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Synthesis of Indigenous callus culture and artificial seeds from *Celastrus paniculatus* Willd.via nodal segments

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Abstract

Celastrus paniculatus Willd. is an Indian medicinal plant which has been used for thousands of years in the traditional Ayurvedic system of medicine. It is fast gaining importance in the primary healthcare systems as well as in herbal drug formulations. In the present investigation an efficient and reproducible *in vitro* protocol for large scale callus cultures and artificial seeds of *Celastrus paniculatus* by nodal segments as explant has been done on MS medium containing different combination like BAP+NAA(1+0.5mg/ml), BAP+NAA(1+0.2mg/ml) ,2,4-D+Kn (0.5+1.0mg/ml) and 2,4-D+BAP (0.5+0.5mg/ml).Best response in terms of callus cultures was obtained in IAA+Kn (1+0.5mg/ml) under controlled condition of 16 h of photoperiod and 8 h dark period at a temperature of 25 ± 2°C. Further synthetic seed production was also investigated using the sodium alginate (NaAlg) encapsulation technique. This study might provide a new insight through protocol development for micropropagation and synthetic seed production of many important plant species with economical relevance.

Key words: *Micropropagation, nodal segments, Celastrus paniculatus.*

Abbreviations: BAP, -6-benzylamino purine**; Kn**, kinetin**; NAA**, α-naphthalene acetic acid**; IBA**, indolebutyric acid, **IAA**- Indole acetic acid

1. Introduction

Celastrus paniculatus Willd.(Celastraceae) commonly known as Malkangni, Jyotishmati, Bitter sweet black oil plant, climbing staff tree and intellect tree is a rare and endangered important medicinal plant with vine like habit reaching up to a height of 10 m. It is distributed throughout India upto an altitude of 1200 m, mainly in deciduous forests. The species is vulnerable in Western Ghat of South India (Rajesekharan and Ganeshan, 2002). Seed oil is often the characteristic of ***Celastrus paniculatus Willd.*** which is an important ayurvedic medicinal plant gaining popularity in the primary healthcare system and in herbal drug formulations.Its seed oil is reported to be beneficial in stimulating intellect and sharpening the memory. It has also been reported in cognitive impairment (Gattu M.*et al.*1997), sedative property (Gatinode BB*et al.* 1957),free radical scavenger (Russo A *et al.*1957), anti-spermatogenic (Wangoo1988 and Bidwai(1990),anti-oxidant(Russo *et al.*2001), analgesic and anti-inflammatory (Ahmad F *et al.* 1994), hypolipaedemic (Mathur*et al.* 1993), anti-malarial activity (Pavanandt K *et al.* 1989),Anti-bacterial activity (Patel RP. *et al.*1962),Pandya KK *et al.* (1990).Bark is reported to be abortifacient, depurative and a brain tonic and taken internally for snake bite (Govil, 1993).The seed oil is useful for treating abdominal disorders, beriberi and sores. Leaf sap is an emmenagogue and antidote for opium poison- ing (Warrier et al., 1994). Root-bark extract also shows antimalarial activity (Rastogi and Mehrotra, 1998). The powdered root is considered useful for the treatment of cancerous tumours (Parotta, 2001). Chemical constituents of seeds as revealed by phytochemical analysis were sesquiterpene alkaloids like celapagine, celapanigine and celapanine (CSIR, 1992).

The conventional method of propagation of this medicinally important plant is through seeds. Poor seed viability and germination (11.5%) restricts the use of seeds in multiplication (Rekha et al., 2005). Indiscriminate over exploitation from natural source to meet the growing demand by pharmaceutical industry coupled with low seed viability, lack of vegetative propagation methods and insufficient attempts for replenishment of wild stock of this medicinally important plant species have contributed to its threatened status. So realizing the threat of extinction and to meet the growing need of pharmaceutical industry, a mass multiplication protocol was developed for its better future supply.

