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Research Article

IN VITRO CALLUS INITIATION AND ORGANOGENESIS FROM SHOOT TIP EXPLANTS OF *TINOSPORA CORDIFOLIA* (WILLD.) MIERS EX HOOK. F & THOMS

Hemant Sharma[1] and Vashistha B.D. [2]
[1]Department of Botany, Dayanand P.G. College, Hisar-125001, Haryana, India
[2]Department of Botany, Kurukshetra University, Kurukshetra-136119, Haryana, India
[*]Author for Correspondence

ABSTRACT
In the present study, *in vitro* callus initiation and organogenesis of *Tinospora cordifolia* using different plant growth regulators has been studied. Callus was induced from shoot tip explants on MS medium and woody plant medium supplemented with IAA, NAA and 2, 4-D at 0.5, 1.0, 2.0 and 4.0 mg/l of concentrations. After sixty days, average fresh and dry weights of calli were measured. Maximum fresh and dry weights were obtained at a concentration of 4 mg/l of plant growth regulator. Shoots were developed on woody plant medium containing 0.5, 1.0, 2.0 and 4.0 mg/l of BAP and Kn.

Keywords: *Shoot Tips, Callus, Organogenesis, Tinospora cordifolia*
Abbreviations: **MS**- Murashige and Skoog, **WPM**- Woody plant medium, **BAP**- 6 benzylaminopurine, **Kn**- Kinetin, **IAA**- Indole-3-acetic acid, **NAA**- a-Naphthalene acetic acid; **2,4-D**- 2,4-Dichloro phenoxy acetic acid

INTRODUCTION
Tinospora cordifolia (Menispermaceae), commonly known as Giloy, is a woody climber known for its valuable medicinal properties. In Ayurveda, it is given to the patient suffering from fever, diabetes, jaundice, skin diseases, urinary disease and piles. Its decoction is well-known for improving immune system of the body. Propagation of plant takes place mainly through stem cuttings. Since, the availability of seeds is very poor and climatic conditions do not favor the growth of stem cuttings. Therefore, its propagation has limited potential for large scale production. *In vitro* culture method is considered as most suitable for the propagation of *T. cordifolia*.
Plant regeneration under *in vitro* condition occurs through direct and indirect organogenesis. The indirect organogenesis takes place via callus formation. There are some reports on direct organogenesis in *T. cordifolia* through nodal explants only (Kumar *et al*., 2003; Raghu *et al*., 2006; Gururaj *et al*., 2007). In the present work, shoot tips were employed for indirect organogenesis through callus under *in vitro* condition.

MATERIALS AND METHODS
Explants were collected from the mature plants grown in Kurukshetra City, Haryana. A careful sterilization procedure of explants was done. Therefore, shoot tips were washed under running tap water and followed by liquid detergent. Then, three to four times of washes were given with distilled water. Thereafter in aseptic conditions, explants were sterilized with 0.1 % (w/v) mercuric chloride solution for 5 minutes and gave three to four rinses with sterilized double distilled water. In the present research, shoot tips were cultured on MS medium (Murashige and Skoog, 1962) and woody plant medium (Llyod and McCown, 1980). The pH of the media was adjusted to 5.8 by using 0.1 N NaOH or 0.1 N HCl before autoclaving at 121 ºC temperature and 15 psi pressure for 20 min. All the cultures were maintained at 25±2 ºC under a 16 hours photoperiod with 30 µmol m^{-2} s^{-1} irradiance provided by cool white fluorescent tubes.
Single shoot tip of 0.5 cm was inoculated in each test tube having culture medium supplemented with 0.5, 1.0, 2.0 and 4.0 mg/l concentrations of IAA, NAA and 2, 4-D individually. MS and WPM without growth regulators were served as control. Observations like percent callus induction, number of days required for

callus initiation, colour and texture of callus were recorded after 60 days of inoculation. A mean of 10 replicates per treatment was taken. To find out the growth of callus on different concentrations of above mentioned growth regulators, the calli were harvested after 60 days of initiation and measured in terms of fresh and dry weights. Then, the callus was transferred to the medium with BAP and Kn (0.5, 1.0, 2.0 and 4.0 mg/l) for organogenesis. Observations such as percent shoot initiation; number of days required for shoot initiation, number of shoots and shoot length were recorded.

