ISSN No:-2456-2165

Impact of Bap and Iaa in Various Media Concentrations and Growth Analysis of Eucalyptus Camaldulensis

Anisha Parveen.K,
M.Sc (Biochemistry),
Bharathidasan College of Arts and Science,
Erode

Senthamarai Selvi.V
Assistant Professor, PG and Research Department of Biochemistry, Bharathidasan College of Arts and Science,
Erode

Abstract:- Eucalyptus species, commonly referred to as Eucalyptus, are natives to Australia and the islands of north Eucalyptus is grown as an exotic plant species in tropical and subtropical regions of Africa, South America and Asia. The Present study was to analyze the effect of Indole 3 Acetic Acid on contamination and shoot elongation of Eucalyptus Camaldulensis in different concentration of MS media, to estimate the biochemical components in the leaves of Benzyl Amino Purine induced Eucalyptus Camaldulensis, to estimate the biochemical components in the shoot of Indole 3 Acetic Acid induced Eucalyptus Camaldulensis. The Eucalyptus Camaldulensis have potential biochemical metabolites in their leaves and shoot and has been proven to be effective for various purposes. Hence, the present study has been made to analyze the impact of Benzyl Amino Purine and Indole Acetic Acid on growth and shoot elongation of Eucalyptus Camaldulensis in various MS Media Concentration.

I. INTRODUCTION

Plants are used as building materials, medicine, paper etc. The word Papyrus refers to a thick type of paper made from the pith of the papyrus plant, Cperus papyrus. Papyrus can also refer to a document written on sheet of papers joint together side by side and rolled up into a scroll, an early from of a book. The first paper machine was invented by a French man, Nicholasolvis Robert in 1709. Paper has a long history, beginning with the ancient Egyptians and continuing to the present day (Hon, 1994). Paper could be made from cereal straw, reeds, esparto grass and even from wood by using these new chemical methods. In the 20th century, wood became the main raw material for paper (Pahkala et al., 2014).

Eucalyptus species, commonly referred to as Eucalyptus, are natives to Australia and the islands of north. They occur naturally from sea level to the alpine tree line, from high rainfall to semi-arid zones and from the tropics to latitudes as high as 43°C south. Eucalyptuses are dominant and co-dominant in almost all vegetation types where they occur and are considered keystone species for ecological studies in their natural ranges.

Fig 1:- Buds, capules, flowers and foliage of Eucalyptus Camaldulensis

Eucalyptus camaldulensis, the river red gum, is a tree of the genus Eucalyptus. It is one of around 800 species within the genus. It is a plantation species in many parts of the world, but is native to Australia, where it is the mostly widespread natural distribution, especially beside inland water courses. The tree produces a welcoming shade in the extreme temperatures of central Australia, and plays an important role in stabilizing river banks.

Indole 3-acetic acid (IAA, 3IAA) is the most common, naturally occurring, plant hormone of the auxin class. It is the best known of the auxins, and has been the subject of extensive studies by plant physiologists. IAA is a derivative of Indole, containing a carboxymethyl substituent. It is a colorless solid that is soluble in polar organic solvents.

The Present study was to analyze the effect of Indole 3 Acetic Acid on contamination and shoot elongation of Eucalyptus Camaldulensis in different concentration of MS media, to estimate the biochemical components in the leaves of Benzyl Amino Purine induced Eucalyptus Camaldulensis, to estimate the biochemical components in the shoot of Indole 3 Acetic Acid induced Eucalyptus Camaldulensis.

II. MATERIALS & METHODS

A. *Sample*

The Eucalyptus Camaldulensis was selected as the explant source of the present study. It was collected from Garden of Tamilnadu Newsprint and Papers Ltd Karur . The auxiliary buds were used as explants.

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B. *Preparation of Explants*

Sprouted branches (approximately 100 mm) of Eucalyptus Camaldulensis with apical and auxiliary buds were harvested and cut into explants of about 1 cm,, bearing nodal segments with apical or auxiliary buds. The leaves were surface sterilized by rinsing in running tap water for an hour. Then they were washed with distilled water for 5 to 6 times. After thorough washing, the materials were taken into the laminar flow chamber where they were disinfected with 70% Alcohol for 60 seconds then disinfected with 0.1% Mercuric chloride for 3 minutes. Finally the materials were thoroughly rinsed with 2 sterile distilled water for 6-7 times to remove the trace of Mercuric chloride and Alcohol.

