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In Vitro Propagation of Nadun (*Pericopsis mooniana*) Through Callus Culture

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ABSTRACT. Cotyledon and hypocotyl explants of Nadun (Pericopsis mooniana) were cultured in a MS medium supplemented with cytokinin (BAP) and auxins (2,4-D or NAA) for callus establishment. The highest response in callus formation from both explants was observed in the media supplemented with BAP and 2,4-D. The callus was compact, nodular and brown in colour. However, shoot formation occurred only in cotyledonary callus grown in the medium supplemented with BAP (2.0 mg/l) and NAA (5.0 mg/l). The highest response in shoot formation was obtained in the medium supplemented with 5.0 mg/l BAP (with or without CH). The isolated shoots produced roots when the concentration of macro-elements of the basal medium, supplemented with IBA and NAA (5.0 mg/l) was reduced to half and was used for culturing shoots.

Key to abbreviation:

AS: adenine sulphate, BAP: 6-benzyl amino purine, CH: casein hydrolysate, CM: coconut milk, 2,4-D: 2,4-dichloro phenoxy acetic acid, IBA: indole-3-butyric acid, MS: Murashige and Skoog (1962) medium, NAA: naphthalene acetic acid, YE: yeast extract.

INTRODUCTION

The world demand for wood and wood products continues to grow. Present tree improvement and production programmes are not adequate to meet the growing demand. Further, large populations of quality planting materials are needed for reforestation purposes. In order to meet this increasing demand for planting material, attempts have been made recently to mass propagate commercially important tree species.

A large number of woody plants belonging to angiosperms and gymnosperms have been grown in culture from a variety of explant

sources, resulting in several advancements of organogenesis and embryogenesis (Winton and Huhtinen, 1976). But only a few have been successfully regenerated from tissue cultures. In many species the frequencies of regeneration were very low, and not of commercial value (Winton, 1978). In the case of rubber (*Hevea brasiliensis*), Paranajothy (1974) has reported adventive embryos in very low frequencies. Later Chinese scientists have established plantlets of rubber from cultured pollen (Han and Shui, 1980). Plantlets of sandalwood (*Santalum album*) has also been raised by somatic embryogenesis of shoot callus cultures of mature trees of 20 - 25 years (Lakshmi *et. al.*, 1980).

The tropical legume tree species are well known to provide food, forage, timber, firewood and manure. Some of them are best suited for reforestation programmes. Leguminous timbers (*Babhia*, *Pterocarpus*, *Dalbergia*) have been in the world market for centuries and command a high price in international trade (Panel, 1979). Nadun (*Pericopsis mooniana*) too belongs to the family Leguminosae. It is indigenous to Sri Lanka and is distributed in a small area in the low country wetzone (Trimen, 1894). Nadun provides valuable timber for the furniture industry. Mature trees required for this purpose are very rare in the forests and hence it is now thought that it could be included among the endangered plant species. The objective of this study was to investigate the feasibility of *in vitro* propagation of Nadun through callus as a means of rapid propagation.

MATERIALS AND METHODS

Nadun seeds collected from the Royal Botanical Gardens, Peradeniya were dipped in 70% (v/v) ethanol (2 sec), then mechanically scarified and surface sterilized for 20 minutes using 10% clorox to which two drops of Tween - 20 were added. The seeds were then rinsed thrice in sterilized distilled water and grown on a hormone - free Murashige and Skoog (1962) medium with 3% w/v sucrose (MS basal medium) under a light (1000 lux, 16h).

Cotyledon and hypocotyl explants (1 cm length) were obtained from the 15 - 20 day old seedlings, grown in the basal medium. The explants were introduced into MS basal medium modified either with all possible combinations of BAP (0, 0.5 and 2.0 mg/l) and 2,4 - D (0, 0.5, 2.0 and 5.0 mg/l) or all possible combinations of BAP (0, 2.0, 5.0 and 10.0 mg/l)

and NAA (0, 0.5, 1.0 and 5.0 mg/l) for the establishment of callus cultures. The cultures were kept in the dark. The growth of explants, cultured in media with BAP and 2,4 – D was observed following 30 days of culture. The explants in media supplemented with BAP and NAA was observed after a growth period of 60 days.

Hypocotyl and cotyledonary callus (60 day old) grown on BAP (2.0 mg/l) and 2,4 – D or NAA (5.0 mg/l) were introduced into different nutrient media for shoot formation. The shoot regeneration media were also derived from MS basal medium. These media contained BAP (2.0 or 5.0 mg/l) with or without AS (100 mg/l), CH (100 mg/l), CM (10% v/v) or YE (100 mg/l). At 90 days in culture, the percentage of cultures which produced shoots were recorded.

