Kong. Res. J. 2(2) : 146-150, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

DOI:10.26524/krj112

EFFECT OF INDOLE -3- ACETIC ACID ON CALLUS INDUCTION FROM LEAF EXPLANT OF *OCIMUM BASILICUM* LINN.

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ABSTRACT

Ocimum basilicum L. belongs to the family Lamiaceae; contains plenty of phytochemicals with significant nutritional as well as health benefits. Antioxidant activity of methanol extract of *Ocimum basilicum* callus was evaluated using various antioxidant assays. Proliferated callus was obtained on half-strength MS media supplemented with 1.40µM IAA. The callus extract showed considerable DPPH radical scavenging, hydroxyl radical scavenging and ferrous ion chelation activity with IC_{50} value of 16.05, 170.7 and 29.16µg/mL respectively.

Keywords: *Ocimum basilicum,* Lamiaceace, *in vitro* callus production, free radical scavenging activity.

1. INTRODUCTION

Ocimum basilicum L. commonly called as Sweet Basil belongs to the family Lamiaceace is a native plant of Indo Malayan region. It is called the "king of herbs" which contains plenty of phytochemicals with significant nutritional as well as health benefits (Jayasinghe *et al.*, 2003). Sweet basil is cultivated for production of essential oils, dry leaves as a culinary herb, condiment/spice or as an ornamental plant (Zheljazkov *et al.*, 2007). Sweet Basil has shown unique health protecting effects due to its important flavonoids and volatile oils. The unique array of active constituents called flavonoids found in basil provides protection at cellular level. Orientin and vicenin are two water soluble flavonoids that have been of particular interest in basil (Nyak and Uma, 2005). Aromatic leaves and flowering parts of *O. basilicum*are traditionally used as stimulant and tonic in folk remedies to treat various ailments such as poor digestion, stomach ache, feverish illnesses, nausea, abdominal cramps, gastro enteritis, migraine, insomnia, depression, gonorrhoea, dysentery and chronic diarrhoea exhaustion (Chopra *et al.*, 1986). Externally, they have been applied for the treatment of acne, insect stings, snake bites and skin infections (Martin and Ernst, 2004).

2. MATERIALS AND METHODS

2.1. Tissue culture studies

2.1.1. Explants selection and mode of sterilization

Leaf explants were collected from actively growing plants and washed thoroughly in running tap water followed by Teepol treatment for 5-10 min.The explants were subsequently surface sterilized with 0.1% (w/v) mercuric chloride solution for 2-3 min and washed 3-4 times with sterile double distilled water for duration of 15 min with an interval of 5 min for each wash.

2.1.2. Method of media preparation

MS (Murashige and Skoog) medium (half strength) was employed in the present study. For the preparation of medium only analytical reagents of "Hi-media" grade chemicals and Borosil glassware's were used. Double distilled water was used for preparing the media. The nutrient media basically consists of inorganic salts, carbon source, vitamins and amino acids. Stock solutions were prepared separately for macronutrients, micronutrients, iron, potassium iodide and vitamins. All the chemicals were weighed accurately in electronic weighing machine (And Electronic balance, ER-182 A). All the stock solutions were poured in to well stoppered sterilized bottles and preserved in a refrigerator at 4^{o}C. Specific quantity of the stock solutions and growth regulators were pipetted onto a little beaker. The final volume was made up to one litrewith distilled water.

To the above said media, 3% sucrose was added and pH was adjusted to 5.8 with either 0.1N NaOH or 0.1N HCl using a pH meter (ELICO), further 0.8% agar (extra pure gelling point 32-35°C, Hi media, Bombay) was added, melted in a water bath and the medium was dispensed into 25 mL (25 x 150 mm) test tubes (10-15 mL medium). The tubes after covering with cotton plug were autoclaved at 1.06 kg pressure/sq cm for about 20min at 121°C. The autoclaved medium in the culture tubes were cooled and allowed to solidify as slants and were stored at 25ºC in the dark for future use. The inoculation was done after five days to ensure that the tubes were free from contamination.

2.2.3. Growth regulator and its preparation

Growth regulatornamely indole 3-acetic acid was used in the experiments. The growth regulator was stored at 4° C until use.

a) Auxins and their preparation

Auxin namelyindole 3-acetic acid (IAA) was used in this experiments. The stock solution was prepared by dissolving 10 mg of IAA in 1mL of ethanol.Then the volume was made up to 100 mL with sterile double distilled water and different concentrations (0.28 - 2.8 µM) were used.

2.2.4. Culture conditions

The cultures were maintained at 25±2°C under a 16 hr photoperiod of 50-60 µ mol m^{-2} S^{-1} flux intensity provided by cool white fluorescent tubes. Each treatment consisted of five replicates and experiment was repeated thrice.