Statistical Analysis

All the data were analyzed by analysis of variance (One way ANOVA) followed by Duncan Multiple Range Test (DMRT) at P = 0.05.

RESULTS AND DISCUSSION

Callus Initiation

Shoot tips were cultured on MS and WPM supplemented individually with IAA, NAA and 2,4-D at different concentrations (0.5, 1.0, 2.0 and 4.0 mg/l).

Table 1: Fresh and dry weights of callus produced by shoot tip explants of *T. cordifolia* cultured on MS & WPM supplemented with IAA, NAA and 2,4-D

Medium	Concentrations of auxins	IAA		NAA		2,4-D	
		FW (g)	DW (g)	FW (g)	DW (g)	FW (g)	DW (g)
MS	Control	0.0 ± 0.00^{g}	0.0 ± 0.00^{e}	0.0 ± 0.00^{g}	0.0 ± 0.00^{f}	0.0 ± 0.00^{h}	0.0 ± 0.00^{g}
	0.5	0.07 ± 0.01^{f}	0.02 ± 0.005^{d}	0.47 ± 0.03^{f}	0.10 ± 0.01^{e}	0.63 ± 0.05^{g}	0.12 ± 0.01^{f}
	1.0	0.13 ± 0.01^{e}	0.03 ± 0.005^{cd}	0.62 ± 0.05^{e}	0.11 ± 0.02^{de}	0.86 ± 0.05^{f}	0.15 ± 0.02^{ef}
	2.0	0.20 ± 0.02^{d}	0.04 ± 0.007^{c}	0.71 ± 0.04^{e}	0.14 ± 0.03^{d}	1.02 ± 0.06^{e}	0.17 ± 0.02^{de}
	4.0	0.42 ± 0.03^{b}	0.06 ± 0.008^{b}	1.14 ± 0.05^{c}	0.20 ± 0.02^{c}	1.55 ± 0.07^{c}	0.25 ± 0.03^{c}
WPM	0.5	0.10 ± 0.01^{ef}	0.02 ± 0.005^{d}	0.65 ± 0.05^{e}	0.12 ±0.01^{de}	1.03 ± 0.05^{e}	0.20 ± 0.02^{d}
	1.0	0.18 ± 0.03^{d}	0.04 ± 0.005^{c}	0.88 ± 0.06^{d}	0.18 ± 0.01^{c}	1.25 ± 0.05^{d}	0.24 ± 0.02^{c}
	2.0	0.31 ± 0.05^{c}	0.06 ± 0.007^{b}	1.44 ± 0.08^{b}	0.26 ± 0.02^{b}	1.96 ± 0.06^{b}	0.40 ± 0.03^{b}
	4.0	0.62 ± 0.05^{a}	0.12 ± 0.01^{a}	1.90 ± 0.07^{a}	0.36 ± 0.04^{a}	2.38 ± 0.08^{a}	0.45 ± 0.03^{a}

- *Values are means ± S.E. of three independent experiments, each consisted of 10 replicates per treatment. Data from 60 days old culture.*
- *Means followed by the same letter within columns are not significantly different at P = 0.05 according to Duncan's Multiple Range Test.*
- *FW-Fresh weight, DW-Dry weight.*

Cultures raised on basal medium (without any growth regulators) served as control. The response of explants was not observed in control cultures. While, shoot tips de-differentiated to form callus at above concentrations of growth regulators in the medium. This is probably due to the insufficient level of endogenous growth regulators in explants to induce callus and therefore it required an exogenous supply. Further, in *T. cordifolia*, for callus induction and differentiation, WPM was better than MS as a basal

medium. It was due to the low ionic strength of WPM which is suitable for salt sensitive woody plant species (Lloyd and McCown, 1980). Many workers used WPM and found suitable results (Feng *et al*., 2010; Sharma and Vashistha, 2010; Siwach and Gill, 2011). Further, the callus formation started at 0.5 mg/l of IAA, NAA and 2,4-D supplemented medium. The percent response increased with increase in concentration and maximum at 4 mg/l of growth regulators (Figure 1 & 2). Therefore, the maximum fresh and dry weights were observed at this concentration (Table 1). Among the different growth regulators tried in *T. cordifolia*, 2,4-D was most effective for callus induction. According to Murashige (1974) 2,4-D is a most potent auxin for callus formation. The colour and textures of calli are summarized in Figure 1 to 3.

