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IN VITRO CALLUS INDUCTION AND HISTOCHEMICAL LOCALIZATION STUDIES IN HIBISCUS MICRANTHUS L.

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ABSTRACT

Hibiscus micranthus (L.) is an important medicinal plant which belongs to the family Malvaceae. It is widely used as folk medicine and treating for many diseases. Leaf explants were cultured on MS medium with different concentrations of NAA AND 2, 4-D alone in combination with BAP and KIN for callus induction. Callus initiation was observed in all concentration with huge amount. Highest percentages of callus (87.3%) induction were observed in the combination of BAP (2.0mg/l) NAA (1.0mg/l) 2, 4-D (0.5mg/l) for explants, node respectively. The experimental result of calli was shown as green friable callus, green yellow friable callus and green compact nodular callus of the and nodal segment were cultured on Murashige and Skoog (MS)medium supplemented with BAP (6-benzyl amino purine) NAA naphthalene acetic acid and IAA Idol acetic acid 2,4-Dichlorophenoxyacetic acid (2,4-D). The protocol might be useful for the production of disease free and healthy plant materials. Histochemical localization of *H. micranthus*. The thin even section were treated with histochemical stains to used by localize substance of carboxylate polysaccharide and lignin indicated giving in specific colors formed the callus. *H. micranthus* is a medicinal plant leaf explant culture good amount results from callus for the histochemical.

KEYWORDS: *Hibiscus micranthus In vitro* callus induction, plat growth regulators Histochemical studies.

INTRODUCTION

The family Malvaceae is one of the most important family consisting of 82 genera and 1,500 species with *Hibiscus* over 200 species. Is a shrub up to 3 m, stem erect, branched, usually

with stiff, slender and stellately hairy plant (Berhan *et al*., 2017). The family is worldwide in distribution but is mostly represented in the tropical and subtropical region. Members may be herbs, shrub or the trees with mucilage. *Hibiscus micranthus* is a slandered shrub grow up to 2.5 meters tall. The plant is sometimes harvested from the forest for its edible leaves, Normal tap root system without any modifications. They wereusually aerial and erect but decumbent in some plants, herbaceous or woody, cylindrical and branched, Carline, alternate, stipulate etiolate dorsiventral Mosley simple (*Hibiscus*) or palmate lobed or multifoliate, reticulate benation. *H. micranthus* Linn. (Malvaceae) is a shrubby, erect, branched, slender and stellate hairy plant. It is widely distributed in hotter parts of India, Ceylon, Saudi Arabia and tropical Africa. In India, the plant is known by different vernacular names in different regions as Chalabharate in Telugu, Sittamutti in Tamil, Chanakbhindo in Gujrati and as order in Sanskrit. this plant has been scientifically validate for its antipyretic, anti-inflammatory, hematological effects (Ashok Kumar *et al*., 2010).

MATERIAL AND METHODS

Plant material

Healthy plants of *Hibiscus micranthus* were collected from Inamkulathur Tiruchirappalli district, Tamilnadu. The collected explants were washed thoroughly in running tap water for 30 minutes. Then the explants were rinsed with 1% salon solution containing 6-8 drops of 20 for 15 minutes and again washed with double distilled water to remove the traces of detergent solution. Then the explant were washed with 70% alcohol for 30 seconds followed by washing with distilled water and were placed inside the laminar air flow chamber. Next, the explants rinsed with 100mg mercuric chloride solution for 5 seconds and again washed with sterile distilled water for 6-8 times. Then the explants were placed in sterile petri plates for inoculation. The sterilized leaves were used as explant source for in vitro culturing. (Murashige and Skoog 1962) medium with various combinations of BAP, NAA and 2, 4-D were used for callus induction. The MS medium consists of macronutrients, micronutrients, iron source and vitamins supplemented with sucrose (15g) as a carbon source and agar (8g) as a solidifying agent. BAP Benzyl amino purine and NAA naphthalene acetic acid, 2, 4-D Dichlorophenoxy acetic acid, in different concentrations were also supplemented in to the MS medium for callus induction. The BAP concentrations were constant (0.5 to 3.0mg/l), NAA concentrations were ranged from (1.0mg/l), 2, 4-D concentrations were ranged from (1.0mg/l). Callus cultures were maintained on solid MS medium and sub cultured with frequent intervals and used for further studies.

