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Topic : Study on compatibility of natural IAA isolation from bacteria (a potential) in tissue culture growth *Bryophyllum* plant

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Abstract

Plant tissue culture is a method used to grow and propagate plants outside of their native habitat in a controlled laboratory setting. The purpose of the current investigation was to clarify the potential function of bacterial auxin in plant tissue culture. In order to achieve this, MS basal medium was supplemented with bacterial supernatant containing auxin, and the effect of this addition on in vitro plant growth was investigated. With internodal explants inoculated on MS + BS, callus induction was observed at the apex of explant at 1 and 2 ml, where adventitious root induction is observed In DS1, DS2 and DS-3. In treatment plant DS3 shows significant increase of carbohydrate and root length with number of advantage root number was increase as compared to other DS1. In comparison of control plant no one is increase root num or length, were not reported increase of in terms of biomass carbohydrates and protein.

INTRODUCATION

Succulent plants in the genus *Bryophyllum* are frequently employed in plant tissue culture because of their capacity to regenerate entire plants from tiny explants or callus tissues. Somatic embryogenesis is the term for this process. Undifferentiated cells are propelled into the formation of embryos during somatic embryogenesis(1), which can later grow into whole plants. *Bryophyllum* is especially helpful in this process because it is simple to regenerate and may quickly produce a large number of plants. Moreover, *Bryophyllum* can be used in plant tissue culture to examine how different growth regulators affect the growth and development of plants. Due to its therapeutic benefits, *Bryophyllum* has been utilised in traditional medicine, particularly for the treatment of infections and wounds (2, 3).

Plant tissue culture is a method used to grow and propagate plants outside of their native habitat in a controlled laboratory setting. The growth medium used to assist plant development and growth is one of the essential elements in plant tissue culture. The Murashige and Skoog (MS) medium, created in

1962 by plant scientists Toshio Murashige and Folke Skoog, is one of the most frequently used growth media. (4,5)

In order to produce a balanced and optimal nutrient environment for plant tissue culture, the MS medium is a sophisticated blend of salts, vitamins, and plant growth regulators(6,7). It has the ideal ratios of macronutrients like nitrogen, phosphorous, and potassium, as well as micronutrients like iron, manganese, and zinc, to support the growth of plants(8). A variety of vitamins, including thiamine, pyridoxine, and nicotinic acid, which are necessary for plant growth and development, are also included in the medium.

The primary endogenous regulators of numerous aspects of plant growth and development are plant hormones. Auxin, one of the most thoroughly investigated hormones, controls In plants, cells divide, lengthen, differentiate, and form patterns (9).Both naturally occurring chemicals and closely related manufactured compounds with comparable actions are classified as auxins. The hormones auxin and cytokinin regulate the growth of plants through a variety of intricate interactions. Auxin-cytokinin ratio influences the development of lateral roots and the growth of shoot axillary buds (10). (11).

Auxin can also be biosynthesized in lower plants(12). IAA that is physiologically active and produced by organisms like bacteria may have significant effects on plant growth and development. According to (13), over 80% of bacteria isolated from plant rhizospheres may produce indole-3-acetic acid. Because its addition to bacterial cultures that produce IAAs encourages and boosts IAA synthesis, L-tryptophan is also regarded as the IAA precursor in bacteria, just like it is in plants (13,14). For the rhizosphere microflora, root exudates constitute a natural supply of L-tryptophan, which may improve auxin biosynthesis in the rhizosphere (15,16). The purpose of the current investigation was to clarify the potential function of bacterial auxin in plant tissue culture. In order to achieve this, MS basal medium was supplemented with bacterial supernatant containing auxin, and the effect of this addition on in vitro plant growth was investigated.