2. Materials and Methods

Nodal explants from mature plants growing in wild in Jaipur were collected. The explants were initially washed under running tap water. Finally these were surface sterilized under aseptic conditions with freshly prepared 0.1% (w/v) mercuric chloride solution for 3 - 5 min and then given a dip in absolute alcohol. After this, these explants were washed with sterilized double distilled water 4 - 5 times. The surface sterilized explants (10 mm long) were inoculated on MS medium (Murashige and Skoog, 1962) containing 3% (w/v) sucrose and 0.8% (w/v) agar-agar supplemented with 0.2 ,0.5, 1.0 mg l-1 cytokinins (BAP and Kn) and auxins (IAA, NAA and 2, 4-D) individually as well as in various combinations. The cultures were incubated in culture room under controlled condition of 16 h of photoperiod and 8 h dark period at a temperature of 25 □□2°C. The intensity of light was approximately 2000 lux.

2.1 Preparation of plant hormones

Different concentrations and combinations of various auxins and cytokinins were dissolved in distill water and pre added in the media before autoclave. Initially auxins were dissolved with one drop of ethanol and then volume were raised with distill water while cytokinins were dis-solved in one drop of ethanol and HCl and volume was make up with distill water.

2.2 Incubation

Cultured flasks were incubated in culture chamber. The temperature of chamber was maintained at 25 ± 10 LC and 1,200 lux light intensity. A photoperiod of 16 h light was provided. The cultures were observed and examined every week and final morphogenetic data were recorded.

2.3 Subculturing procedure

Explant About six explants from ten replicates including germinated seeds, nodal explants and in vitro grown seedlings were inoculated to fresh MS medium without hormone and later MS medium (Murashige and Skoog 1962) supplemented with various concentrations of hormones.

3. Result

3.1 Callus induction

Nodal explants showed initial swelling followed by initiation of callus from the cut ends within 2nd day of inoculation on media [MS_7] supplemented with IAA:Kn (1:0.5 mg l^{-1}) and best response in terms of undifferentiated mass of callus (about 95 %) was

Table 1- Callus production from nodal segment of *Celastrus paniculatus* Willd on MS medium supplemented with varied concentration of growth hormones

S. No.	Growth hormones	Modified MS media	Concentration (mg l-1)	Explant used Nodal segment Frequency (in %)	Morphological appearance	Nature of callus	Days of Initiation
1.	(i) Without hormones						
	MS	MS_{00}		-	–	–	
	(ii) Callus induction						
2.	2,4-D+BAP	MS_1	2 + 1	–	–	–	–
3.	2,4-D+BAP	MS_2	0.5 + 0.5	60	GY	FG	12
4.	BAP+Kn	MS_3	1+ 0.5	22	BN	FG	21
5.	BAP+NAA	MS_4	1 + 0.2	75	BL	HD	16
6.	BAP+NAA	MS_5	1+0.5	88	BL	HD	7
7.	2,4-D+Kn	MS_6	0.5+1	20	WH	FG	25
8.	IAA+Kn	MS_7	1+0.5	95	GN	FG	2
9.	BAP+NAA	MS_8	0.5 + 0.5	15	BN	FG	28
10.	BAP+IAA+Kn	MS_9	1+1+0.2	50	BN	FG	11
11.	BAP+IAA	MS_{10}	1	65	BN	FG	4

GY – Grey, BN – Brown, BL – Black, WH – White, FG – Fragile, HD – Hard., "–"no response

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obtained on media [MS_7] followed by another induction of callus from nodes was observed on media supplemented with BAP:NAA (1:0.5 mg l^{-1}; MS_5) and BAP: NAA (1:0.2 mg l^{-1}; MS_4). The texture of callus became green and fragile when media [MS_7] was supplemented (1.0:0.5 mg l^{-1}) with IAA:Kn and Brown and fragile when media [MS_{11}]were supplemented with BAP:IAA (1.0:0.2 mg l^{-1}).When media was supplemented with BAP:NAA (1.0:0.5 mg l^{-1} ;1.0:0.2) the texture of callus became black and hard. Callus turned grey and fragile when proliferated from nodal segments grown on media (MS_2) supplemented with 2,4-D:BAP (0.5:0.5 mg l^{-1}). Fragile and whitish callus induction from nodal segment was observed when media [MS_6] was supplemented with 2,4-D:Kn (0.5:1.0 mg l^{-1}). Growth of the callus was slow during first 4 weeks and later the proliferation of callus increased for next 2 weeks and finally from 7th week the growth became stationary and then declined. During 8th week the media started to turn brown, which might be due to leaching out of phenols and accumulation of phenolic compounds in the medium. The probability of survival and proliferation of C.paniculatus callus tissue on subculturing the callus during 5–6th week was higher in comparison to subculturing after 7–8th week (Table1)