C. *Preparation of MS Stock Standard (Media A)*

Both macronutrients and micronutrients was dissolved separately in a beaker and then made up to 1 L with distilled water and were used as stock solution.

- *Preparation of Culture Media Using BAP*

BAP, CAP, Sucrose, Riboflavin was added to five different concentration of media and it was named as A1, B1, C1, D1 and E1. The media was made into gel by adding gel rich. The pH was adjusted to 5.7 by using NaOH.

- *Preparation of Culture Media Using IAA*

BAP, CAP, Sucrose, Riboflavin was added to five different concentration of media and it was named as A2,B2,C2,D2 and E2.The media was made into gel by adding gelrich. The pH was adjusted to 5.7 by using NaOH.

III. METHODOLOGY

- *Inoculation Procedure*

The surface sterilized explants were aseptically transferred to the 10 different culture media (A1 to E1 and A2 to E2) in the laminar flow chamber. The explants with nodal region were inserted vertically using forceps and then the bottles were closed. After a week, the growth and the elongation rate was measured and the best results were taken for further studies.

- *Culture Conditions*

The cultures were maintained in a culture at 25± 2°C under 16 hour light and 8 hours dark photoperiod with light intensity of 1350 lux supplied by a cool white fluorescent tube. Their growth conditions were referred to as standard culture condition for in vitro studies.

- *Analysis of growth Rate in BAP Culture Media*

The contamination and number of leaves and bunches were monitored as growth parameters. Data was taken from the five different Media concentrations (A1, B1, C1, D1 and E1).

- *Analysis of Shoot Elongation in IAA Culture Media*

The Contamination and shoot height, shoot diameter were monitored as growth parameters. Data was taken from the five different Media concentrations (A2, B2, C2, D2 and E2).

- *Quantitative determination of the Biochemical Components*

Estimation of protein (Lowry et al., 1951), Estimation of total soluble sugars (Dubioi's et al., 1951), Determination of total phenols by spectra photometric method (Mahadev, 1996).

IV. RESULTS AND DISCUSSION

In the present investigation, the Eucalyptus camaldulensis were evaluated for growth factor by MS medium with various concentrations in BAP and IAA hormone and subsequently the ethanol extract of shoot and leaf for its phytochemical analysis. Tissue culture method with enhanced auxiliary bud breaking was used to achieve high frequency of shoot induction. Plant clones derived from shoot apices and auxiliary buds often have been observed to be uniform suggesting genetic stability as in Ecucalyptus camaldunlensis. (Murshige 1972, Gupta and Masearenhar., 1982).

Plant growth regulators and nutrients are the two major consideration for the medium preparation when optimizing the tissue culture medium.BAP at 0.00mg/Lto 0.5mg/L for multiple leaves production were used for the micro propagation of Eucalyptus tereticornies (Lakshmi sit and shoba rani., 1985).

S. No.	Concentration of Media	Number of Leaves (in numbers)			
		7th day	14th day	21st day	28th day
1	A1	0 ± 0	4 ± 0	8 ± 0	12 ± 1
2	B1	3 ± 0	5 ± 1	7 ± 1	10 ± 1
3	C1	5 ± 0	8 ± 1	12 ± 1	14 ± 0
4	D1	6 ± 1	12 ± 1	16 ± 0	20 ± 1
5	E1	4 ± 1	8 ± 1	14 ± 1	16 ± 1

Table 1 :- Analysis of different concentration in BAP media growth rate of Eucalyptus Camaldulensis

The number of leaves and bunches were monitored as growth parameters measured at the 7th day in all five culture media (A1, B1, C1, D1 and E1). D1 showed more number of leaves (6) compared to the other culture media. A1 did not show any leaf development. BAP was added in different concentrations to the shoot induction media and its effect was studied. It was seen that the best result from leaf callus was obtained at 5 mg/l of BAP and in case of callus obtained from nodal cuttings, at 3 mg/l of BAP comparatively a high number of shoot proliferation was observed. No shoots were produced in BAP concentrations lower than 3 mg/l or higher than 5 mg/1 (Natasha Mahajan *et al*., 2012).

The number of leaves and bunches were monitored as growth parameters measured at the 14th day in all five culture media (A1, B1, C1, D1 and E1). D1 showed more

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number of leaves (12) compared to the other culture media. A1 showed the least number of leaves. Endang *et al*., 2016 have used BAP medium for the growth of shoots, leaves and roots on Sapodilla (Achras zapota) and have reported that leaf explants gave the best results in terms of maintaining the genetic information of the plant.