Material Method

Explant preparation and culture establishment: *Bryophyllum* leaf cultures were created using the method (17). Plants in good health were purchased at the botanical garden. Fresh leaves were chosen, trimmed, and rinsed with tap water to remove dust before going through two detergent washes and being sterilised by soaking them in 70% ethanol for one minute. By treating the explant with 1% sodium hypochlorite for 10 minutes and then rinsing it three to four times in sterile distilled water, the explant was further sterilised. The standard Murashige and Skoog's (1962) medium, which contains 3.0 mg L-1 BAP, 0.01 mg L-1 NAA, 0.8% agar, and 3.0% sucrose, was used for aseptically inoculating the explant. Every three weeks after the initial establishment of cultures, routine sub-culturing was carried out. Cultures were maintained

Experiment design: The MS medium (Murashige and Skoog, 1962) was the base medium utilised in this investigation. It contained 8 g agar l-1, 30 g sucrose l-1, 100 mg myoinositol l-1, and 0.4 mg thiamine-HCl l-1 as carbon sources. Each test tube (2.5 15 cm) received five millilitres of MS medium, which was utilised in various combinations. Filter-sterilized bacterial supernatants were added to three groups of

combinations in varying amounts—1, 2, and 5 ml each in 50 ml of MS media. Three different MS medium combinations were used: DS1: MS + BS(1 ml), DS2: MS + BS(2 ml), DS3: MS + BS(5 ml), and DS4: MS + IAA(2 mg).(18)

Measurement of growth parameters: Average root length, total root number and total biomass in terms of fresh and dry weight were measured. For measurement of biomass (fresh weight and dry weight), propagules obtained from each treatment were taken out and the fresh weight was measured using an electronic top pan balance. For dry weight calculation, after measuring the fresh weight those fresh shoots were kept in an oven at 62˚C for drying till constant weight.

Chlorophyll contents: The chlorophyll contents were calculated as per the method described by (19). 500 mg of shoots were weighed and ground in pestle and mortar with 80 % acetone under dark conditions. Extracts were centrifuged at 10, 000 rpm and the supernatant was used to measure absorbance on spectrophotometer (UV-1800 Shimadzu, Japan).

Total phenols: The total phenol content was measured as per the method described (20) 500 mg of shoots were weighed and crushed in pestle and mortar in 70 % methanol. The extracts were centrifuged at 10, 000 rpm for 15 minutes. The clear supernatants were used for quantitative determination of total phenol content. For each reaction 500 µL methanolic extract was taken in a test tube and to this 1.0 mL suitably diluted (1:1 ratio of reagent and DDW) Folin Ciocaltaeu's reagent was added followed by 2.0 mL of Na2CO3 (20 % w/v) solution. The test tubes were heated in boiling water bath with intermittent shaking for about 1.0 min. Tubes were subsequently cooled under running tap water. The blue colored product was diluted to 25 mL by adding DDW and the percent transmittance was measured at 650 nm in a spectrophotometer (UV-1800 Shimadzu, Japan). The total phenol concentration in each sample was estimated with the help of standard curve prepared using different concentrations (10-100 µg) of caffeic acid.

Total carbohydrates: Quantitative estimation of total carbohydrate content was carried out as per method described by Tandon (21). In vitro derived propagules were homogenized in 0.1 M phosphate buffer (pH 7.0) and the homogenates were centrifuged at 10, 000 rpm for 15 min. For each reaction 15 µL of supernatant was mixed with 4.0 mL of 0.2 % Anthrone reagent (in conc. H2 SO4) and placed in boiling water bath for five minutes. The absorbance was recorded at 610 nm wave length. The total carbohydrate contents were determined using standard curve prepared from various concentrations of glucose.

Total protein: Quantitative estimation of total protein was performed as per (22)One mL of the suitably diluted crude tissue extract (the supernatant) was mixed with 5.0 mL of Coomassie Brilliant Blue G–250 dye (Bradford reagent) and transmittance of the resultant solution (coloured complex) was read with spectrophotometer (UV-1800 Shimadzu, Japan) at 595 nm. The amount of protein was determined using standard curve prepared using various concentrations of albumin protein.

Table 1 IAA Composition Different Concentration

Media	PGRs	Isolated PGRs
DS4 (Control)	IAA-2mg/L	-
DS1	-	20 ml/L
DS2	-	40 ml/L
DS3	-	100 ml/L

Table 2: Ingredient of MS Media

Ingredients	Quantity Required or 1000 ml
Stock A	50 ML
Stock B	5 ML
Stock C	5 ML
Stock D	5 ML
Agar Agar	0.8%
Sucrose	0.3%
BAP	2 mg
IAA	0.75 mg

Result and discussion

With internodal explants inoculated on MS + BS, callus induction was observed at the apex of explants at 1 and 2 ml as compared to 5 ml, where adventitious root induction was observed In DS1 , DS2 and DS3. In MS medium supplemented with standard IAA adventitious root induction was observed on all treatments with maximum induction a compare to treatment plant. whereas in MS + IAA can also use for shoot induction was IAA alone can

Table no.3 Effect of bacterial Auxin on In vitro plant root growth and biochemical parameters.