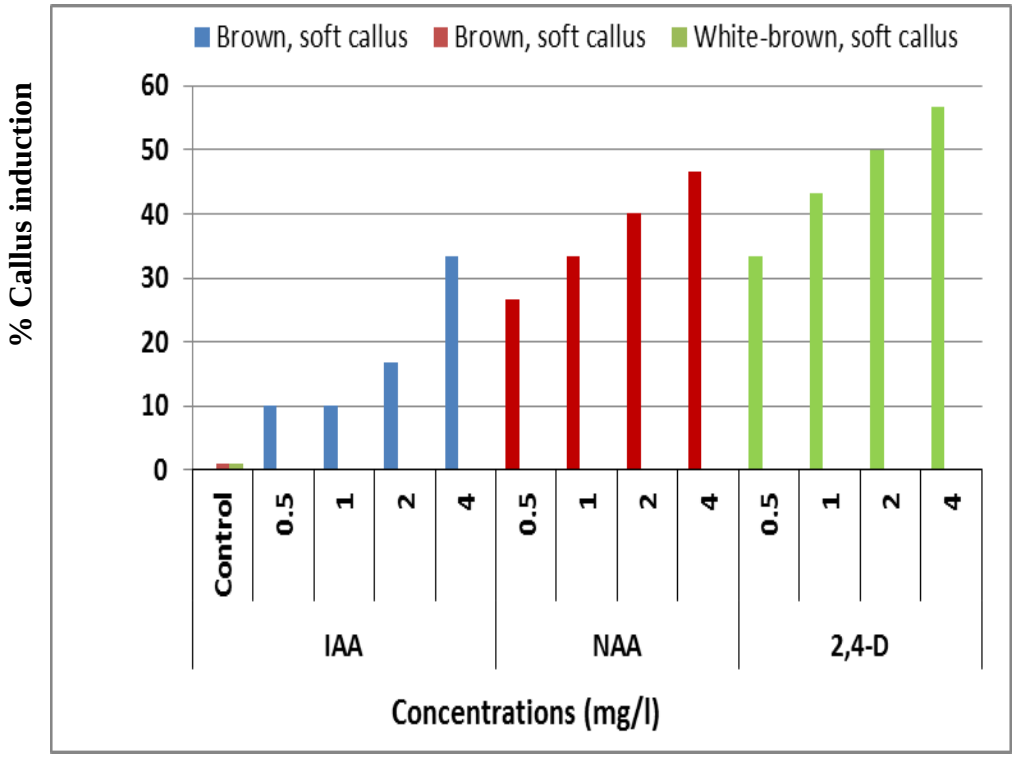
Callus Organogenesis

The callus so obtained were divided into small pieces and cultured on WPM supplemented with different concentrations (0.5, 1.0, 2.0 and 4.0 mg/l) of BAP and Kn individually for shoot formation. The results are summarized in Table 2. From the table, it is clear that the concentration of 2 mg/l BAP was optimum for callus organogenesis. Only this very concentration was effective in inducing shoots on callus. BAP at this level resulted in 43.3 % response with 1.5 ± 0.09 shoots per culture whereas Kn failed to induce shoots from callus.

