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Topic : Effect of different cheaper Nitrogen source as replacement of Ammonium Nitrate in bryophyllum tissue culture

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Abstract

The aim of this in vitro study was to investigate the effects of different concentrations of KNO_3 and NH_4Cl_2 on shoot growth, fresh weight, and dry weight of bryophyllum plant leaf in invitro condition. The plants were treated with 200 mg and 400 mg nitrate, whereas the control group had the highest fresh weight among all the treatments. The control group showed a decrease in dry weight, while the shoots showed an increase in shoot growth with higher ratings of ++ and +++ at 200 mg, 400 mg, and 200 mg N, respectively. In terms of biomass total carbohydrate, it was observed that the treatment at higher concentrations could potentially enhance the growth and biomass of the plants. The results of this study provide valuable insights into the role of different nitrogen atoms in plant regeneration, indicating that further optimization may be needed for its effective use in in vivo regeneration.

Keywords : KNO_3, Nitrogen, Ammonium nitrate, Urea, BAP

INTRODUCTION :-

Plants serve as sources of direct medicinal agents, models for novel synthetic chemicals, and taxonomic markers for the discovery of novel molecules in contemporary medicine[1]. They act as a starting point for the development of more sophisticated semi-synthetic chemical substances. Chemical synthesis of bioactive substances is challenging due to their expensive and complex nature[2][3]. Herbal preparations made from field-grown plants are prone to bacterial, fungal, and insect infestations, which might change the preparation's therapeutic potency[4]. The demand for plants used to make traditional remedies is not being met by the supply.[5]

Bryophyllum pinnatum belongs to genes *Bryophyllum* and crassulacease family. This family plant have their traditional medicine value, *bryophyllum pinnatum* is used to cure earaches, burns, ulcers, abscesses, insect bites, diarrhea, and lithiasis [6]. It has been documented as being used in Trinidad and Tobago as a traditional therapy for hypertension and kidney stones [7]. in

this family many species become endangered plant which needs to be conserved as well as explored for its significant green chemistry [8]. This herb is employed in Southeastern Nigeria to aid in the placenta drop of a newborn infant [9]. It is helpful for treating conditions including infections, rheumatism, and inflammation as well as for preventing toxic, viral, and alcoholic liver damage**.** It show the anticancer property [10].It can also lower blood pressure and blood sugar levels and has antioxidant properties, making it a health-promoting agent[11]. The creation of *in vitro* systems for the production of medicinal plants and their extracts is a practical and ideal alternative solution to the issues the pharmacological business faces [12].

Plant tissue culture is a set of techniques for maintaining or growing plant cells, tissues, or organs in sterile conditions on a known nutrient culture medium. The growth media containing different a organic and in organic salt and vitamin and plant growth regulators.in this media have a wide role of Nitrogen source. It will be required for plant growth and include in plant building block and important material for genetic make-up. The amount of nitrogen as a form of nitrate play significant role on in vitro plant growth the influence differentiation of plant cell has been reported many paper (Halperin and Wetherell 1965,was observe the amount of ammonium nitrate effect morphological change in plant in invitro condition. Many at a time use a ammonium nitrate as a nitrogen source in media. But due to some restriction and explosive nature it will ban by government in this presence report we are investigate different source of Nitrogen like "Urea, KNO_3 and NH_4Cl_2 in growth medium.

Material and method

Explant preparation and culture establishment: *Bryophyllum* leaf cultures were created in accordance with the Carelli and Echeverraray technique.[15]. Plants in good health were purchased at the botanical garden. Young leaves were chosen, trimmed, and rinsed with tap water to remove dust before going through two detergent washes and being sterilized 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 sterilized. The standard Moorashig and Skoog's (1962) medium, which contains 2.0 mg/l BAP, 0.01 mg/l NAA, 0.8% agar, and 3.0% sucrose, was used for aseptically inoculating the explant. After initial culture establishment, routine sub-culturing was carried out every three days.

Experiment and design The MS medium (Morishige and Skoog, 1962) was the base medium utilized in this investigation. It contained 0.8% agar, 3% sucrose, 100 mg/l myoinositol, and 0.4 mg/l thiamine-HCl as carbon sources. Each culture bottle contains 50 ml of MS medium, which was utilized in various combinations. (MS media with NH_4NO_3 (Control), MS1: KNO_3-100 mg/l , MS2 KNO_3 -200 mg/l, MS3: KNO_3-400 mg/l; MS4: Urea-100 mg/l , MS: Urea 200 mg/l, MS6 Urea-400mg/l; MS7 NH_4Cl_2-100 mg/l , MS8 NH_4Cl_2 200 mg/l, MS9 NH_4Cl_4 400 mg/l)[16]. Bryophyllum plant leaf were used as a plant material for in vitro regeneration to find out the effect of different Ammonium Nitrate substrate concentration.

Measurement of growth parameters: Growth characteristics were measured, including average root length, total number of roots, and total biomass in terms of fresh and dry weight. Propagules from each treatment were removed and the fresh weight of the biomass (fresh weight and dry weight) was assessed using an electronic top pan balance. Following the measurement of fresh weight, those fresh shoots were dried in an oven at 62 °C until they reached a constant weight in order to calculate dry weight.