2.2.5. Callus induction

Leaf explants were used for callus induction on MS (half strength)medium supplemented with various concentrations of IAA (0.28 - 2.8 µM)individually. Percentage of callus induction was recorded after 15 days of culture.

2.2.6. RAPD analysis

DNA isolation was performed with modified protocol of Padmalatha and Prasad (2006). Freshly collected leaf and callus sample (both *in vivo* and *in vitro*respectively) was ground in CTAB extraction solution using a mortar and pestle along with 0.1% of PVP. The pulverized leaves were quickly transferred to centrifuge tubes. The tubes were incubated at 65° C in hot air oven or water bath for 60-90 min with intermittent shaking and swirling for every 30 min. Equal volume of chloroform: isoamylalcohol (24:1) was added and mixed properly by inversion for 30 min and centrifuged at 12,000 rpm for 15 min at room temperature. The supernatant was carefully decanted and transferred to a new tube. 1/10th volume of CTAB/NaCl solution was added and mixed by inversion. Chloroform: isoamylalcohol extraction step was repeated. Aqueous phase was transferred to fresh tubes and equal volume of CTAB precipitation solution was added and centrifuged at 3000 rpm for 5 min at 4ºC. Supernatant was removed and the pellet was washed with high salt TE buffer. 0.6 mL of isopropanol was added and centrifuged at 10,000 rpm for 15 min at 4°C. Supernatant was removed and the pellet was washed with 70% ethanol, centrifuged at 10,000 rpm for 2 min at 4º C. Supernatant was removed and air dried the pellet. The pellet was resuspended in TE buffer.

2.2.6.1. Preparation of Agarose gel

Agarose gel (1.5%) was prepared by adding 0.75 g of agarose (low EEO grade, HiMedia, India) in 50 mL of 1X TAE buffer. It was heated to dissolve agarose. Ethidium bromide (0.5 mg/mL) was added, mixed well and poured into the gel casting platform with well former. The gel was allowed to polymerize at room temperature.

2.2.6.2. PCR amplification

The extracted DNA was amplified using PCR technique in the Eppendorf gradient thermal cycler with the aim of studying genetic variability of *in vitro* callus of *O.basilicum*. The genomic DNA was amplified using four primers. Each primer is a 10-mer of arbitrary sequence: 1(5'-TGCCGAGCTG-3'), 2(5'-TCGTTCCGCA-3'), 3(5'-CACCTTTCCC-3'), and 4(5'-GTGCAACGTG-3') (GE healthcare, UK). PCR amplification was performed in 20µL reaction mix containing 40 ng genomic DNA for 45 cycles. The following conditions were followed: i) 92ºC initial denaturation for 5 min, ii) 92º C denaturation step for 30 s,iii) 33º C, 33.6º C, 32.5º C and 32º C annealing for 1 min for each primer respectively and iv)72ºC extension for 2 min followed by a final extension of 72° C for 5 min. Reactions were carried out in a volume of 20 µL containing 10 µL of PCR master mix (2x) (Merck Specialties, Mumbai) solution with 4 µL of nuclease free water, 3 µL of primer and 3 µl of template DNA. The final product was separated and visualized using agarose gel electrophoresis.

2.2.6.3. Electrophoresis of genomic DNA

The PCR product was separated by horizontal electrophoresis through 1.5% agarose gel

mixed with ethidium bromide (0.5 mg/mL) for 45 min at 50 V in tris acetate EDTA buffer (40 mM Tris; 2 mM EDTA; 20 mM Glacial acetic acid pH8) (Sambrook*et al*., 2001). The samples were mixed well with loading dye (1:1) and the samples were gently loaded on to the wells using a disposable micropipette tip. The bands were visualized using gel documentation system (Biorad, Italy).

3. RESULTS AND DISCUSSION

3.1. Callus induction

Leaf explant of *Ocimum basilicum* was cultured on half strength MS medium supplemented with IAA at different concentrations. Medium devoid of hormone did not show any response on callus induction. The inoculated leaf explants of *O. basilicum* on medium containing IAA induced callus after a week of culture. Out of ten different concentrations tested, 0.28 µM failed to induce callus. Good callus proliferation was observed on medium containing 0.56 µM to 1.40 µM IAA. Formation of anthocyanin like pigments from callus was recorded at concentration 0.84 µM and 1.12 µM IAA (Fig. 1). Root induction was observed with increase in concentration (Fig. 2). *In vitro* culture of medicinal plants (Shohael *et al.,*2006; Shin *et al.,*2008; Jeong *et al.,*2009; Park *et al.,*2012) with the objective to isolate secondary metabolites or altering or enhancing the concentration of secondary metabolites (Schijlen *et al.,*2006, Dorais *et al.,*2008) has been well established.Owing to callus itself had the ability to produce secondary metabolites, plant tissue culturists use this capacity to gain many useful chemicals via callus culture *in vitro* such as flavor and fragrance, pigment and pharmaceutical compounds (Ram *et al*., 2011).