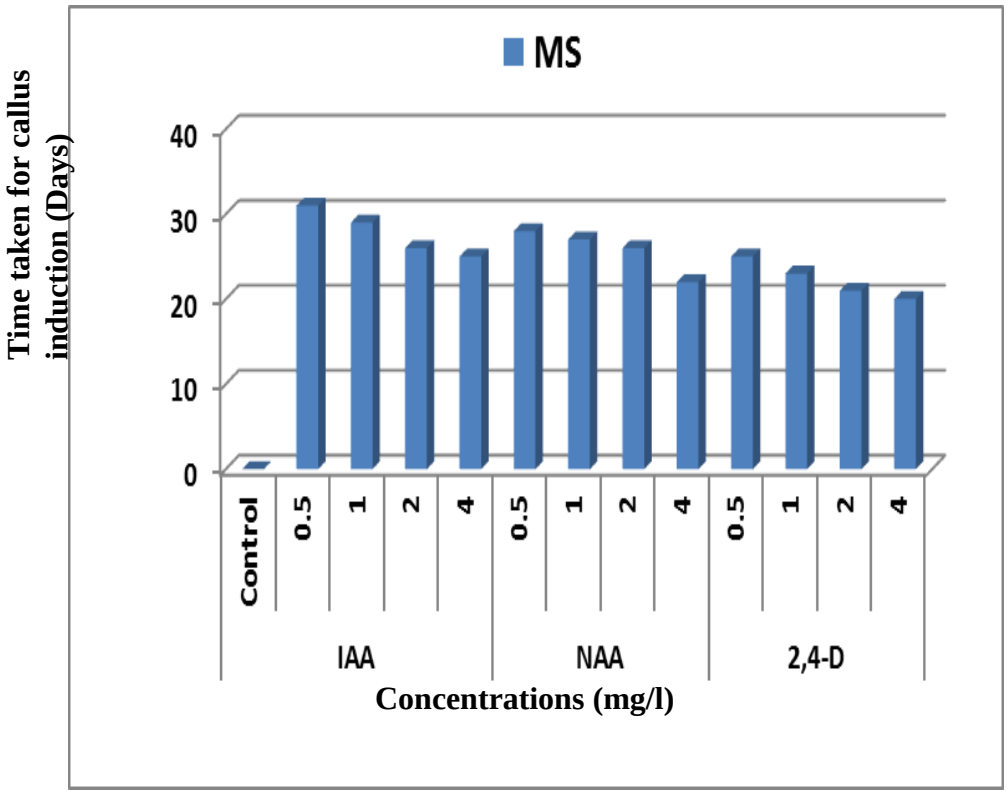
Table 2: Effect of different concentrations of BAP and Kn on shoot formation on calli induced from shoot tip explants of *T. cordifolia* cultured on WPM.

Growth Regulators	Concentrations (mg/l)	% response	Time taken for shoot formation (Days)	Average number of shoots per culture	Average shoot length (cm)
	0.0	-	-	0.0 ± 0.00[b]	0.0 ± 0.00[b]
BAP	0.5	-	-	0.0 ± 0.00[b]	0.0 ± 0.00[b]
	1.0	-	-	0.0 ± 0.00[b]	0.0 ± 0.00[b]
	2.0	43.3	27	1.5 ± 0.09[a]	0.8 ± 0.04[a]
	4.0	-	-	0.0 ± 0.00[b]	0.0 ± 0.00[b]
Kn	0.5	-	-	0.0 ± 0.00[b]	0.0 ± 0.00[b]
	1.0	-	-	0.0 ± 0.00[b]	0.0 ± 0.00[b]
	2.0	-	-	0.0 ± 0.00[b]	0.0 ± 0.00[b]
	4.0	-	-	0.0 ± 0.00[b]	0.0 ± 0.00[b]

- *Values are means ± S.E. of three independent experiments, each consisted of 10 replicates per treatment. Data from 60 days old culture.*
- *Means followed by the same letter within columns are not significantly different at P = 0.05 according to Duncan's Multiple Range Test.*
- *(-) No response*