Chlorophyll content: Using Arnon's approach, the chlorophyll content was determined [17]. Under cover of darkness, 500 mg of shoots were weighed and pulverised in a pestle and mortar with 80% acetone. The supernatant from extract centrifugation at 10,000 rpm was used to measure absorbance with a spectrophotometer. (UV-1800 Shimadzu, Japan).

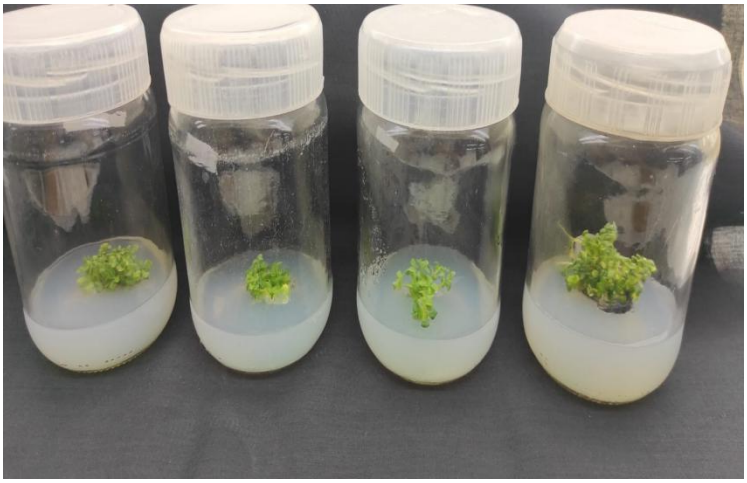
Total phenols: Using Folin Ciocalteu's reagent, the total phenol content was determined in accordance with Mahadevan's [18] method. Weighed and crushed in a mortar and pestle with 70% methanol were 500 mg of shoots. The extracts were centrifuged for 15 minutes at 10,000 rpm. The total phenol concentration was quantitatively determined using the clear supernatants. A test tube containing 500 mL of methanolic extract was used for each reaction. To this, 2.0 ml of Na2CO3 (20% w/v) solution was added after 1.0 mL of Folin-Ciocaltaeu reagent had been properly diluted (1:1 ratio of reagent and DDW). For about one minute, the test tubes were heated in a pot of boiling water while being periodically shaken. After that, tubes were cooled under flowing water

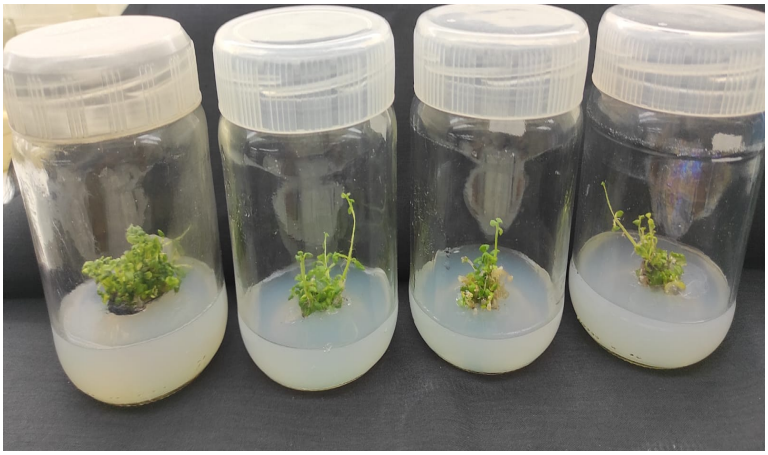
Total carbohydrateas: The approach outlined by Tandon was used to quantitatively estimate the level of total carbohydrateS [19]. In vitro propagules were homogenised in 0.1 M phosphate buffer (pH 7.0) and centrifuged at 10,000 rpm for 15 minutes. Each reaction required 15 L of

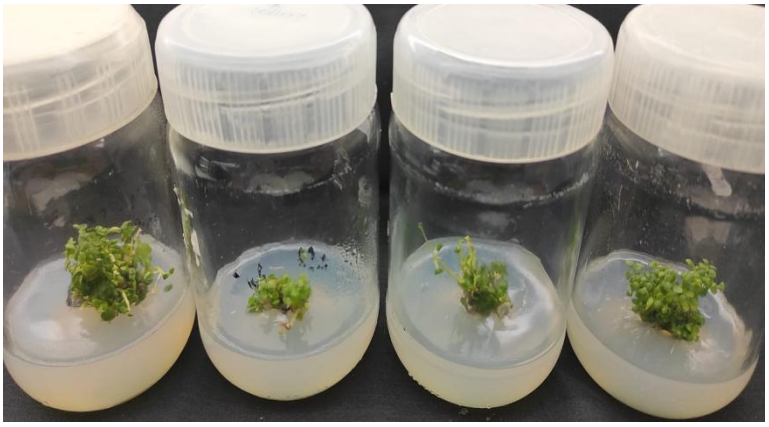
supernatant, 4.0 mL of 0.2% Anthrone reagent (in concentrated $H_2 SO_4$), and five minutes in a boiling water bath. The absorbance was measured at a wavelength of 610 nm. The total amount of carbohydrates was calculated using a standard curve derived from varied glucose concentrations.