Subsequently the concentration of macro – elements of MS basal medium was reduced to half and the sucrose to 2% w/v. This medium was modified with different levels (0 – 0.5 mg/l) of IBA and NAA (alone or in combination), and was used for induction of roots from shoots, isolated from the callus cultures. At 60 days in culture percentage of rooting of these shoots was recorded.

After adjusting the pH to 5.6 using 0.1 N NaOH or 0.1 N HCl, each nutrient medium was solidified with 0.75% w/v agar and was sterilized by autoclaving at 15 PSI for 20 minutes. Unless otherwise mentioned all the cultures were incubated in an air – conditioned culture room at 26 $\pm$ 1 C and illuminated (1500 – 2000 lux) with white fluorescent tubes for 16 hours daily.

RESULTS AND DISCUSSION

Callus formation from both explants was influenced by the hormonal composition of the nutrient medium. It appeared within 10 – 15 days of culture, when the nutrient media were supplemented with both BAP and 2,4 – D. The media with BAP and NAA induced callus formation within 14 – 20 days of inoculation. The growth responses of cultured explants are summarized in Table 1 and 2. From the tissue, cultured hypocotyl explants did not produce any adventitious organs in the media used. However, cotyledons produced roots and/or shoots in some of the media tested (Table 2).

Table 1. Effect of BAP and 2,4 – D on Callus formation in hypocotyl and cotyledon explants at 30 days in culture.

Growth regulators (mg/l)		Callus formation	
BAP	2,4 – D	Hypocotyl	Cotyledon
0	0	–	–
0	0.5	+	+
0	2.0	–	+ +
0	5.0	–	+
0.5	0	–	–
0.5	0.5	+	+
0.5	2.0	+ + +	+ +
0.5	5.0	+ + +	+ + +
2.0	0	–	–
2.0	0.5	+ +	+
2.0	2.0	+ + +	+ +
2.0	5.0	+ + +	+ + +

(Average of 10 replicates)

– no callus
+ poor callus
+ + fair callus
+ + + good callus

Table 2. Effect of BAP and NAA on callus formation and organ formation in hypocotyl and cotyledon explants at 60 days in culture.

Growth regulators (mg/l)		Callus formation		Organogenesis in Cotyledon callus (%)	
BAP	NAA	Hypocotyl	Cotyledon	Rooting	Shooting
0	0	–	–	0	0
2.0	0	–	–	0	0
5.0	0	–	+	0	10
10.0	0	–	+	0	10
0	0.5	–	–	20	0
2.0	0.5	–	+	0	10
5.0	0.5	–	+	0	10
10.0	0.5	–	+ +	0	10
0	1.0	–	–	30	0
2.0	1.0	+	+ +	0	0
5.0	1.0	+	+ +	0	30
10.0	1.0	+	+	0	10
0	5.0	–	–	10	0
2.0	5.0	+ +	+	30	30
5.0	5.0	+ + +	+ +	0	10
10.0	5.0	+ + +	+ +	0	0

(Average of 10 replicates)

– no callus
\+ poor callus
\+ + fair callus
\+ + + good callus

The cut ends of the cultured explants began to swell during the first few days. The epidermis was intact but uneven, covering the tissue bulges. The swelling of the tissue may have been due to cell division and enlargement. The epidermal layer ruptured in several places (2–3), exposing proliferating masses of cells within 10–20 days of culture. These masses of cells gave rise to callus. The callus was compact, nodular and light brown at the early stage of development. At later stages (after 60 days) it gradually became dark brown.

In Nadun, either 2,4–D or NAA combined with BAP was used to induce callus from hypocotyl and cotyledon explants, and rapid callus induction was achieved from the media supplemented with 2,4–D and BAP. 2,4–D has been widely used to induce callus in forest tree species such as *Acacia koa* (Skolmen and Mapes, 1976), *Arterocarpus heterophyllus* (Narasimhan *et. al.*, 1970) *Santalum album* (Rao and Bapat, 1978) and *Tectonia grandis* (Narasimhan *et. al.*, 1970). Several researchers have reported the use of BAP in callus formation (Zaerr and Mapes, 1982) and results with Nadun also indicated that BAP enhanced the callus formation in both explants in the presence of either 2,4–D or NAA.

