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Effects of Various Growth Regulators on Callus Induction of *Justicia sps.* and its Co-relation with Total Phenolic and Carbohydrate Content

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Abstract

Justicia sps. is known for their immense medicinal property and pharmaceutical applications. Callus was induced using leaves as explant derived from pre-established axenic culture of *Justicia* grown in MS medium supplemented with IBA (0.5 mg/l) + Kn (1.0 mg/l). Effect of various PGRs on callus induction and biomass generation as well as on production of total phenolic content was investigated. Best growth and maximum biomass of callus was obtained in medium supplemented with BAP (2.0mg/l). The present study revealed that medium supplemented with BAP (1.5mg/l) and BAP (2.0mg/l) produced high amount of phenols with more carbohydrate content. It is also inferred that MS medium supplemented with cytokinins like BAP can enhance secondary metabolite production. The enhanced phenol and carbohydrate content may be co-related with the fact that polyphenols increases with increase in carbohydrate content as the excess of carbohydrate is diverted to the secondary metabolite pathways producing more phenols.

Keywords: Callus*, In-vitro, Justicia,* PGR, Phenolic content, Carbohydrate content.

INTRODUCTION

Justicia is the largest genus of Acanthaceae, constituting 600 species, found in pantropical as well as tropical southern regions of India[1]. It is a shrub and widely used in the Indian system of medicine for respiratory and gastrointestinal diseases, inflammation, rheumatism, arthritis, hallucinogens, sedatives, epilepsy, mental disorders, headache, fever, cancer, diabetes, HIV [2], expectorant, antispasmodic and good blood purifier and also used for speeds up the child birth. People mostly use extracts from leaves followed by the roots[2]. Various polyphenolic compounds like

alkaloids, lignans, flavonoids, and terpenoids are present in *Justicia sp*. Among these lignans are the major constituents possessing antiviral, antitumoral, anti-inflamatory, and antiplatelet aggregation activities. Other chemicals like essential oils, vitamins, fatty acids and salicylic acid are also found [2,3,4]. Many species of *Justicia* (*J. gendarussa L., J. insularis T. Anderson* and *J. tenella* (Nees) *T. Anderson*) have shown antisickling activity [5]. The present study was aimed to evaluate the effect of PGRs (Auxins and Cytokinins) alone and in combinations on callus induction and callus biomass. Callus induction can facilitate extraction of secondary metabolites from the plant.

MATERIALS AND METHODS

Source of Explant material: *Justicia sps.* plant was obtained from Andhra Pradesh.

Induction of caullogenesis: The callus was induced from the leaves of pre-established axenic culture of *Justicia* sps. grown in Murashige and Skoog[6] (MS) medium supplemented with Plant Growth Regulators (PGRs) namely Indole 3-butyric Acid (IBA- 0.5 mg/l) + Kinetin (Kn - 1.0 mg/l). The above induced calli was the further grown in MS supplemented with different concentrations/combinations of PGRs namely 6-Benzyl Amino Purine (BAP), Kn and α-Napthyl Acetic Acid (NAA) (Table:1).

Incubation and Maintenance: The culture tubes were maintained in growth room at 25±2ºC, 75% relative humidity and a photoperiod cycle of 16hrs light/8hrs dark regime and light intensity of 2000 lux. Sub culturing of the callus was carried out after 30-40 days.

Statistical Analysis: ANOVA was used to determine the effect of various PGRs on caullogenesis and % response of callus induction to assess significance of calli growth and biomass response (at $p<5\%$). All statistical analysis was performed by using SPSS software program.

Plant extract: One gram of ground tissue samples was extracted in 10ml of ethanol on an orbital shaker for 120 minutes at 50^0C, subsequently filtered and filtrate was used for quantification of total phenolic content and total soluble sugar determination.

Determination of total phenolic content: Phenolic content was determined using the Folin–Ciocalteu method[7]. 200 µL of extract (1 mg/mL) was mixed with 1.5 ml of Folin–Ciocalteu reagent and incubated at 22°C for 5 min followed by addition of 1.5 ml of $NaNO_3$ and incubation at 22°C for 2 hours. The absorbance at 725nm was measured. The results are expressed as mg g^{-1} Gallic acid equivalent (mg GAE g^{-1} dry sample). Phenol content was calculated[7,8,] by C (GAE) = c x V/M (Where- c = concentration of standard (mg/ml) at a particular OD, V= volume used during the assay (ml), M= mass of extract used during the assay (g).