Total protein: Using Bradford's method, a quantitative estimate of total protein was made[20]. The supernatant from the appropriately diluted crude tissue extract was combined with 5.0 mL of Coomassie Brilliant Blue G-250 dye (Bradford reagent), and the resulting solution's transmittance (or colour complex) was measured at 595 nm using a spectrophotometer (UV-1800 Shimadzu, Japan). A standard curve created using varying albumin protein concentrations was used to calculate the amount of protein.

Result:







Control	MS7	MS8	MS9

Sr. No.	Treatment	Concentration	Shoot Growth	Fresh Weight (mg)	Dry Weight (mg)	Total carbohydrate	Total Protein
MS1	KNO_3	100	+	7	1.19	89	10
MS2		200	++	10	1.7	95	12
MS3		400	+++	15	2.55	105	15
MS4	Urea	100	+++	14	2.38	102	14
MS5		200	++	12	2.04	96	12
MS6		400	+	10	1.7	88	8
MS7	NH_4Cl_2	100	+	7	1.19	92	13
MS8		200	++	10	1.7	100	15
MS9		400	+++	20	3.4	109	17
Control			++++	25	3.74	115	22

The table presents the effects of different treatments and their concentrations on shoot growth, fresh weight, and dry weight, Total carbohydrate , total protein in a study. The treatments include KNO_3, Urea, and NH_4Cl_2, at concentrations of 100 mg, 200 mg, and 400 mg, along with a control group. The results indicate varying levels of response to the treatments.[21]

In terms of shoot growth, it was observed that increasing concentrations of KNO_3 and NH_4Cl_2 resulted in improved shoot growth, with higher ratings of ++ and +++ at 200 mg and 400 mg concentrations. However, the response to Urea was different, with the highest shoot growth observed at 100 mg concentration and a decrease in growth at higher concentrations of 200 mg and 400 mg. Notably, the control group showed the highest shoot growth with an excellent rating of ++++.

Fresh weight generally followed a similar trend as shoot growth, with increasing concentrations of KNO_3 and $_{NH4C442}$resulting in higher fresh weights, whereas Urea showed a decrease in fresh weight at higher concentrations. The control group had the highest fresh weight among all the treatments.

Dry weight also showed a similar trend, with KNO_3 and NH_4Cl_2 treatments at higher concentrations resulting in higher dry weights, and Urea showing a decrease in dry weight at higher concentrations. The control group had the highest dry weight.

In term of biomass total carbohydrate with with KNO_3 and treatments at higher concentrations resulting in higher amount, and Urea showing a decrease in at higher concentrations. The control group had the

highest carbohydrate and other biomolecule protein is also showing significantly increase in when increase the concentration of KNO_3 and NH_4Cl_2 treatment plant, but in urea treated plant it was totally different increase the concentration of urea suppress the growth of plant and it was responsible to low amount of protein in plant.

Discussion

The findings of this study are consistent with the known role of nitrogen as an essential macronutrient required for plant growth and development. KNO_3 and NH_4Cl_2 are both sources of nitrogen, and the higher concentrations of these treatments may have provided an ample and readily available source of nitrogen, resulting in increased shoot growth, fresh weight, and dry weight of the plants. Nitrogen is a key component of amino acids, proteins, and other essential plant compounds, and its availability can significantly impact plant growth and biomass accumulation.

On the other hand, urea is a common source of nitrogen in many fertilizer formulations, but its effectiveness in promoting plant growth can be influenced by various factors, including its conversion into ammonium by the enzyme urease. The variable results observed with urea treatment in this study, with the highest shoot growth at a lower concentration and a decrease in growth at higher concentrations, may indicate that the conversion of urea into ammonium was not optimized under the experimental conditions of this study. This could have led to suboptimal nitrogen availability for the plants, resulting in inconsistent and less significant effects on shoot growth, fresh weight, and dry weight compared to KNO_3 and NH_4Cl_2 treatments.

The decrease in growth observed with urea treatment at higher concentrations compared to the control group suggests that excessive levels of urea may have adverse effects on plant growth and biomass accumulation. This could be due to the potential toxicity of urea at higher concentrations, as excess nitrogen can disrupt the nutrient balance and metabolic processes in plants, leading to reduced growth and productivity.

In conclusion, the results of this in vitro study highlight the significant effects of KNO_3 and NH_4Cl_2 treatments at higher concentrations on shoot growth, fresh weight, and dry weight of the plants. These findings suggest that these treatments can potentially enhance the growth and biomass accumulation of in vitro plants. On the other hand, the urea treatment showed variable and less significant effects, indicating that further optimization may be needed for its effective use in in vitro plant cultures. The results of this study provide valuable insights into the role of different nitrogen sources in promoting plant growth under in vitro conditions, Further analysis and interpretation of the data, along with statistical analysis, would be required to draw more conclusive findings. Additionally, the underlying mechanisms behind the observed responses would require further investigation and discussion.

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