secondary metabolites. Moreover, tissue culture technologies open the possibility to manipulate the biosynthesis pathways of plant cells to produce derivatives of secondary metabolites with improved features for the market. Furthermore, biotransformation reactions can be used to convert specific substrates to desired end products (Efferth, 2019) [3].

Micropropagation has now become the method of choice for the propagation of several important plant species. The plant selected here for micropropagation is of great relevance to the economy of Kerala and the well being of people especially in the rural areas.

W. tinctoria is a medicinal plant that have been affected by rapid loss in the recent decades in the state. Large scale planting of these plants will thus be necessitated if continued availability is to be ensured. However problems in mass propagation of the plant *W. tinctoria* create hurdles in their cultivation on a big scale. An attempt to standardise callus induction protocol for this important species has been made in this study.

Table 1: Response of nodal segments of *W.tinctoria* to varying combinations of hormones in the induction of callus

Sl. No	Media	Rate of callusing	Nature of callusing
1	MS + 1.0 BA + 0.5 2,4-D	++	Friable and white
2	MS + 1.0 BA + 1.0 2,4-D	++	Compact and White
3	MS + 1.0 BA + 1.5 2,4-D	+++	Compact and White
4	MS + 0.5 NAA + 0.5 2,4-D	++	Friable
5	MS + 1.0 NAA + 2.0 2,4-D	+++	Compact and White
6	MS + 0.5 IAA + 0.5 2,4-D	++	Friable
7	MS + 1.0 NAA + 1.5 2,4-D	++	Compact and White
8	MS + 0.5 NAA + 2.5 2,4-D	+++	Compact and White
9	MS + 1.0 IBA + 0.5 2,4-D	++	Friable
10	MS + 1.0 NAA + 2.5 2,4-D	+++	Compact and White

Table 2: Response of leaf segments of *W.tinctoria* to varying combinations of hormones in the induction of callus

Sl. No	Media	Rate of callusing	Nature of callusing
1	MS + 1.0 BA + 0.5 2,4-D	++	Friable and white
2	MS + 1.0 BA + 1.0 2,4-D	+	Compact and pale yellow
3	MS + 1.0 BA + 1.5 2,4-D	+++	Compact and pale yellow
4	MS + 0.5 NAA + 0.5 2,4-D	++	Friable and white
5	MS + 1.0 NAA + 2.0 2,4-D	+++	Compact and pale yellow
6	MS + 0.5 IAA + 0.5 2,4-D	++	Friable and white
7	MS + 1.0 NAA + 1.5 2,4-D	+++	Compact green
8	MS + 0.5 NAA + 2.5 2,4-D	+++	Compact and pale yellow
9	MS + 1.0 IBA + 2.0 2,4-D	+++	Compact and pale yellow
10	MS + 1.0 NAA + 2.5 2,4-D	+++	Compact pale yellow

Responses
+ - low, ++ - medium, +++ - high
Observation recorded after 3 months

Plate 1: Callusing of nodal segments in MS+BA 1.0 mg l^{-1} +2,4-D 1.5 mg l^{-1}

Plate 2: Callusing of leaf segments in MS + 1.0 mg l^{-1} NAA + 2 mg l^{-1} 2,4-D

Plate 3: White and friable callus of nodal segments in MS + 1.0 BA + 0.5 2,4-D

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