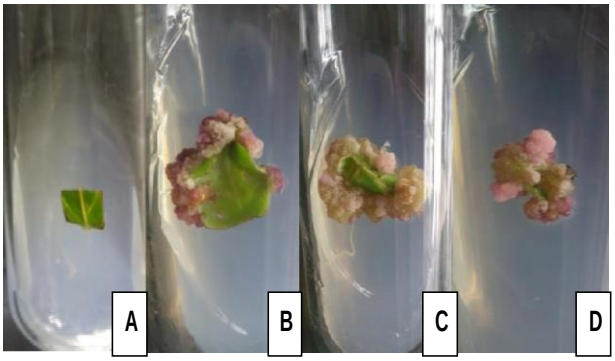

Fig. 1. Formation of Anthocyanin like pigments on Callus.

In the present study, *O. basilicum* callus induced on the MS medium containing lower concentrations of IAA released anthocyanins. This result indicated that plant growth regulator is necessary for the production of valuable secondary metabolites.

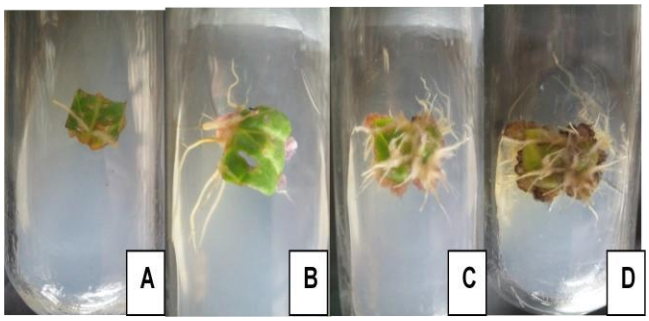

Fig. 2. Root induction from Leaf explants

3.2. RAPD analysis

The genetic level variation of *in vitro* developed callus was accessed through RAPD analysis (Fig 3). The result of RAPD analysis indicates *in vitro* developed callus was slightly polymorphic. The polymorphic banding pattern possibly indicates that there was a genetic level variation.

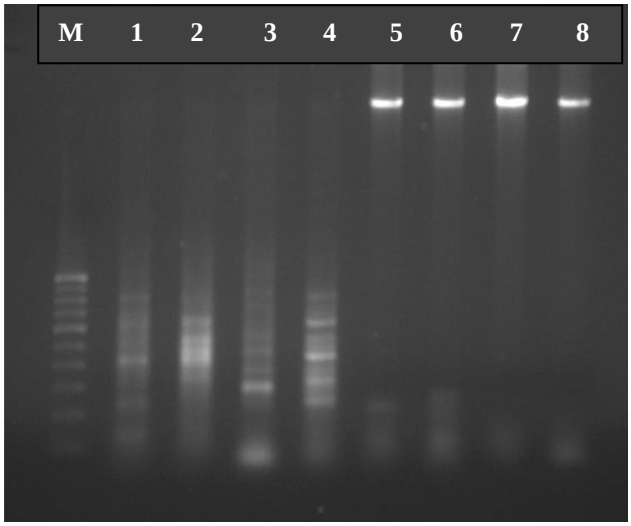

Fig. 3: RAPD analysis of *in vitro* callus.

Random amplified polymorphic DNA (RAPD) markers have proved to be very useful tool providing a convenient and rapid assessment of the genetic differences between genotypes (Williams *et al*., 1990). Moreover, RAPD use arbitrary primers that provide a large number of multilocus markers and can be applied to analyze almost any organism even those for which no previous genetic or molecular information are available. RAPD is referred as an appropriate tool for certification of genetic fidelity of *in vitro* propagated plants (Gupta

and Rao, 2002). The polymorphic banding pattern in the callus of *O. basilicum*possibly indicates that there is a negligible level of genetic variation. Bernhardt *et al*. (2014) have evaluated the RAPD banding pattern of eight different *O. basilicum* gene bank accessions and have reported similar result. Anamika *et al*. (2010) reported the same condition in *Pogostemoncablin*. Rady and Nazif (2005) reported that *in vitro* shoots of *O. americanum* gave polymorphic bands by using the random primers.

The current outcome for *O. basilicum* pointed out that IAA exactly had the influence on the antioxidant property of the callus. Similar results for antioxidant activity of *O. basilicum*have been reported in the literature (Javanmardi *et al*., 2003; Seung-Joo Lee *et al*., 2004; Politeo *et al*., 2007; Gulcin *et al*., 2007).

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