Determination of total soluble sugar: Sugar content was measured by Anthrone method[8]. Carbohydrate content was calculated by $A_S/A_{STD}\times D.F/W\times 100$ (Where-A_S=

Absorbance of sample, A_{STD}= Absorbance of standard, D.F= Dilution factor, W= Weight of sample)

Determination of total protein content: Protein was extracted using phosphate buffer saline (PBS) and total protein content was measured by Lowry method[9].

RESULTS AND DISCUSSION

1. Induction, effect of various PGRs on callus growth and morphology of callus: Variation in the calli type (Table 1) was obtained in different combinations of PGRs (Fig1). On the basis of maximum % response and biomass, four calli were chosen for further phenolic, carbohydrate and protein quantification. Callus induction is vital for many processes like establishment of cell suspension cultures[10,11], indirect somatic embryogenesis [12,13] and other biochemical test. Profuse callus induction was observed with (BAP 2.0mg/l) alone and BAP (1.0mg/l) + NAA (1.0mg/l). Maximum calli biomass was obtained when the medium was supplemented with BAP (2.0mg/l) followed by BAP (1.5mg/l), BAP (1.0mg/l) and NAA (1.0mg/l), BAP (0.5mg/l). ANOVA (Table 2) at 95 percent significance level shows that the data is significant for comparing mean weight of calli (Fig 2). Though auxins are known to be the main inducer for callus development [14], in the present study cytokinin (BAP) induced callusing was observed as compared to auxin. Similar results have been reported by Greco *et.,al* where they obtained better callusing with BAP [15]. The cytokinin induced callusing in *Justicia sps.* may be attributed to variable response of the genotype to the different PGRs.

Table 1: Callus induction from the leaf explants of *Justicia sps.* in MS medium supplemented with different hormone condition

PGR Concentration	Morphology and colour of callus	Degree of callus formation	Explanation of Sign
BAP (0.5mg/l)	Non green friable	+ + +	* no callus was formed, + very few callus formation, ++ minor callus formation, +++ slight callus formation, ++++ moderate callus formation, +++++ profuse callus formation.
BAP (1.5mg/l)	Compact green	+ + + + +	
BAP (2.0mg/l)	Compact green	+ + + + +	
BAP (3.0mg/l)	Browning	*	
BAP (1.0mg/l) NAA(1.0mg/l)	Green friable	+ + + + +	
Kn (1.0mg/l)	Browning	*	
BAP (0.5mg/l) Kn (0.5mg/l)	Green compact	+ + +	

Table 2: Comparison of mean weight of calli grown in medium supplemented with various PGRs concentration/combination using one way ANOVA.

Source	SS	df	MS	F	P	Explanation of Sign
Between the groups	2.028622	3	0.676207	15.07	<0.0001	SS = Sum of squares df = Degree of freedom MS= mean square * One tailed tabulated value ** Two tailed tabulated value
Error	1.645223	36	0.044867			
Total	3.643845	39				

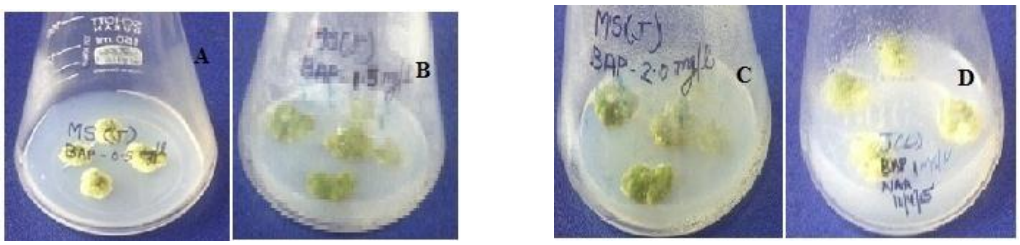

Fig:1 A)Friable calli (BAP-0.5mg/l), B) Compact calli (BAP-1.5mg/l), C) Compact calli (BAP-2mg/l), D) Friable calli (BAP-1mg/l ;NAA-1mg/l)