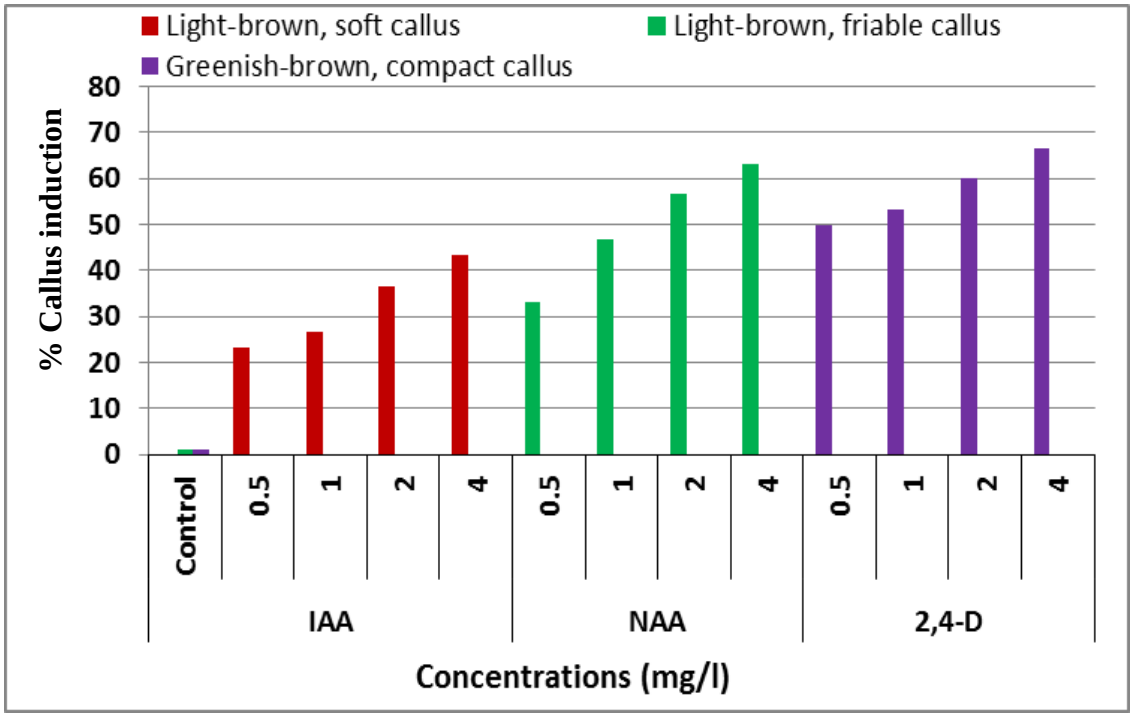


(A)

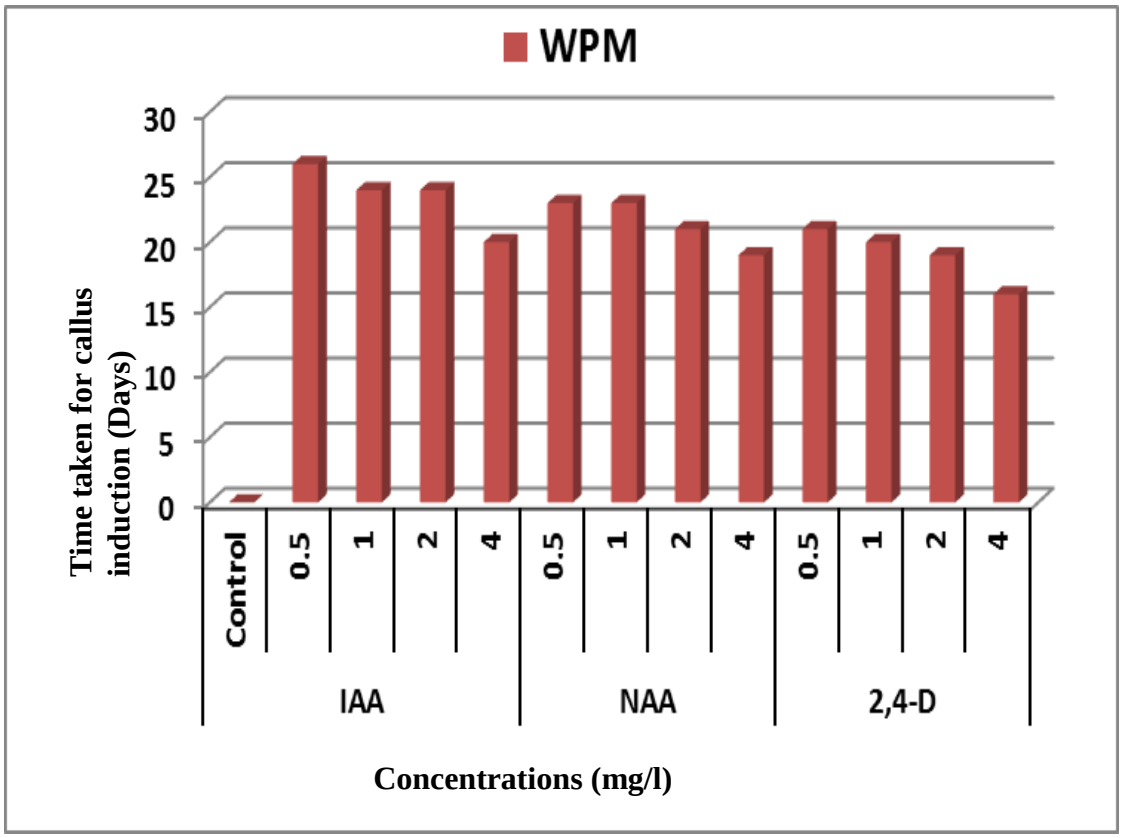


(B)

Figure 1: Effect of plant growth regulators on shoot tip explants of *T. cordifolia* cultured on MS medium- (A) Percent callus induction (B) Time taken for callus induction