The number of leaves and bunches were monitored as growth parameters measured at the 21st day in all five culture media (A1, B1, C1, D1 and E1). D1 showed more number of leaves (16) compared to the other culture media. B1 showed the least number of leaves (7). Cathleen *et al*., 2011 reported the effect of light on explants grown in BAP medium, inducing shoot formation.

The number of leaves and bunches were monitored as growth parameters measured at the 28th day in all five culture media (A1, B1, C1, D1 and E1). D1 showed more number of leaves (20) compared to the other culture media. B1 showed the least number of leaves (10). When MS medium was fortified with BAP (3.5 mg/L), NAA (2.5 mg/L), 2, 4-D (0.5 mg/L) and the best response was observed on intermodal callusing (80%) when the MS medium contained 3.0 mg/L BAP, 2.0 mg/L NAA and 0.5 mg/L 2, 4-D (M. Elamvaluthi *et al*.,2016).

- *Analysis of growth response on shoot height of Eucalyptus camaldulensis in different concentrations of IAA media*

The shoot elongation was monitored as a growth parameter. The shoot elongation growth rate was measured at 7th day, 14th day, 21stday and 28th day in all five culture media (A2, B2, C2, D2 and E2). Compared to other concentrations, D2 and E2 media showed best shoot height. But E2 plant was retrified and showed a glossy look. The shoot was very soft and it had less fibre content. So D2 plant was best and was taken for further studies. Shoot formation can be optimized by manipulation of medium phosphate and nitrogen level.

S. No.	Concentration of Media IAA	Shoot Height (in cm)			
		7th day	14th day	21st day	28th day
1	A2	0.29±0.02	0.42±0.02	0.61±0.03	0.76±0.01
2	B2	0.44±0.02	0.64±0.01	0.84±0.06	1.48±0.08
3	C2	0.67±0.08	0.98±0.06	1.89±0.3	2.22±0.06
4	D2	0.81±0.01	1.53±0.06	2.06±0.10	3.09±0.14
5	E2	0.75±0.04	1.02±0.01	1.93±0.3	2.33±0.02

Table 2 :- Analysis of growth response on shoot height of Eucalyptus Camaldulensis in different concentrations of IAA Media

The shoot elongation was monitored as a growth parameter measured at the 7th day in all five culture media (A2, B2, C2, D2 and E2). D2 media showed the highest growth response (0.81cm) compared to the other culture media. A2 media showed the least growth response (0.29cm). Logeswari et al., 2014 carried a weekly measurement of the shoot growth in Eucalyptus tereticornis.

The shoot elongation was monitored as a growth parameter measured at the 14th day in all five culture media (A2, B2, C2, D2 and E2). D2 media showed the highest growth response (1.53cm) compared to the other culture media. A2 media showed the least growth response (0.42cm). This study revealed that the plant reacts to different concentrations of IAA for their metabolic differential activities especially on their metabolites. However, the results of the study contrast with the report of Ahuzi on the vegetative propagation of Pinus caribaea. The report revealed that 25 ppm should be used in the propagation of Pinus caribaea.(Mbagwu FN,2017).

The shoot elongation was monitored as a growth parameter measured at the 21st day in all five culture media (A2, B2, C2, D2 and E2). D2 media showed the highest growth response (2.06cm) compared to the other culture media.

The shoot elongation was monitored as a growth parameter measured at the 28th day in all five culture media (A2, B2, C2, D2 and E2). D2 media showed the highest growth response (3.09cm) compared to the other culture media. A2 media showed the least growth response (0.76cm). Two different types of explants (apical shoots and lateral shoots) were cultured on MS media containing different concentrations of BAP, NAA, or KIN to evaluate their effects on shoot initiation. Explants grown on plant growth regulator-containing media showed varying success in shoot initiation depending on the type of explant and the growth regulators added. The response of explants cultured in MS media supplemented with BAP, IAA, and KIN are shown the best results (Zuraida Ab Rahman *et al*., 2015).

The biochemical compounds like Protein, Sugar and Phenol were estimated in the Eucalyptus camaldulensis grown in D1 and D2 media. Stino et al., 2009 providing plants with essential nutrient elements required for protein formation.

Proteins are essential compounds for plant growth as protein has high fiber content, it plays an important role in making a paper. In this study the protein content is more in D1 (30.4mg/l) when compared to that in D2 (26.6 mg/l). . Omoyeni *et al.*, 2011 reported the screening showed the presence of alkaloids, flavonoid, steroid, protein, and carbohydrate whereas Saponins, Tannins, Terpenoids, Quinine and Glycosides are absent. Protein having polymers it will helps to builds up the wall of plant and helps to morphologically study.

