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Investigating the Role of Plant Growth Regulators in the Induction of Calluses in Cissus quadrangularis Linn.

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Abstract:

Cissus quadrangularis Linn belongs to family Vitaceae. It is commonly known as 'Hadjod' due to its bone healing properties. It is also useful in various disorders like osteoporosis, diabetes, survey, menstrual disorders etc. β- sitosterol is an active principle present in C. *quadrangularis*. As the plant is slow growing, plant tissue culture is one of the methods used for propagation of plant. Different plant parts are used as explants for callus induction from the plant.MS medium fortified with various combinations of auxins and cytokines is used in present study for callus induction.

Keywords: *C. quadrangularis*, β- sitosterol, callus induction

Abbreviations: MS medium: Murashige & Skoog's medium, NAA: Naphthalene Acetic Acid, BAP: Benzyl Amino Purine, Kin: Kinetin

Introduction: Plants play very important role in human life. They fulfil our basic needs as well as act as rich source of medicines. According to World Health Organization over 80% of world's population relies on traditional forms of medicines obtained from plants and is dependent on these medicines for primary health care (Ghosh, 2008). 11% of the 252 drugs considered as basic and essential by WHO are exclusively derived from flowering plants (Rates S.M.K. 2001). Chemical synthesis of these drugs is difficult and costly, and many plants containing these drugs are becoming endangered due to overharvesting and improper methods of collections. Hence,

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various biotechnological approaches are now used for cultivation of these important medicinal plants. Plant Tissue culture is one of the approaches commonly used for same.

Cissus quadrangularis, commonly known as 'Hadjod' is the plant belonging to family Vitaceae. The plant is probably native to India and Srilanka, but also found in Africa, Arabia and South East Asia (Vijayalaxmi A. *et. al.*, 2013). The plant contains various compounds like alkaloids, resvertol, piceatannol, palidol, patthenocission, qudrangularins, ascorbic acid, carotene, phytosterol substances, calcium, flavonoids, vitamins, enzymes, nicotinic acid, tyrosine, triterpenoids iridoids, stilbenes, quercetin, β - sitosterol, δ – amyrin, δ – amyron, glycoside, triterpenes', fatty acid, methyl esters, glycerolipids, steroids, phytol's, and stigmasterol (Pathom W. *et. al*., 2015). Phytosterols, keto steroids and flavonoids present in *C. quadrangularis* exhibit magnificent antioxidant properties. Studies have revealed that many polyphenolic compounds, such as phenolic acids, flavonoids, anthocyanidins, and tannins are present which possess remarkable antioxidant and anticancer properties (Vijayalakshmi A. *et. al*., 2013). Phytochemical analysis has revealed that the plant contains high number of dietary antioxidants that include vitamin C, β carotene and polyphenols (Oben J. *et. al*., 2007).

The conventional uses noted for this herb in the management of bone fractures, scurvy, tumors, hemorrhoids, peptic ulcer (Jainu and Shyamala Devi, 2004), menstrual disorders (Jainu M. *et.al*., 2006), gout, syphilis, venereal disease, piles, as an aphrodisiac, diarrhea, dysentery (Rex and Ravi, 2022), leucorrhoea in the animals (Chopra S. *et. al*., 1958; Yoganarsimhan S., 2000*)*. The active principle in plant is β-sitosterol which plays important role in bone healing process. The root and stem extract of plant is traditionally used in healing of bone fractures. The plant is documented in Ayurveda for the treatment of osteoarthritis, rheumatoid arthritis and osteoporosis. The stem juice of plant is used to treat scurvy, menstrual disorders, otorrhea and epitaxis. Decoction of shoots with dry ginger and black pepper is given for body pain the infusion of plant is anthelmintic. The fleshy stout stem is used for treatment of gastritis constipation, eye disease, chronic ulcers, piles and anaemia.

Cissus quadrangularis is generally propagated by vegetative propagation method (Patel D.K., 2014), but it is slow growing. Plant tissue culture is one of the biotechnological methods for large scale production of plant in lesser time. Various attempts are for micropropagation of

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plants using different media compositions (Nathawat R.S. (2011); Singh P., 2014); various media compositions to enhance beta sitosterol in suspension culture. Present study mainly focused on is development of callus using various explants along with various media compositions for induction of callus.

