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In vitro Callus Induction and Shoot Multiplication from Nodal Explants and Leaves of *Memecylon edule*

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Abstract: A protocol for callus induction and shoot multiplication was developed for leaf and nodal segments of *Memecylon edule* (Melastomataceae). Leaf and nodal explants were cultured on MS medium supplemented with different phytohormones combinations IAA, NAA, 2,4,D, BAP, KIN, GA_3 with different concentrations (0.5-6 mg) for callus induction and shoot multiplication. Callus was initiated from cut ends of green tender leaves and nodal segments of *Memecylon edule* in both dark and light condition. Green friable callus and light brown calli was derived from nodal segments of MS ½ strength medium supplemented with 0.7% agar and 30% sucrose with GA_3+BAP+NAA, BAP+NAA+KN+GA_3, BAP+KN+NAA, BAP+GA_3+NAA, BAP+GA_3+2,4,D, IAA+NAA+ 2,4,D+KN+BAP, BAP+KN+NAA+2,4,D+GA_3 promotes the callogenesis in nodal explants. A best combination for shoot multiplication was MS medium supplemented with IAA+BAP+GA3 from the nodal segments and the nodal calli and IAA+BAP combination with 1 to 3 mg concentration was best for leaf callus. Percentages of callus induction were higher in nodal segments than leaf. In both leaf and nodal explants callus was developed in the MS medium containing BAP with IAA and GA_3 combinations.

Key words: *Memecylon edule*, callogenesis, nodal explants, GA_3, full strength MS, Melastomataceae

INTRODUCTION

Memecylon edule belongs to the family Melastomataceae is one of the most species-rich groups among the woody plants. *M. edule* is known in Tamil as Kayam. It's a small sized evergreen trees found in many regions of world (Natarajan *et al*., 2004). In India it's widely distributed in Andhra Pradesh (Nellore district, Chinglepet) Karnataka and Tamil Nadu. In Tamil Nadu mostly found in Chennai, Vandalur, Navalur and Maduranthakam (Ranjit *et al*., 2007). It is commonly used for folk medicine as anti-inflammatory, anti analgesic (Nualkaew *et al*., 2007) and hypoglycemic effects (Amalraj and Ignacimuthu,1998). Very few numbers of species in this family takes high aluminium above the ground tissues and these plants are termed as accumulators since, they store atleast 1000 mg kg^{-1} in leaves (Steven *et al*., 2002). Flowers in Melastomataceae are oil producing (Buchmann and Buchmann, 1981) and the tree has thin bark and timbers were used for building houses, boats. The fruits of *M.edule* are small green (about 1 cm) turning red then black. It's most valuable dye yielding medicinal plant and these dye used for coloring leather, silk, cotton and

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wool (Paisan *et al*., 2002). The tissue culture of Melastomataceae in *Miconia* sp. was first reported by Luis and they were used leaves, nodes and intermodal explants for plant regeneration. High percentage of callus was observed in Thidiazuron (TDZ) at dark condition (Luis *et al*., 1997).

Regeneration of multiple shoots and callus induction was achieved from leaves of *Trigridiopalma magnifica* (Melastomataceae) using NAA (Napthalene acetic acid) BAP (6, Benzyl amino purine) and TDZ (Thidiazuron) in the MS basal medium at (0.1-4 mg) concentrations (Song *et al*., 2008).

To our knowledge callus induction and shoot multiplication of *Memecylon edule* using leaves and nodal segments has not been reported so far. In present study, this is the first report for standardized hormone combinations and concentrations for *M. edule* and we established a new methods for callus induction and shoot multiplication in *Memecylon edule* using nodal explants and leaves. Callus induction and shoot multiplication are not same for all medium and phytohormones.

MATERIALS AND METHODS

Collection of Plant Material

Memecylon edule (Melastomataceae) were collected in Potheri forest, Kanchipuram district, Tamil Nadu, India. This research project was conducted from July 2007 to 2009. The plants were authentified by taxonomist Dr. Narasiman, Madras Christian College, Chennai. Leaf and nodal segments of *Memecylon edule* were collected and used for this study.