Sugars play an important role in the metabolism of a plant. If the sugar content is more, then the plant growth gradually increases. In this study the Sugar content is more in D1 (20.2mg/l) when compared to that in D2 (3.97mg/l). Rajinikanth Marka et al., 2013 reported as Phytochemical analysis of various solvent extracts of leaf of A. hypogaea revealed the presence of alkaloids, glycosides, fats, oils, phenols, lignins whereas tannins, flavonoids, quinones and saponins were completely absent. The extraction medium,

ISSN No:-2456-2165

methanol extracts of stem bark of Eucalyptus Camaldulensis displayed maximum antibacterial activity against B.subtilis bacterial species.

Phenol is one of the most important aromatic compounds present in a plant. It plays a very important role in antibiotic activity. In this study the phenol content was more in D1 (26.3mg/l) when compared to that in D2 (15.4mg/l). The percentage yield of extracts and the phytochemical constituents of the plant are shown high in Eucalyptus camaldulensis (Bhawana Pandey *et al*., 2014). The components, anthraquinones, hydrolysable tannin, phenol, having volatile compound which is used for preparing eucalyptus oil. *Eucalyptus* oil is a crid and bitter and is a ruptured astringent, thrmogenic, antiseptic properties.

S. No.	Biochemical Compounds	Leaves of D1 BAP media (mg/g)	Shoot of D2 IAA media (mg/g)
1.	Protein	30.4	26.6
2.	Sugar	20.2	3.97
3.	Phenol	26.3	15.4

Table 3 :- Estimation of Biochemical compounds in BAP and IAA cultures stage of Eucalyptus camaldulensis studies

Eucalyptus oil is a crid and bitter and is a ruptured astringent, thrmogenic, antiseptic, Deodorant, stimulant, carminative, and digestive, cardio phonic, diuretic, expectorant, insect repellant, Rubefacient and antipyretic. Eucalyptus traditionally is strong wood that has been used in heavy construction and for building interiors, wooden rollers, short fiber pulp, paper, plywood, and agglomerate boards. It is used in cabinetmaking and carpentry and for crossbeams, transmission poles, firewood, and charcoal. It is also planted to shade and protect and to produce excellent-quality honey. its having essential oil for muscular pain .it is also having antimicrobial activity. Plant protein widely available , have low potential to be immunogenic and can be made into fibers, films, hydogels and medicinal applications.

Plant growth regulators are BAP, IAA and nutrients are the two major consideration for the medium preparation when optimizing the tissue culture and medium. it gives shoot elongation and growth rate of leaves in this study it was gradually increased and it will measured at growth parameter.

The phytochemical screening of the ethanolic leaves of Eucalyptus camaldulensis showed significant increase in proteins, sugars, phenols. The result of the present study signifies the use of Eucalyptus camaldulensis as exerting various medicinal activities since it contains various bioactive components.

This study indicates increase in BAP and IAA concentration and fibre source in protein content and hence can be used for pulp and paper manufacturing industries. This new work focuses mainly on eucalyptus and the environmental and industrial benefits arising from sustainable forest management. Its content is based on the compiling of independent reports and studies prepared by prestigious scientists in research centers worldwide.

Micro propagation generally involves four distinct stages: initiation of cultures, shoot multiplication, rooting of in vitro grown shoots, and acclimatization. The first stage: culture initiation depends on Explant type or the physiological stage of the donor plant at the time of excision. Explants from actively growing shoots were generally used for mass scale multiplication. The second stage: shoot multiplication is crucial and achieved by using Plant Growth Regulators i.e., auxin and cytokinin. The third stage: the elongated shoots, derived from the multiplication stage, are subsequently rooted either ex vitro or in vitro. In some cases, the highest root induction occurs from excised shoots in the liquid medium when compared with semi-solid medium. The fourth stage: acclimatization of in vitro grown plants is an important step in micro propagation.

The Indian Paper Industry started using recycled fiber as a raw material for paper making in early 70's only when Government took a conscious decision to increase domestic paper production capacity to meet the sudden spurt in domestic pulp & paper demand.

V. CONCLUSION

The Eucalyptus Camaldulensis have potential biochemical metabolites in their leaves and shoot and has been proven to be effective for various purposes. Hence, the present study has been made to analyze the impact of Benzyl Amino Purine and Indole Acetic Acid on growth and shoot elongation of Eucalyptus Camaldulensis in various MS Media Concentration.