Fig. 1, Green fragile callus in IAA+Kn (1+0.5 mg l^{-1})

Fig. 3, Blackish and hard callus in BAP+NAA (1+0.2mg l^{-1})

Fig. 2, Brown colored fragile callus BAP+IAA (1+0.2 mg l^{-1})

Fig. 4, Blackish and hard callus in BAP+NAA (1+0.5mg l^{-1})

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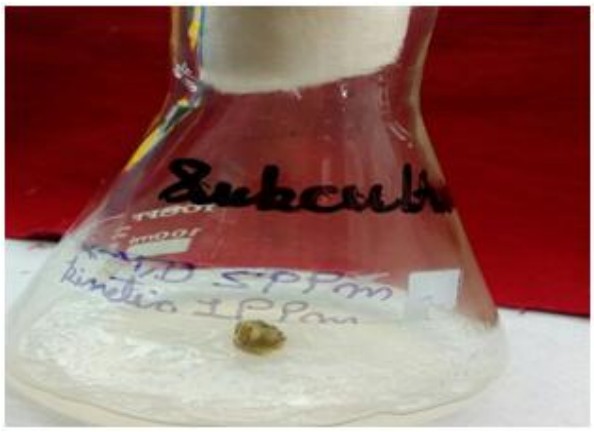

Fig. 5, Fragile and whitish callus in 2,4-D+Kn (0.5+1.0mg l^{-1})

Fig. 6. Grayish and fragile callus in 2,4-D+BAP (0.5+0.5mg l^{-1}l)

4. Discussion

In the present investigation callus was raised from nodal segments on various combination doses of IAA:Kn (1:0.5 mg l^{-1}), BAP:NAA (1:0.2 mg l^{-1}), BAP:NAA (1:0.5 mg l^{-1}) unlike other reports mentioned above. The presence of auxins and cytokinin in the culture medium regulates various aspects of dedifferentiation and differentiation (Woodward and Bartel 2005) at cellular levels. Generally, auxins have been used for callus induction and proliferation, and both cytokinins and auxins were required for redifferentiation of callus into organized cell (Wang et al. 2008).

In the present investigation, selected experimental plant will be practicised in concern of the polycystic ovary syndrome problem of females as it is reverence to them.

If ***Celastrus paniculatus Willd.*** plant will grow in Kota region of Rajasthan then peoples which have problems related to irregular menstrual cycle, polycystic ovary syndrome, neurodegenerative disorder, memory loss, epilepsy, insomnia, hypertension etc. can take benefits from this plant.

The results of these experiments demonstrated the via-bility of the micropropagation technique for the mass reproduction and it can be useful as a tool for in vitro germplasm conservation of the Celastrus species.

5. Conclusion

Nodal explants showed initial swelling followed by initiation of callus from the cut ends within 2nd day of inoculation on media [MS_7] supplemented with IAA:Kn (1:0.5 mg l^{-1}) and best response in terms of undifferentiated mass of callus (about 95 %) was obtained on media [MS_7] followed by another induction of callus from nodes was observed on media supplemented with BAP:NAA (1:0.5 mg l^{-1}; MS_5) and BAP: NAA (1:0.2 mg l^{-1}; MS_4) and the plantation of this plant can cure such disease related to irregular menstrual cycle as it's caused no side effects and have marvelous medicinal property, it is very much commercially exploitable. Hence, it will be chosen as a source for the development of drug for treatment of Polycystic Ovary Syndrome and this use of medicinal plant will also give boon to the future researchers. Many more such researches are still going on and the potential health benefits of ***Celastrus paniculatus Willd.*** looks very promising. .

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