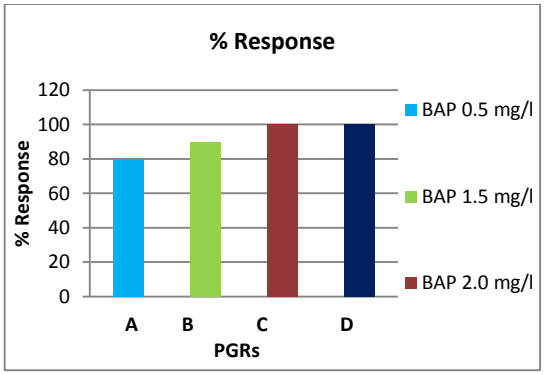

Fig 2. Percent response of calli in MS supplemented with different PGRs.

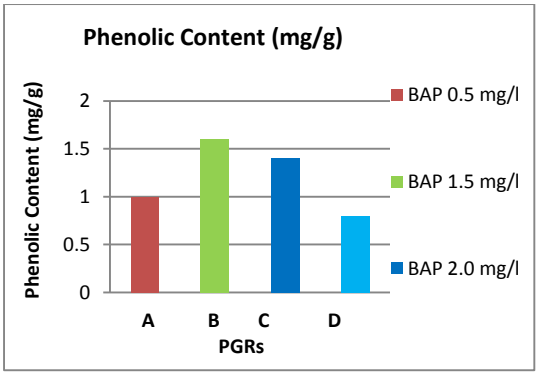

Fig 3: Total phenolic content of calli grown in MS supplemented with different PGRs.

2. Total phenolic content: Calli supplemented with BAP (1.5mg/l) showed the highest phenolic content (1.6mg/g) among the four chosen combinations followed by BAP (2.0mg/l) which gave 1.4mg/g of phenolic content. BAP (0.5mg/l) and BAP (1.0 mg/l) with NAA (1.0mg/l) showed near similar phenolic content, 0.8mg/g and 1.0mg/g respectively (Fig 3). Calli with high phenolic content were further analyzed to check the variation in amount of phenols using different amount of calli (1.0, 2.0,

3.0 and 4.0 gm). Interestingly, there was significant increase in the phenolic content with increase in the weight. When grown in presence of FAP (KN) in medium, browning of tissue was observed. There is some evidence that phytohormone FAP causes browning of tissue due to excision in explant resulting in secretion of polyphenols in the medium[16].When the calli grown in medium supplemented with four different combinations of PGRs was compared for total phenol and carbohydrate content, highest amount was found in BAP (1.5mg/l) and minimum in BAP (0.5mg/l).

3. Carbohydrate content: Calli supplemented with BAP (1.5mg/l) showed the highest carbohydrate pecentage i.e. 97% among the four chosen combinations followed by BAP 2mg/l which gave 90% carbohydrate content. BAP (1 mg/l) with NAA (1mg/l) showed 89% whereas BAP (0.5mg/l) had 82% of carbohydrate content. The medium supplemented with BAP (1.5mg/l) and BAP (2.0mg/l) produced higher amount of phenolic and carbohydrate content. This indicates that there may be a positive co-relation between sugars and phenolic content. Plants produce a wide range of carbon based secondary metabolites, among which polyphenols are predominant and are derived from phenyl propanoid pathway. Phenyl propanoid pathway is the continuation of the Shikimate pathway which uses phosphoenolpyruvate (PEP) as a precursor. When carbohydrate content is more, excess PEP is formed which is an intermediate product of glycolysis cycle and is then diverted to the secondary metabolite pathway, specifically shikimate pathway specific to plant system.

4. Total protein content: Protein content of 30 and 60 days old calli (BAP 1.5mg/l) was 0.2mg/ml and 0.65mg/ml respectively. Increase in protein content and browning with ageing of calli is due to release of polyphenols in the medium. As mentioned polyphenol are phenyl propanoids derived from the carbon skeleton of phenylalanine and other aromatic amino acids. Increase in the protein content can be correlated with increase in phenol content, and attributed to the fact that, with the increased protein there may be increase in various amino acids including aromatic amino acids which are the precursors for Phenylpropanoid pathway and thus increase in polyphenols. To confirm this fact, further molecular investigations on expression of enzymes have to be carried out.