Medium Preparation

The medium was prepared by adding the stock solutions in the respective order and water was added to the prepared medium and made up to 1 L. The pH of the medium was adjusted to the required level (5.8), 0.7% Agar and 3% sucrose were dissolving in the medium. Heat the medium solution while stirring until all the agar is dissolved. After dissolving the agar, medium was poured into pre sterilized test tubes, conical flask, with sterilized cotton plugs and then autoclaved for 121°C for 15 min and medium was cooled at room temperature and then transferred to the culture racks at 25°C of 2000 lux (Majib, 2005).

Explant Source

Young tender green leafs, nodes were selected as a explants for inoculation.

Surface Sterilization

Before incubation tissue culture glasswares was kept separately in hot air oven and again sterilizing in laminar airflow UV for 15 min. Leaf and nodal explants were separately washed with teepol for 3 min and then washed with distilled water for 3 times (Al-Khalifah, 2004). Washed explants taken inside the laminar airflow hood and explants were cut into 2 mm size, surface sterilized with 70% ethanol for 30 sec, followed by seeds were sterilized for 5 min, leaves were sterilize for 2 min, nodal explants for 5 min in 0.1% mercuric chloride, then rinsed four times with sterile distilled water (Anis *et al*., 2003).

Inoculation and Incubation

Explants were inoculated in the sterilized test tubes containing medium with phytohormones. Inoculated test tubes were labeled with name of the medium, hormone

combination, concentrations, with date and kept in tissue culture racks. The cultures were maintained under a white cool, fluorescent light of 2000 lux at photoperiod of 16 h light and 8 h darkness at 24±2°C.

Callus Induction

Leaves and nodal explants were aseptically placed on test tubes with 20 mL of (half strength MS) and (Full strength MS) (Thengane *et al*., 2006) supplemented with different combinations of BAP (0.5-5 mg L^{-1}), NAA(0.5-4 mg L^{-1}, KN (0.5-4 mg L^{-1}) KN (0.5 -4 mg) IAA (0.5-5 mg), 2,4,D(0.5-3 mg) and GA3 (0.5-4 mg). The cultures were maintained under a photoperiod of 16 h light and 8 h darkness and at 24±2°C. After 5-6 weeks of culture well developed callus was selected and sub cultured on fresh medium of the same composition at an interval of 3 weeks. Percentage of tubes responded, color and nature of callus was recorded.

Multiple Shoots from Nodal Explants

Multiple shoot induction from the nodal explants of ½ strength MS containing 3% sucrose and 0.7% agar supplemented with different combinations of IAA+BAP+GA^3 (1-4 mg). Cultures were incubated under a photoperiod of 16 h light and 8 h darkness and at 24±2°C. Percentage of shoot formation was observed after 4 weeks of culture.

RESULTS AND DISCUSSION

Effect of Bap and Kinetin with NAA and GA_3 on Callogenesis of Nodal Explants of *Memecylon edule*

Sterilized nodal explants were cultured on ½ strength MS solidified with 0.7% agar, 30% sucrose and supplemented with different hormone combinations and concentrations of auxins and cytokinins (Fig. 1). The effects of addition of BAP, NAA and GA_3 on MS medium at different levels (1, 2, 3, 4 and 5 mg L^{-1}) for callus induction. Table 1 shows higher number of calli induced on the MS medium containing 1 mg BAP, 2 mg NAA, with 1 mg GA_3. One milligram to 2 mg concentration of BAP, NAA and 1 mg of GA_3 is the optimum level for callus induction. Growth rate of callus was high in combination of 1mg kinetin with 1 mg

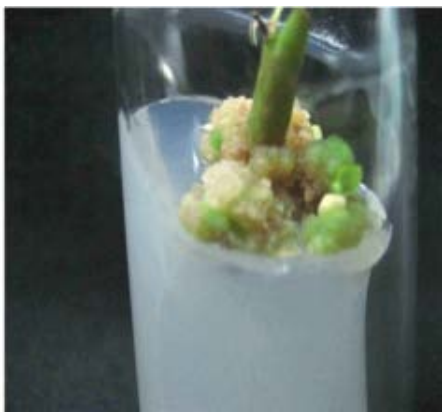

Fig. 1: Effect of BAP and Kinetin with NAA and GA_3 on Callogenesis of Nodal explants of *Memecylon edule*

Table 1: Effect of BAP, NAA AND GA_3 on callogenesis of nodal explants with half-strength MS medium of *Memecylon edule*