Materials and method: The plant material is procured from Botanical Garden, S.P. College Pune. Aerial roots, apical meristems, leaf, nodal regions are used as explant for callus induction. The media formulation described as Murashige and Skoog (1962) was selected as the optimal culture medium. The ready to use dehydrated MS media were used along with 3% sugar, 0.8% agar with different concentration of growth hormone (auxin and cytokine). The pH of the media was adjusted 5.8 to 6.0 with 0.1N NaOH or 0.1 N HCl. Media and Glassware were sterilized by autoclaving at 121°C and 15 lbs pressure for 20min. The procured explants are initially washed under running tap water for 10 min to remove all dust and other particles followed by washing with Tween 20 for 10 min. Then explants were washed with Sterile Distilled Water (SDW) two times and were transferred to Laminar Airflow Cabinet for inoculation. Further, the explants were washed with 70% ethanol. Then explants were treated with 0.1% $HgCl_2$ for 5 min followed by for 4-5 times washing with SDW. The explants were dried with sterile tissue papers and cut into small pieces (around 1 to 1.5 cm). These are inoculated in tube containing various media combinations. The Inoculated cultures were transferred to light intensity (100 µmol $m^{-2}s^{-1}$), 16 h light / day at 25 ± 2°C and 55 – 60 % relative humidity.

Result & Discussion:

Table 1: Time required for callus induction and maximum callus formation on different PGR compositions when nodal regions were used as explant

Sr No	MS+PGR concentration	Callus Initiation	Maximum Callus
1	MS + 1.5mg/lit NAA +1.5mg/lit BAP	9 Day	14 Day
2	MS + 1.5mg/lit 2,4 D + 1.5mg/lit BAP	12 Day	24 Day
3	MS + 2.09mg/lit 2,4 D	7 Day	12 Day
4	MS + 1.99mg/lit BAP + 1.99 mg/lit Kin	9 Day	13 Day

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Fig 1(A) Fig 1(B) Fig 1(C)

Fig 1(A): Inoculation of nodal region of *Cissus qudrangularis* L (MS + 1.5mg/lit NAA + 1.5mg/lit BAP). Fig 1(B): - Callus initiation, Fig 1(C): Maximum Callus (after 14 Days) when nodal regions were used as explant.

Fig 2(A) Fig 2(B) Fig 2(C)

Fig 2(A): - Inoculation of nodal region of *Cissus qudrangularis* Linn (MS + 1.5mg/lit 2, 4 D + 1.5mg/lit BAP). Fig 2(B): - Callus initiation, Fig 2(C): - Maximum callus growth (after 24 Days).

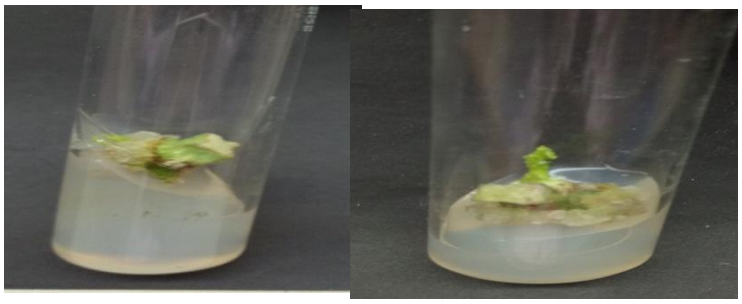

Fig 3 (A) **Fig 3(B)**

Fig 3(A): - Callus initiation (MS + 2.09mg/lit 2, 4 D); Fig 3(B): - Maximum Callus (after 12 Days).

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The MS + 1.5mg/lit NAA + 1.5mg/lit BAP showed callus induction in 9 days after inoculation (Fig 1(A) to Fig 1(C)) and it showed maximum callus after 14 days of inoculation. Callus initiation was observed on MS + 1.5mg/lit 2,4 D + 1.5mg/lit BAP after 12 days of inoculation and maximum callus was observed after 24 days (Fig 2 (A) to Fig 2(C)). MS + 2.09mg/lit 2, 4 D showed callus initiation 9 after inoculation and maximum callus was after 13 days (Fig 3 (A) to Fig 3 (C)). Leaves and tendrils did not give response on the above-mentioned media compositions and no callus formation was observed.

Conclusion:

The medium composition MS + 2.09mg/lit 2,4 D is comparatively gives early callus initiation than the MS + 2.00mg/lit 2,4 D used by Pragat Singh (2014). The later medium composition MS + 2.00mg/lit 2, 4 D showed callus induction after 21 days of inoculation. While the medium composition MS + 2.09mg/lit 2, 4 D showed callus induction after 7 days of inoculation. So, we conclude that the concentration of 2, 4 D slightly increases it give early callus induction. The high concentration of 2, 4 D is also affecting the callus growth. Sharma N. *et. al.* (2011) used the medium composition MS + 2, 4 D (2.5mg/lit, 3.0mg/lit) these two medium composition showed callus induction after 14 to 16 days of inoculation. MS + 2.09mg/lit 2, 4 D used in present study it gives callus induction in 7 days after inoculation.

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