(A)



(B)

Figure 2: Effect of plant growth regulators on shoot tip explants of *T. cordifolia* cultured on WPM: (A) Percent callus induction (B) Time taken for callus induction

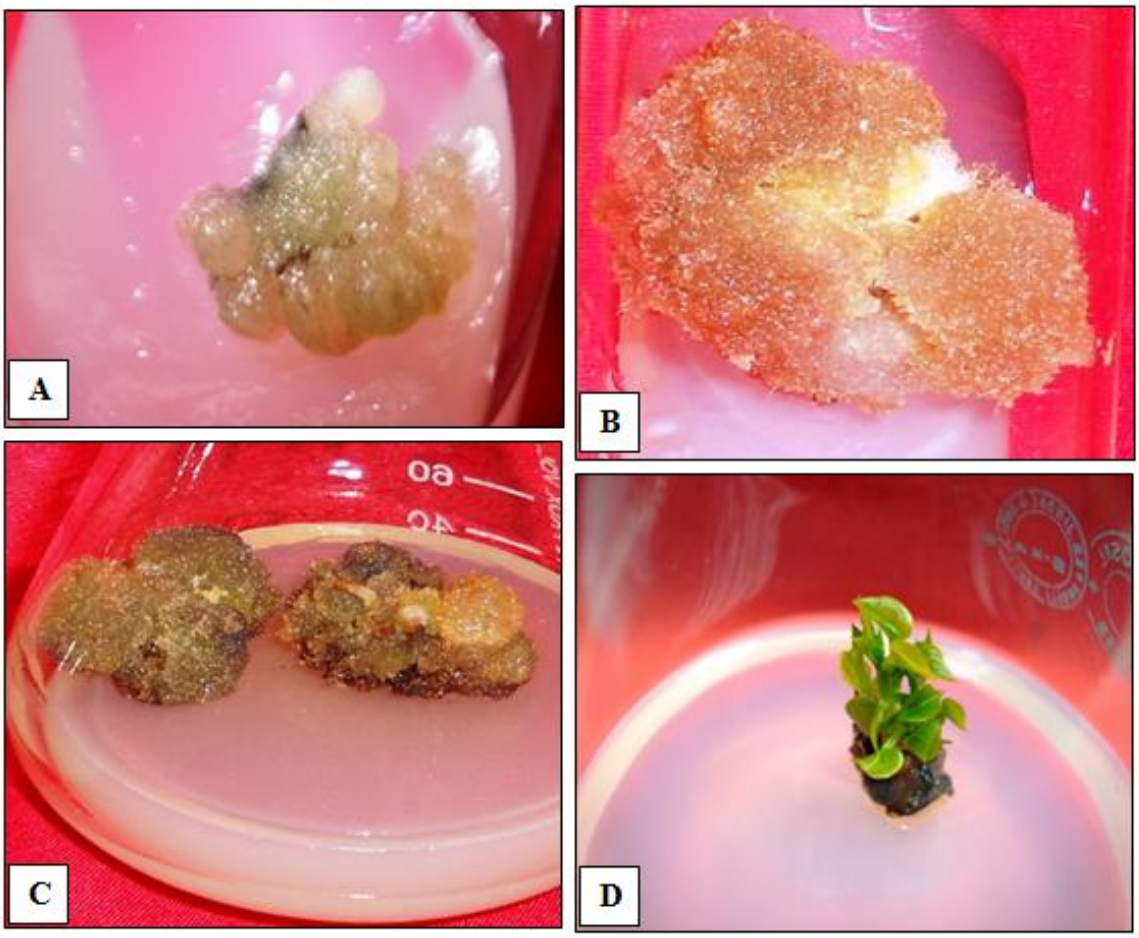


Figure 3: Effect of plant growth regulators on shoot tip explants of *T. cordifolia*: A. Callus on WPM + 4 mg/l IAA; B. Callus on WPM + 4 mg/l NAA; C. Callus on WPM + 4 mg/l 2,4-D; D. Shoot differentiation from callus on WPM + 2 mg/l BAP

Therefore, BAP was superior over Kn for shoot regeneration (Figure 3.D). Similar opinions were made by other authors (Wu *et al*., 2009; Sharma and Vashistha, 2010; Tan *et al*., 2011; Savita *et al*., 2011).

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