Type of explants	Medium	Phytohormones combination in MS medium (mg L^{-1}) BAP	GA_3	NAA	Degree of callus formation	Morphology of callus
N	½ MS	1	1	0.5	---	---
N	½ MS	1	1	1.0	---	---
N	½ MS	1	1	1.5	+	Green, Friable
N	½ MS	1	1	2.0	+++	Green, Friable
N	½ MS	1	1	2.5	++	Green, Friable
N	½ MS	1	1	3.0	++	Green, Friable

N: Node, ½ MS: Half strength MS: Medium, NAA: Naphthalene acetic acid, BAP: Benzyl amino purine, GA_3: Gibberellic acid, ---: No callus formation, +: Slight callus, ++: Moderate callus, +++: Massive callus

Table 2: Effect of BAP, NAA GA_3 and KINETIN on callogenesis of nodal explants with half strength MS medium of *Memecylon edule*

Type of explants	Medium	Phytohormones combination in MS medium (mg L^{-1}) BAP	GA_3	NAA	K	Degree of callus formation	Morphology of callus
N	½ MS	1	1	0.5	1	---	---
N	½ MS	1	1	1.0	1	+	Green, Friable
N	½ MS	1	1	1.5	1	+	Green, Friable
N	½ MS	1	1	2.0	1	++	Green, Friable
N	½ MS	1	1	2.5	1	+++	Green, Friable
N	½ MS	1	1	3.0	1	++	Green, Friable

N: Node, ½ MS: Half strength MS: Medium, NAA: Naphthalene acetic acid, BAP: Benzyl amino purine, GA_3: Gibberellic acid, K: Kinetin, ---: No callus formation, +: Slight callus, ++: Moderate callus, +++: Massive callus

BAP, 2.5 mg NAA and 1 mg GA_3 as shown in Table 2. Instead of 1 mg of kinetin, concentration of kinetin was raised to 6 mg L^{-1}. But the callus induction was observed only in the Kinetin at 1-2 mg L^{-1} concentration. But callus was not induced well at kinetin with 3-4 mg L^{-1} concentrations. The NAA, BAP with GA_3 also induced the callus but showed slower growth rate than NAA, BAP, GA_3 with Kinetin combination. Percentage of calli was high in Kinetin combinations. But callus was not induced and no changes in explants at kinetin with 3-4 mg L^{-1} and NAA with 3-4 mg L^{-1} concentrations. Green calli was observed in BAP, NAA, GA_3 combinations and also green color calli were observed on the BAP, NAA and GA_3 with Kinetin combinations but green calli slightly changes to brown.

Effect of BAP, NAA and Kinetin on Callogenesis of Nodal Explants of *Memecylon edule*

Half strength MS medium supplemented with BAP/NAA, calli was produced from the nodal explants. Table 3 shows callus growth was observed in BAP combination with NAA and Kinetin (1+2+1 mg L^{-1}) shows better result than BAP/NAA combination. When concentration of NAA was constant 2 mg k^{-1} and slightly concentration of KN was changed from (0.5-3 mg) but the percentage of callus response is high for BAP 1 mg L^{-1} and KN 0.5-2 mg L^{-1}. The NAA concentration was increased to 2-5 mg L^{-1} percentage of callus response is very low and initiation time also very high, more than 2 months (Chandra and Bhanja, 2002). The Green and friable (Ahmed *et al*., 2007).

Effect of BAP, GA_3 with IAA and 2,4,D on Callogenesis of Nodal Explants of *Memecylon edule*

Leaf and nodal explants were cultured on ½ strength MS medium supplemented with effects of various concentration of IAA and 2,4,D, with BAP and GA_3 hormone combination. Among several combinations of growth regulators were used. Table 4 shows percentage of callus induction was found to be higher on MS medium with 1mg BAP+ 1.5 mg

Table 3: Effect of BAP, NAA AND kinetin on callogenesis of nodal explants half strength MS medium of *Memecylon edule*

Type of explants	Medium	Phytohormones combination in MS medium (mg L^{-1}) BAP	NAA	KN	Degree of callus formation	Morphology of callus
N	½ MS	1	1	0.5	---	---
N	½ MS	1	1	1.0	---	---
N	½ MS	1	1	1.5	+	Green, Friable
N	½ MS	1	1	2.0	+	Green, Friable
N	½ MS	1	2	0.5	+	Green, Friable
N	½ MS	1	2	1.0	++	Green, Friable
N	½ MS	1	2	1.5	---	---
N	½ MS	1	2	2.0	---	---