The organogenesis of shoot buds were observed only with the cotyledonary callus in the medium supplemented with BAP (2.0 mg/l) and NAA (5.0 mg/l), while other callus types failed to produce shoots even after 90 days in culture. The highest response was observed from the media supplemented with 5.0 mg/l BAP and 5.0 mg/l BAP plus 100 mg/l CH in which 60% of the cultures produced shoots (Table 3).

In *Eucalyptus* too the callus cultures derived from hypocotyls did not differentiate (Lakshmi, 1981) and this finding is similar with the results in organogenesis of shoot buds from hypocotyl callus of Nadun. In certain woody plants eg. *Acacia koa* (Skolmen and Mapes, 1976), *Santalum album* (Rao and Bapat, 1978), BAP has been used to induce shoot differentiation. Supplementing additives such as AS, CH, CM and YE in tissue culture techniques, have been shown to have varying effects on shoot formation. Mridula *et. al.*, (1983) reported that supplementing the medium with CH resulted in callus differentiation giving rise to many shoots in *Sapium sebiferum*. Mathews and Rangen (1981) observed in pineapple that transfer of callus cultures to a MS medium supplemented with CH and CM, induced shoot generation and that the effect of CM on regeneration was not influenced by either the presence or absence

Table 3. Influence of composition of nutrient media on shoot formation in hypocotyl and cotyledonary callus, established in media containing BAP and 2,4 – D or NAA (at 90 days in culture).

Composition of medium (Amounts in parenthesis are in mg/l except in CM)	Percentage cultures which produced shoots			
	Callus grown in 2,4 – D + BAP		Callus grown in NAA + BAP	
	Hypocotyl	Cotyledon	Hypocotyl	Cotyledon
MS + BAP (2)	0	0	0	0
MS + BAP (2) + AS (100)	0	0	0	0
MS + BAP (2) + CH (100)	0	0	0	40
MS + BAP (2) + YE (100)	0	0	0	0
MS + BAP (2) + CM (10% v/v)	0	0	0	0
MS + BAP (5)	0	0	0	60
MS + BAP (5) + AS (100)	0	0	0	40
MS + BAP (5) + CH (100)	0	0	0	60
MS + BAP (5) + YE (100)	0	0	0	40
MS + BAP (5) + CM (10% v/v)	0	0	0	10

(Average of 10 replicates)

of CH in the medium. However, Noh *et. al.*, (1988) observed that CM at concentrations 0.1 – 5% (v/v) inhibited the formation of adventitious shoot primodia as well as shoot elongation of *Pinus resinosa*. Organogenesis in *Eugenia grandis* callus was not obtained in a media supplemented with AS at concentrations of 100 – 400 ppm (Lee and Rao, 1981). In this experiment of organogenesis in Nadun callus, AS and CM inhibited the regeneration of shoots and these results are comparable with the observation made by Noh *et. al.*, (1988) and Lee and Rao (1981).

The influence of different combinations of IBA and NAA on adventitious root formation of cultured Nadun shoots is given in Table 4. After 10 – 14 days in culture, emergence of root primodia was best

Table 4. Effect of different combinations of IBA and NAA on rooting of shoots at 60 days in culture.

Growth regulator (mg/l)		Frequency of rooting (%)
IBA	NAA	
0	0	–
1.0	0	–
2.0	0	–
5.0	0	–
0	1.0	20
1.0	1.0	–
2.0	1.0	–
5.0	1.0	–
0	2.0	–
1.0	2.0	–
2.0	2.0	20
5.0	2.0	–
0	5.0	20
1.0	5.0	20
2.0	5.0	–
5.0	5.0	60

(Average of 5 replicates).

observed in shoots that were cultured on the medium supplemented with 5.0 mg/l IBA and 5.0 mg/l NAA. At 60 days 70% of shoots produced roots.

Since auxin is essential for *de novo* regeneration of adventitious roots from shoots, most of the media have been supplemented with this hormone. Indole – 3 – butyric acid is particularly associated with rooting of shoots of species such as *Cerotinia siliqua* (Sebastian and McComb, 1986) and *Eucalyptus grandis* (Lee and Rao, 1981). However, the highest response on root induction was obtained in cultured Nadun shoots when a mixture of two auxins (IBA and NAA) were added to the media. There is some evidence that a mixture of auxins has been tried with success on inducing roots of cultured shoots (Mridula *et. al.*, 1983).

Since there are a few problems, such as low frequency of shoot regeneration and establishment of plantlets in soil, remaining to be solved yet, a future programme will include the results from this study. However, it can be concluded that Nadun can be propagated *in vitro* through callus cultures.

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