Effect of different cheaper Nitrogen sources as replacement of Ammonium Nitrate in the *bryophyllum* tissue culture

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This is to certify that the work for this manuscript entitled " Effect of different cheaper Nitrogen sources as replacement of Ammonium Nitrate in the *bryophyllum* tissue culture" was successfully carried out by Miss **Shraddha Baxi** towards the partial fulfillment of requirements for the degree of Master of Science in Biotechnology of Atmiya University, Rajkot. It is an authentic record of his/her work, carried out by him/her under the guidance of **Dr.Preetam Joshi** for a period of three months during the academic year 2022–23. The content of this manuscript, in full or in parts, has not been submitted for the award of any other degree or certificate in this or any other university.

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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 the *bryophyllum* plant in leaf *in vitro* conditions. 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 the genes *bryophyllum* and the crassulacean family. This family plant has its 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 that needs to be conserved as well as explored for their significant green chemistry [8]. This herb is employed in Southeastern Nigeria to aid in the placenta drop of a newborn infant [9]. It helps treat conditions including infections, rheumatism, and inflammation as well as preventing toxic, viral, and alcoholic liver damage**.** It shows 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 contains different organic and inorganic salt and vitamin and plant growth regulators. these media have a wide role as a Nitrogen source. It will be required for plant growth and included in plant building blocks and important material for genetic makeup. The amount of nitrogen as a form of nitrate play a significant role *in vitro* plant growth the influence differentiation of plant cell has been reported in many papers (Halperin and Wetherell 1965, observed the amount of ammonium nitrate effect morphological change in plant in *vitro* condition. Many a time use ammonium nitrate as a nitrogen source in media. But due to some restrictions and its explosive nature it will ban by the government in this present report we are investigating different sources of Nitrogen like Urea, KNO_3, and NH_4Cl_2 in the growth medium.

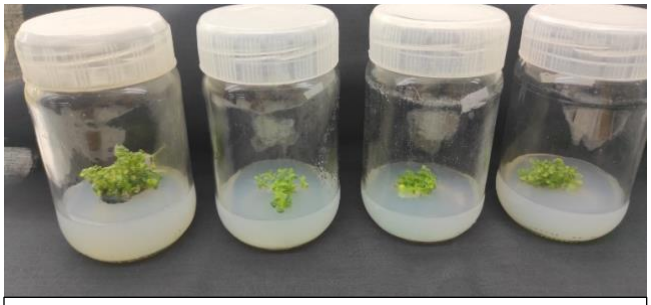
MATERIAL AND METHOD

Explant preparation and culture establishment: *Bryophyllum* leaf cultures were created by the Carelli and Eccehyerrey 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 Murashige and Skoog (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 the initial culture establishment, routine sub-culturing was carried out every three days.

Experiment and design The MS medium (Murashig 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 leaves were used as plant material for in vitro regeneration to find out the effect of different Ammonium Nitrate substrate concentrations.

Measurement of growth parameters: Growth characteristics were measured, including average root length, the 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 to calculate the dry weight.

RESULT



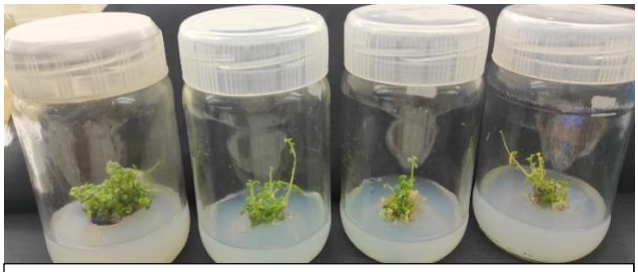
Control MS1 MS2 MS3

In image control (MS media with NH_4NO_3

MS1: KNO_3 100 mg/l

MS2: KNO_3 200 mg/l

MS3: KNO_3 400 mg/l



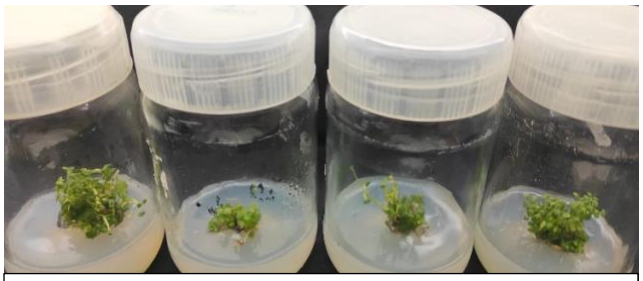
Control MS4 MS5 MS6

In image control (MS media with NH_4NO_3

MS4: Urea 100 mg/l

MS5: Urea 200 mg/l

MS6: Urea 400 mg/l



Control MS7 MS8 MS9

In image control (MS media with NH_4NO_3

MS7: NH_4Cl_2 100 mg/l

MS8: NH_4Cl_2 200 mg/l

MS9: NH_4Cl_2 400 mg/

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 terms of biomass total carbohydrates with KNO_3 and treatments at higher concentrations resulted in higher amounts, and Urea showed a decrease at higher concentrations. The control group had the highest carbohydrate and other biomolecule proteins also showing a significant increase when increasing the concentration of KNO_3 and NH_4Cl_2 treatment plant, but in urea treated plant it was a different increase in the concentration of urea suppress the growth of the plant and it was responsible to the low amount of protein in the plant.

DISCUSSION

The results of this study are in line with nitrogen's well-known function as a crucial macronutrient needed for plant development and growth. The greater concentrations of both treatments may have supplied an abundant and easily accessible source of nitrogen, resulting in a rise in shoot development, fresh weight, and dry weight of the plants. KNO_3 and NH_4Cl_2 are both sources of nitrogen. Because nitrogen is a crucial component of proteins, amino acids, and other vital plant components, its availability can have a big impact on how quickly plants grow and how much biomass they produce on the other hand, urea is a frequent source of nitrogen in many fertilizer formulations, but its ability to stimulate plant growth can be affected by several elements, including the enzyme urease's conversion of urea to ammonium. The inconsistent results with urea treatment seen in this study, with the highest shoot growth occurring at a lower concentration and a decline in growth occurring at higher concentrations, may suggest that the conversion of urea into ammonium was not optimized under the experimental circumstances of this study. This might have caused the plants' access to nitrogen to be less than ideal, which would have had uneven and less significant impacts on shoot development, fresh weight, and dry weight compared to treatments with KNO_3 and NH_4Cl_2 The lower growth rates seen with greater urea concentrations compared to the control group indicate that using too much urea may be harmful to plant growth and biomass buildup. This could be a result of urea's potential toxicity at greater concentrations,

as too much nitrogen can disturb the metabolism and balance of nutrients in plants, resulting in slower growth and lower output.

The findings of this in vitro investigation demonstrate the considerable impacts of greater doses of KNO_3 and NH_4Cl_2 treatments on plant growth, fresh weight, and dry weight. These results imply that these treatments may improve the development and accumulation of biomass *in vitro* plants. The urea treatment, on the other hand, demonstrated variable and less

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