HISTOCHEMICAL STUDIES

The thin even section were treated with histochemical stains to localize various constituents the quantities of different constituents like carbohydrates proteins lipids polyphynenolic lignin and alkaloid localized in the plant material were indicated by the symbol cods +, ++, +++, ++++ (-) to show the plant presence of constituents in intense moderately intense more intense strongly intense strongly intense and not detectable amounts especially when quantitative comparison is warranted the details of the procedure followed and the specific colors formed are formed are given in.

RESULT AND DISCUSSION

The node explant of *H. micranthus* were cultured on Murashige and Skoog medium supplemented with different concentration of BAP (0.5 to 3.0 mg/l), NAA (1.0 mg/l), and 2, 4-D (0.5 mg/l). The effect of BAP (2.0 mg/l) with varying concentration of NAA and 2,4-D were tried. The highest percentages of callus induction wereobserved on MS medium supplemented with BAP 2.0 mg/l + NAA 1.0 mg/l + 2,4-D 0.5 mg/l (**Table-1**). For callus induction, the impact of different plant growth regulator like, BAP in combination with IAA, 2, 4-D and ads were tested. In all the treated of plant growth regulated inducted multiple callus induction was noticed from the explants. Among the different treatment medium compromising of MS salts, B5 Vitamins BAP (2.0 mg/l) + IAA (2.0 mg/l) + 2,4-D (1.0 mg/l) showed the mean value 23/25 (89.8%) has the best result for callus induction in this concentration huge amount of callus was observed from single shoot tip explant. Cotyledonary leaf explants from aseptically grown 7 to 10 days old leaves were collected and cut into small squares of 0.5 × 0.5 cm2 were placed on MS medium by dipping the cut surfaces on medium supplemented with three different combinations of benzyl adenine (BA) and indole-3-butyric acid (IBA) i.e. 1.5 mgL-1 BA + 0.05 mgL-1 IBA, 2 mgL-1 BA + 0.1 mgL-1 IBA and 2.5 mgL-1 BA + 1.00 mgL-1 IBA for callus induction (Samanthi, *et al.*, 2013). Profuse callusing with sparse shoot regeneration on MS minimal organic medium supplemented with KIN 1 × 10-5 M and IBA 5 × 10−6 M (bar8 mm) (TareqWani*et al.*, 2016).The callus proliferation was observed in all the treatments and the callus on subculture at 3 mg/l benzyl amino purine (BAP) and 1 mg/l naphthalene acetic acid (NAA) and at 2 mg/l BAP and 1 mg/l NAA induced shoot buds from hard green compact callus. (Sudarshana Mysore Shankarsingh *et al*., 2016). In leaf, nodal and intermodal explants were showed better response for callus indication on BAP (13.32 µM) + KIN (13.92 µM) + IAA (1.43 µM) + IBA (1.71 µM) + NAA (1.34 µM) + AdS (0.867 µM) and obtained the mean value 100.0 is

the best response. (Ramar 2015). The ranges for callus production from *H. sabdariffa* var. sabdariffa were 8-40, 72-94, and 82-100% for roots, cotyledons, and hypocotyls, respectively while those from *H. sabdariffa* var. altissima for the same explants were 0-33, 69-83, and 67-99%, respectively (Raoul SylvereSie *et al.*, 2010).

HISTOCHEMICAL STUDIES

Callus

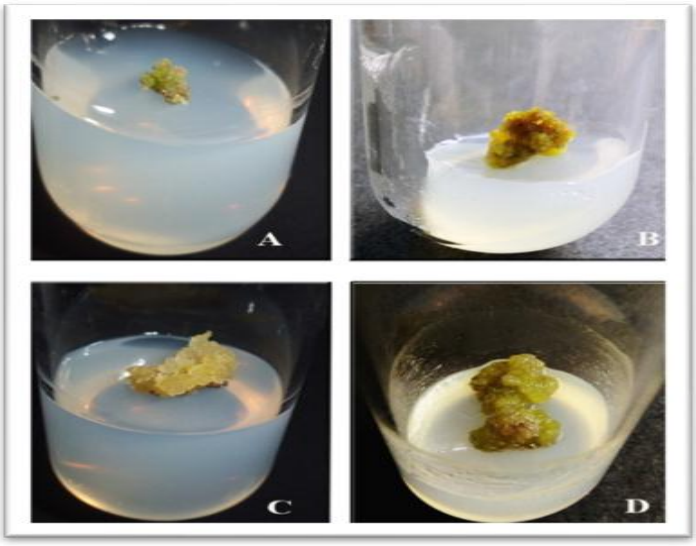
T.S of callus differentiated into two distinct part (inner and outer) the inner zone cells were much differentiated vascular elements the callus cells, thin well walled cells. The outer zone cells showed more dividing cells and cells were loosely arranged (Table 2).