N: Node, ½ MS: Half strength MS medium, NAA: Naphthalene acetic acid, BAP: Benzyl amino purine, K: Kinetin, ---: No callus formation, +: Slight callus, ++: Moderate callus

Table 4: Effect of BAP, GA_3 with IAA on callogenesis of nodal explants half strength MS medium of *Memecylon edule*

Type of explants	Medium	Phytohormones combination in MS medium (mg L^{-1}) BAP	GA_3	IAA	Degree of callus formation	Morphology of callus
N	½ MS	1	1	0.5	---	---
N	½ MS	1	1	1.0	---	---
N	½ MS	1	1	1.5	++	Brown, Hard
N	½ MS	1	1	2.0	+	Brown, Hard
N	½ MS	1	1	2.5	---	---
N	½ MS	1	1	3.0	---	---

N: Node, ½ MS: Half strength MS medium, IAA: Indole acetic acid, BAP: Benzyl amino purine, GA_3: Gibberellic acid, ---: No callus formation, +: Slight callus, ++: Moderate callus

Table 5: Effect of BAP, GA_3 with 2,4,D on callogenesis of nodal explants half strength MS medium of *Memecylon edule*

Type of explants	Medium	Phytohormones combination in MS medium (mg L^{-1}) BAP	GA_3	2,4,D	Degree of callus formation	Morphology of callus
N	½ MS	0.5	1	1	---	---
N	½ MS	1.0	1	1	+	Brown, Hard
N	½ MS	1.5	1	1	+	Brown, Hard
N	½ MS	2.0	1	1	++	Brown, Hard
N	½ MS	2.5	1	1	---	---
N	½ MS	3.0	1	1	---	---

N: Node, ½ MS: Half strength MS medium, 2,4,D: 2,4,Dichlorophenoxy acetic acid, BAP: Benzyl amino purine, GA_3: Gibberellic acid, NAA: Naphthalene acetic acid, ---: No callus formation, +: Slight callus, ++: Moderate callus

IAA+ 1 mg GA_3 L^{-1} of medium and callus formed in 1 mg GA3+2 mg BAP+1 mg 2,4,D shown in Table 5. High concentrations of IAA and 2,4,D at 2-6 mg L^{-1}, explants were turned brown and callus was not produced. Increased the BAP and GA_3 combination to 1-5 mg concentration, Callus growth was obtained only in BAP and GA_3 combination at 1, 2 mg L^{-1}, callus was visible after 26 days of an inoculation day. Combination of 2,4,D with BAP and GA_3 stimulate the growth of callus induction (Sahoo *et al.*, 1997). Callus growths were brown and hard.

Effect of BAP, Kinetin with NAA, IAA, 2,4,D on Callogenesis of Nodal Segments with Full Strength MS Medium of *Memecylon edule*

Table 6 shows callus were high in nodal explants of MS with different combination of phytohormones at 1 mg BAP/2 mg NAA/1 mg IAA, 0.5 mg K/0.5 mg 2,4,D (Fig. 2). Effects

Table 6: Effect of BAP, kinetin with NAA, IAA, 2,4,D on callogenesis of Nodal segments with full strength MS medium of *Memecylon edule*

Type of explants	Medium	Phytohormones combination in MS medium (mg L^{-1}) BAP	IAA	NAA	KN	2,4,D	Degree of callus formation	Morphology of callus
N	½ MS	1	1	0.5	0.5	0.5	---	---
N	½ MS	1	1	1.0	0.5	0.5	++	Green, Friable
N	½ MS	1	1	1.5	0.5	0.5	++	Green, Friable
N	½ MS	1	1	2.0	0.5	0.5	+++	Green, Friable
N	½ MS	1	1	2.5	0.5	0.5	+	Green, Friable
N	½ MS	1	1	3.0	0.5	0.5	---	---

N: Node, F- MS: Full strength MS medium, 2,4,D: 2,4,Dichlorophenoxy acetic acid, BAP: Benzyl amino purine, IAA: Indole acetic acid, NAA: Naphthalene acetic acid, K: Kinetin, ---: No callus formation, +: Slight callus, ++: Moderate callus, +++: Massive callus