Media	Fresh Weight	Dry Weight	Root Length	Root Number	Total Carbohydrate	Total protein
DS1	8 gm	1.2 gm	2 cm	18	100 mg g-1	68 mg g-1
DS2	8.5 gm	1.5 gm	2 cm	20	110 mg g-1	79 mg g-1
DS3	10 gm	1.9 gm	5 Cm	25	125 mg g-1	75 mg g-1
DS4 (Control)	12 gm	2 gm	3 cm	28	130 mg g-1	85 mg g-1

sinduce callus on leaf segments, and in vitro experiments have demonstrated that coconut water in combination with synthetic auxins can be used for shoot induction and multiplication (Cui et al. 2004; Loc et al. 2005). In term of biochemical total carbohydrate was higher found in conrol plant as compared to treatment plant. In treatment plant DS3 shows significant increase of carbohydrate and root length with number of advantage root number was increase as compare to other DS1 and DS2 but in compare of control plant no one is increase root num or length , were not reported increase of in term of biomass carbohydrate an protein.(table 3)

Fig :1 Effect of Bacterial Auxin on *In vitro* grown plant root growth.

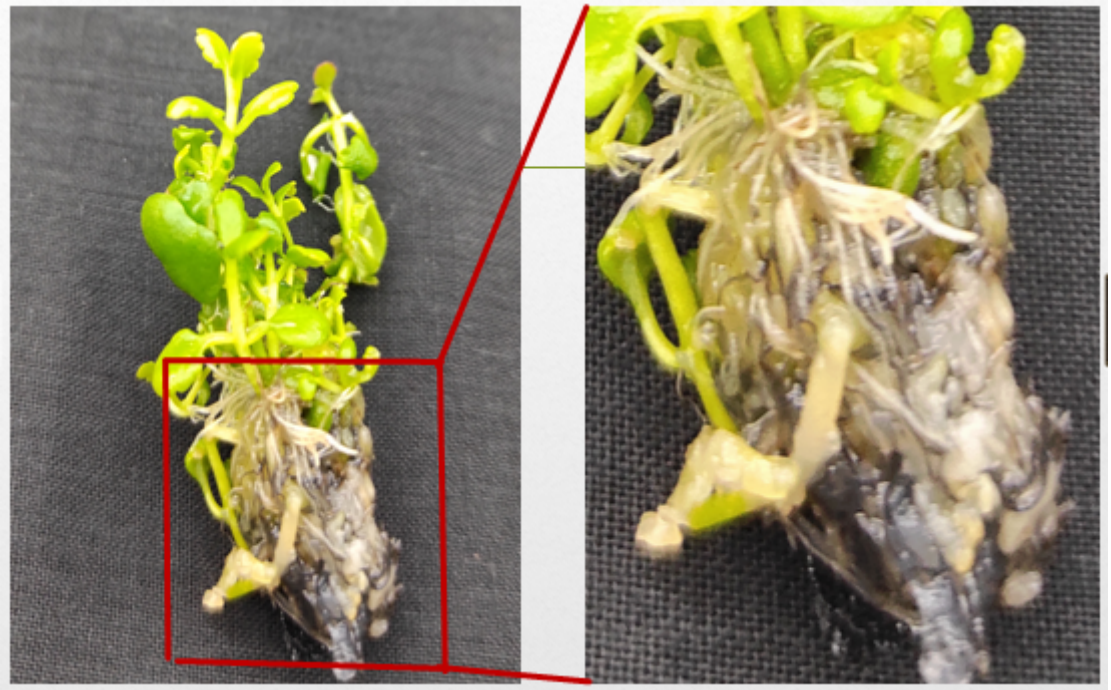

Fig:2 Adventitious root induction from *In vitro* plant growth

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