Formations of vascular elements were showed a continuous active state of callus. The pink colored near to blue colored cells (Trachied) were phloem cells. The proliferation of callus often occurred most in outer zone. It was identified by thin walled cell with dense cytoplasm that produced from Clem of cells. The callus produced from the primary callus known as secondary callus that were very quickly proliferate and to produced the mass of callus. However the differention also occurred in many layers.

T.S of callus showed well distinguished outer and inner region the callus with differenced portion in inner part and a moss of parenchyma cells actively produced new cell in outer region. A portion of callus always contained the meristematic portions that were identification by actively divided cells with prominent nucleus.

In vitro callus induction of *H. micranthus*

A1- Habit



A1 habit of *H. micranthus*

A callus from leaf explants after 25 days

B callus from leaf explants after 40 days

C callus from leaf explants after 52 days

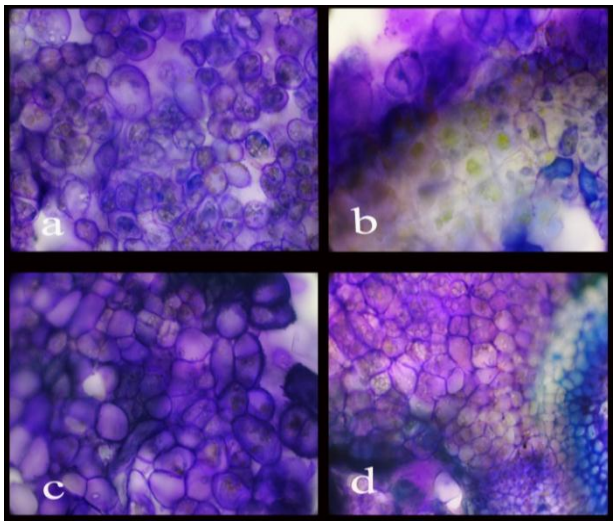
D callus from leaf explants after 60 days

Table.1 Effect of growth concentration of BAP, NAA alone with 2, 4-D on Callus proliferation from leaf and nodal explants of *H. micranthus.*

Explant Leaf and Node	BAP	NAA	2, 4-D	Number of explant responded	Degree of callus formation	% Responded	Nature of callus induction
	0.5	1.0	0.5	16/65	+	67.5	Green friable callus
	1.0	1.0	0.5	19/25	+++	74.4	Yellow friable callus
	1.5	1.0	0.5	21/25	+++	78.5	Green Yellow friable callus
	2.0	1.0	0.5	23/25	++++	87.3	Green compact nodular callus
	2.5	1.0	0.5	18/25	++	71.7	Green compact callus
	3.0	1.0	0.5	20/25	+++	75.6	Green friable callus

Note: small callus (+), moderate callus (++), quite massive callus (+++), very quiet massive callus (++++).

Histochemical localization of *H. micranthus*



A to D T.S of Callus stained with TBO.

Table 2: Histochemical results.

S.no	Histochemical methods	Localized substance	Colour indication	Callus Outer zone PC	Callus Outer zone SC	Callus Inner zone PC	Callus Inner zone SC
1	Toludine Blue O	A)Carboxylate Polysaccharide	Blue to purple	++	+++ +	+++ +++	++++ +
2		B) lignin	Yellow to red	++	++	–	–

CONCLUSION

An efficient callus induction protocol for *Hibiscus micranthus* has been developed in these protocol useful to the propagation studies. Histochemical localization studies also useful to localize the chemical substances.

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