Fig. 2: Effect of BAP, Kinetin with NAA, IAA, 2,4,D on callogenesis of Nodal segments with Full strength MS medium of *Memecylon edule*

of BAP and Kinetin with 2,4,D, NAA and IAA combinations shows callus induction. Green friable calli was observed in NAA combinations. When 2,4,D at low concentration 0.5 mg L^{-1} of medium was observed high percentage of callus induction. (Amoo and Ayisire, 2005). When concentration was tested up to 5 mg L^{-1}, callus was not observed. Auxin induction experiment was carried out to determine the optimal level of concentrations. Effect of each auxins (NAA, IAA, 2,4,D) was tested individually in the medium at (0.5-6 mg L) at different concentrations. But higher number of calli was observed in NAA with Kinetin and BAP combinations. Color of callus is green and friable. Friable callus was sub cultured on same medium containing same hormone combination and concentration. After 3-4 weeks of culture maximum callus was observed in these combinations.

Effect of BAP, Kinetin with NAA, 2,4,D and GA_3 and on callogenesis of Nodal Segments with full Strength MS Medium of *Memecylon edule*

Nodal explants were cultured on MS full strength MS medium with different hormone combinations of BAP and Kinetin with NAA and GA_3. Successful callus induction was observed in MS (full strength) supplemented with 1 mg BAP/3 mg NAA/1 mg GA_3/1 mg Kinetin and 2,4,D 0.5 mg L^{-1} are shown in Table 7. The color of the callus was green and friable, but green color callus were changed to white calli. Callus was sub cultured on the same medium with same combination and concentration.

Table 7: Effect of BAP, Kinetin with NAA, 2,4,D and GA_3 and on callogenesis of Nodal explants with Full strength MS medium of *Memecylon edule*

Type of explants	Medium	Phytohormones combination in MS medium (mg L^{-1}) BAP	GA_3	NAA	KN	2,4,D	Degree of callus formation	Morphology of callus
N	F-MS	1	1	0.5	1	0.5	---	---
N	F-MS	1	1	1	1	0.5	---	---
N	F-MS	1	1	1.5	1	0.5	+	White, Friable
N	F-MS	1	1	2	1	0.5	++	White, Friable
N	F-MS	1	1	2.5	1	0.5	+++	White, Friable
N	F-MS	1	1	3	1	0.5	+++	White, Friable

N: Node, F-MS: Full strength M S medium, 2,4,D: 2,4,Dichlorophenoxy acetic acid, BAP: Benzyl amino purine, GA_3: Gibberellic acid, NAA: Naphthalene acetic acid, K: Kinetin, ---: No callus formation, +: Slight callus, ++: Moderate callus, +++: Massive callus

Table 8: Effects of IAA and GA_3 with BAP for multiple shoots of *Memecylon edule* with ½ strength MS medium of *Memecylon edule*

Type of explants	Medium	Phytohormones combination in MS medium (mg L^{-1}) IAA	BPA	GA_3	Degree of shoot regeneration
N	F-MS	2	0.5	1	---
N	F-MS	2	1.0	1	++
N	F-MS	2	1.5	1	+++
N	F-MS	2	2.0	1	++
N	F-MS	2	2.5	1	---
N	F-MS	2	3.0	1	---

N: Node, F- MS: Full strength MS medium, 2,4,D: 2,4,Dichlorophenoxy acetic acid, BAP: Benzyl amino purine, IAA: Indole acetic acid, NAA: Naphthalene acetic acid, K: Kinetin, +++ : Good shoot formation, ---: No shoot formation, ++: Moderate shoot formation, +: Slight shoot formation

Effects of IAA and GA_3 with BAP for Multiple Shoots of *Memecylon edule* with MS ½ Strength

Nodal explants were inoculated on the MS ½ strength medium supplemented with 0.7% agar and 30% sucrose with different hormone combinations (IAA, GA_3 and BAP) and concentrations (0.1-6 mg). After callus was induced in the 2 mg IAA/1 mgBAP/1 mgGA_3 concentrations. Callus was observed at 28th day after inoculation. Percentage of callus was high in the 1-2 mg L^{-1}. Callus was transferred to the same medium containing same hormone combinations and concentrations. But concentration of BAP was tested at 1-6 mg L^{-1} with IAA and GA_3 (Karthikeyan *et al*., 2009). Multiple shoots was observed in the callus at 2 mg IAA/1.5 mg BAP/1 mg GA_3 shown in Table 8 and slight shoot formation was observed in BAP 1-2 mg concentrations (Fig. 3) and direct shoot regeneration from nodal explants with phytohormones IAA, BAP, GA_3 at (2/2 mg/2 mg L^{-1}) concentrations (Fig. 4) (Loc *et al*., 2005).