Media A is standard MS media concentration. Media B is half the concentration of Media A. Media C, D and E are the half, triple and fouble concentrations of Media A respectively. Then the hormone BAP was added to all the five Medias and named in the order of A1, B1, C1, D1 and E1.

The hormone IAA was added to all five Medias and named as A2, B2, C2, D2 and E2. Then the buds of Eucalyptus Camaldulensis was selected a source of the explants and was made to grown in vitro culture of different MS media concentrations. After every week, the number of leaves and bunches, shoot elongation were monitored as growth parameters in BAP and IAA induced cultured plants. Compare to others D1 and D2 shown best results. D1 shown 6, 12, 16 and 20 leaves at 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively. D2 shown shoot height of 0.81 cm, 1.53cm, 2.06cm and 3.09 cm at 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively. Then the biochemical components in leaves of D1 and shoot of D2 were estimated. The protein content is more D1 (30.4mg/g) than the compared to D2 (22.6mg/g). The sugar content is more in D1 (20.2mg/g) than the compared to D2 (3.97mg/g). The phenol content is more in D1 (26.3mg/g) than the compared to D2 (15.4mg/g). Therefore the study reveals that the plant Eucalyptus Camaldulensis had higher growth rate in triple the concentration of standard MS media.

REFERENCE

[1]. Hon, D. N.-S.Cellulose: a random walk along its historical path. Cellulose. 1994; 1: 1-25.
[2]. Pahkala, L. Paavilainen and Mela T. Grass Species as Raw Material For Pulp And Paper. Institute of Crop and Soil Science, Agricultural Research Centre of Finland, FIN-31600, 2014.
[3]. Murashige, T. and Skoog, F.A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 1962; 15: 473-497.
[4]. Natasha Mahajan, Manoj Kumar, Vikas Shrivastava, Vijayta Sharma, Uma wood clone, international journal of novel trends in pharmaceutical sciences, 2014;(4): 456-468.
[5]. Logeswari N and Kanagavalli J.Influence Of Iaa On In Vitro Shoot Elongation Of Eucalyptus Tereticornis-103; A Potencial Pulp Wood Cloneinternational Journal Of Novel Trends In Pharmaceutical Sciences.2014; 56-58.
[6]. Endang Yuniastuti, Novita Chrisna Wardani and Nandariyah. The Effect of Explant Type and 6-Benzyl Adenine (BAP) in Sapodilla (Achras zapota) Micropropagation. American journal for biochemistry.2016; 6 :2016-2018.
[7]. Cathleen M.A, Raj Deepika, Alexander A Myburg, Martin Ranik, for improved in vitro plant health and transformability, BMC Proceedings, 2011; 5:153.
[8]. Steven H Strauss, Development of Eucalyptus tissue culture conditions Mbagwu FN, Ogbonnaya CI, Umeoka N, and Edoki N, Effects of Indole-3-Acetic Acid (IAA) on the Vegetative Propagation and Phytochemical Properties of Bushbuck (Gongronema Latifolium Benth. Food Process Technol, 2017; 8: 1.
[9]. Zuraida Ab Rahman, Erny Sabrina Mohd Noor, Mohd Shukri Mat Ali, Razali Mirad, Ayu Nazreena Othman. In Vitro Micropropagation of a Valuable Medicinal Plant, Plectranthus amboinicus , American Journal of Plant Sciences.2015; 1091-1097.
[10]. Stino R.G, Mohsen A.T, Maksouds M.A, Abd El-Migeed M.M.M, Gomaa A.M, Ibrahim A.Y. Bioorganic fertilization and its Impact on Apricot young trees in newly reclaimed soil. American-Eurasion. J. Agric. Environ. Sci, 2009; 6(1):62-69.
[11]. Bhawana Pandey, Sheetal Singh, Evaluation of Antimicrobial Potential of Eucalyptus camaldulensis L. Journal of Pharmaceutical , Chemical and Biological Sciences, 2014.; 2(3): 166-171.
[12]. Rajinikanth Marka, Samatha Talari, Srinivas Penchala, Shyamsundarachary Rudroju, Rama Swamy Nanna, Preliminary Phytochemical Analysis of Leaf, Stem, Root and Seed Extracts of Arachis hypogaea L. Int. J. Pharm. Sci. Rev. Res, 2013; 20 (1) : 134-139.