Justicia sps. is a rich source of economically important pharmaceutical secondary metabolites. Based on the results, it can be inferred that MS medium supplemented with cytokinins like BAP can enhance the production of secondary metabolites and increased in vitro biomass production of callus, using various growth regulators can be obtained.

REFERENCES

[1] Durkee,L.H.,1986, "Flora Costaricensis: Acanthaceae,"*Fieldiana Bot.*,18,pp.1-87.

[2] Correa,G.M., and Alcantara,A. F. de C.,2012, "Chemical constituents and biological activities of species of *Justicia* - a review,"*Brazilian J.of Pharmacog.,* 22(1),pp. 220-238.

[3] Angonese,M.T., Moreira,D.L., and Kaplan, M.A.C.,1992,"Perfilquímico da familia acanthaceae,"*Boletim do museu de biologia mello leitão. nova série.*1,pp. 3-6.

[4] Al-Juaid,S.S., and Abdel-Mojib,M.A.,2004, "A novel podophyllotoxin lignan from *Justicia heterocarpa," Chem. Pharm. Bull.*, 52,pp. 507-509

[5] Mpiana,P.T., Bokota,M.T., Ndjele,M.B.L., Mudogo,V., Tshibangu,D.S.T., Ngbolua,K.N., Atibu,E. K., Kwembe,J.T.K., and Makelele,L.K.,2010,"Antisickling activity of three species of Justicia from Kisangani (D.R. Congo): J. tenella, J. gendarussa and J. insularis," *Int. J. Biol. Chem. Sci..* 4(6),pp. 1953-1961.

[6] Murashige, T., Skoog, E., 1962, "A revised medium for rapid growth and bio-assays with tobacco tissue culture," Physiol. Plant, 15, pp. 473-497.

[7] Stankovic,M.S.,2011, "Total Phenolic content, flavonoid concentration and antioxidant activity of Marrubium peregrinum l. Extracts,"*Kragujevac J. Sci.*, 33,pp. 63-72.

[8] 8.Ibrahim,M.H., Jaafar,H.Z.E., Karimi, E., and Ghasemzadeh,A., 2014,"Allocation of Secondary Metabolites, Photosynthetic Capacity, and Antioxidant Activity of Kacip Fatimah (Labisia pumila Benth) in Response to and Light Intensity," *The Sci. World J.*, 2014,pp.1-13.

[9] Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., 1951, *J. Biol. Chem,* 193,pp. 265.

[10] Kumar,S., andKanwar,J.K.,2007,"Plant regeneration from cell suspension in Gerbera gamesonii Bolus,"*J. Fruit Ornamental Plant Res.*, 15,pp.157-166.

[11] Ngara,R., Rees,J., and Ndimba,B.K.,2008,"Establishment of sorghum cell suspension culture system for proteomics studies,"*Afr. J. Biot.*," 7,pp. 744-749.

[12] Kulkarni,V.M.,Varshny,L.R., Bapat, V.A., and Rao,P.S.,2002, " Somatic embryogenesis and plant regeneration in a seeded banana [Ensete superbum (Roxb.) Cheesman]," *Curr. Sci.* 83, pp. 939-941.

[13] Rahman, M., Asaduzzaman,M., Nahar,N., and Bari,M.A.,2006,"Efficient plant regeneration from cotyledon and midrib derived callus in eggplant (*Solanum melongena* L.),"*J. Biosc.*14, pp. 31-38.

[14] Dixon, R.A., and Gonzales,R.A.,1994, "Plant cell culture: a practical approach," *Oxford University Press (2nd Ed.)*: New York.

[15] Greco,B., Tanzarella, O.A., Carrozzo, G., and Blanco, A.,1984,"Callus induction and shoot regeneration in sunflower (Helianthus annuus L.)," *Plant. Sci. Lett.* 36(1), pp. 73–77.

[16] North, J,J, Ndakidemi,P. A.and Laubscher,C. P., 2011, " Effects of antioxidants, plant growth regulators and wounding on phenolic compound excretion during micropropagation of *Strelitzia reginae," Int. J. Phy. Sci.* 7(4),pp. 638 – 646.