Effect of IAA with BAP on Callogenesis of Leaf Explants of *Memecylon edule*

For callus induction seeds were cultured in the dark for two days at ½ strength MS medium containing without phytohormones. From the day of inoculation cotyledonary leaves were observed at third week. Callus was initiated in the half strength MS medium supplemented with IAA and BAP with various hormone concentrations 0.5-6 mg L^{-1}. Callus was induced in 1 mg IAA-3 mg BAP hormone combinations shown in Table 9. Compare to nodal explants, percentage of callus was observed in leaf explants was lower than nodal explants.

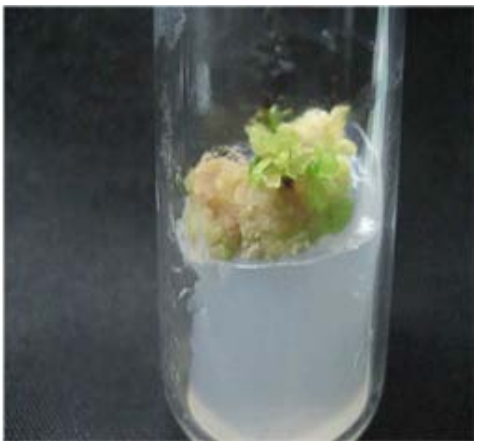

Fig. 3: Effects of IAA and GA_3 with BAP for multiple shoots of *Memecylon edule* with MS ½ strength medium of *Memecylon edule* (Multiple shoots was observed in the callus at 2 mg IAA/1.5 mg BAP/1 mg GA_3).

Fig. 4: Effects of IAA and GA_3 with BAP for multiple shoots of *Memecylon edule* with MS ½ strength medium of *Memecylon edule* (Direct shoot regeneration from nodal explants with phytohormones IAA, BAP, GA_3)

Table 9: Effect of IAA with BAP on callogenesis of leaf explants with ½ strength MS medium of *Memecylon edule*

Type of explants	Medium	Phytohormones combination in MS medium (mg L^{-1}) BPA	IAA	Degree of callus formation	Morphology of callus
L	½ MS	0.5	1	---	---
L	½ MS	1.0	1	---	---
L	½ MS	1.5	1	*	White, Milky
L	½ MS	2.0	1	*	White, Milky
L	½ MS	2.5	1	*	White, Milky
L	½ MS	3.0	1	+	White, Milky

L : Leaf, ½ MS: Half strength MS, ---: No callus formation, *:Slight callus, +: Moderate callus, BAP: Benzyl amino purine, IAA: Indole acetic acid

CONCLUSION

Various combinations of IBA, 2,4,D, NAA, KN, BAP, IAA, GA_3 (0.5-5 mg) were tried for callus initiation. Among these auxins tested NAA showed better result, NAA combination with BAP/KN shows callus formation within 3-4 weeks. GA_3 combination with BAP shows Green friable calli from nodal segments. Callus was not induced well in the medium containing IBA alone and IBA with Kinetin and BAP. Compare to three medium composition (1/2 Strength, Full strength and MS with B5 vitamins), ½ MS is very suitable for callus induction of *Memecylon edule*. Callus was initiated from cut ends of green tender leaves and nodal segments of *Memecylon edule* in both dark and light condition. Cotyledonary leaf segments were derived from the two month old seedlings and callus was initiated from these leaves within 29-32 days of inoculation. Callus was derived from the tender leaves with ½ strength MS medium containing different hormone combinations BAP+NAA+K (1.5+2+1). Explants were cultured on medium (full strength and half strength) and observed highest good green friable callus it was derived from nodal segments of MS ½ strength medium supplemented with GA_3 and NAA Combination and Light brown call was present in without NAA combinations Green tender leaves were better and showed good callus development.

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