Impact of Nitrogen Deficiency on the Growth and Yield of Bacopa monnieri: A Computational and Experimental Study

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ABSTRACT:

Bacopa monnieri (L) Wettst is a plant valued for its extensive use in traditional medicine. However, there is limited understanding of how nitrogen deficiency affects its growth and yield. Nitrogen is a critical micronutrient involved in plant growth, and its deficiency can negatively impact biomass and chlorophyll production. This study investigates the impact of nitrogen deficiency on Bacopa monnieri cultivated in Murashige and Skoog (MS) media by comparing growth under nitrogen-sufficient and nitrogen-deficient conditions. Experimental data was supplemented by a computational model developed using Python to simulate biomass growth and nitrogen uptake. The study revealed a 29.05% reduction in growth yield under nitrogen-deficient conditions, highlighting the importance of nitrogen in Bacopa monnieri cultivation.

Keywords: *Bacopa monnieri*, nitrogen deficiency, growth yield, computational modeling, nutrient management

INTRODUCTION:

Bacopa monnieri is a plant that is commonly used in Ayurvedic medicine for its neuroprotective and memory-improving qualities. Its ability to grow and produce its bioactive components, such as bacosides, depends on careful nutrition management. Nitrogen is one of the key elements that is vital to plant development since it is an essential part of proteins, amino acids, nucleic acids, and chlorophyll. A lack of nitrogen can cause plants to grow more slowly and produce less chlorophyll which lowers yields and compromises the health of the plants. Plant hormones like auxins and cytokinins are essential for controlling growth and development in addition to nitrogen. Increased leaf output and a sustained chlorophyll content are the results of cytokinins' promotion of cell division and shoot development. They also prolong photosynthetic activity by delaying the senescence of leaves[1]. Conversely, auxins control the lengthening and growth of the roots, improving the plant's capacity to take up nutrients and water. Auxins and cytokinins interact to keep shoot and root growth in check, promoting effective nutrient uptake and general plant health. Since the availability of nitrogen influences both hormones' levels and functions, the two are intimately related to each other. Sufficient nitrogen promotes the increased interaction between auxins and cytokinins, which promotes ideal growth. On the other hand, a lack of nitrogen disrupts this equilibrium, hindering plant growth and lowering productivity. It is essential to comprehend how nitrogen and these hormones interact in order to maximize Bacopa monnieri's development and therapeutic potential[2].

LITERATURE REVIEW

Role of Murashige and Skoog (MS) Media in Bacopa Cultivation

In plant tissue culture, **Murashige and Skoog (MS) medium** is one of the most widely used nutrient formulations for the in vitro propagation of various plants, including Bacopa monnieri. The MS medium provides essential macro- and micronutrients necessary for plant growth and development under controlled conditions. It offers a stable platform for studying the plant's metabolic pathways and optimizing the synthesis of its active compounds, such as bacosides[4].Bacopa monnieri's high morphogenetic potential makes it an ideal candidate for in vitro culture using MS media. Explants, like leaves or stems, can be grown in controlled environments to study organogenesis, transgene expression, and functional research on bacoside synthesis. Using MS medium also allows researchers to study the effects of various nutrient compositions, particularly the roles of nitrogen (N), phosphorus (P), and potassium (K), which are critical macronutrients for plant growth and secondary metabolite production.

Importance of Nitrogen, Phosphorus, and Potassium in MS Media

1. **Nitrogen (N):** Nitrogen is a key component of amino acids, proteins, and nucleic acids, playing a fundamental role in the plant's vegetative growth. In Bacopa monnieri, nitrogen is vital for enhancing cell division, promoting chlorophyll synthesis, and supporting the overall biomass accumulation. In MS media, nitrogen is often provided in the form of ammonium (NH_4^+) and nitrate (NO_3^-), and its availability can influence the production of secondary metabolites, including bacosides. A deficiency of nitrogen in the culture medium can lead to stunted growth, reduced chlorophyll content, and a consequent decline in the production of bioactive compounds[3].
2. **Phosphorus (P):** Phosphorus is essential for energy transfer and storage, as it is a critical component of ATP (adenosine triphosphate). It also plays a crucial role in root development and the formation of new tissues. In Bacopa monnieri, phosphorus helps in cell division and root elongation, supporting the plant's structural integrity and its capacity to absorb other nutrients efficiently. Adequate phosphorus levels in MS media can lead to enhanced root growth and improved synthesis of important compounds like

bacosides. A deficiency in phosphorus can impair energy transfer processes, reducing both plant vitality and secondary metabolite production.

3. **Potassium (K):** Potassium is a vital regulator of enzymatic activities and is involved in processes such as osmoregulation, water uptake, and photosynthesis. It helps plants tolerate environmental stress by improving water retention and activating key enzymes involved in carbohydrate metabolism. In Bacopa monnieri, potassium enhances the plant's resilience to abiotic stress and increases its productivity by promoting efficient photosynthesis and nutrient uptake. Potassium in MS media supports the overall growth of the plant, contributing to higher yields of bacosides and other secondary metabolites. A deficiency in potassium can reduce the plant's ability to withstand stress, lowering its medicinal quality.

Optimizing Bacopa monnieri Cultivation Through MS Media and Macronutrients

The combination of nitrogen, phosphorus, and potassium in MS media is crucial for ensuring the healthy growth of Bacopa monnieri in in vitro conditions. Researchers have shown that the balanced application of these macronutrients significantly enhances the yield of secondary metabolites, particularly bacosides[5], by promoting robust vegetative growth, root development, and stress tolerance.In tissue culture, the ability to manipulate nutrient levels allows for precise control over plant growth and secondary metabolite production. Studies have demonstrated that increasing nitrogen levels can promote biomass growth, while higher phosphorus levels are linked to improved root development. Potassium is particularly effective in enhancing the plant's ability to tolerate stress, ensuring higher bacoside content even under suboptimal environmental conditions[6].

In addition to the macronutrient composition of MS media, other factors such as pH, light intensity, and the use of plant growth regulators (PGRs) further influence the in vitro propagation of Bacopa monnieri. The manipulation of these factors in combination with nutrient optimization creates a favorable environment for large-scale production and consistent quality control of Bacopa monnieri plantlets.

Abiotic Factors and Secondary Metabolite Production in Bacopa monnieri

Apart from the nutrient profile in MS media, abiotic factors such as temperature, light, and water availability significantly influence the biosynthesis of secondary metabolites in Bacopa monnieri. Research has shown that optimal light intensity and mild temperature stress can lead to an increase in bacoside content, enhancing the plant's medicinal properties. For instance, controlled environmental stress during tissue culture can act as a trigger for the enhanced production of bioactive compounds like Bacoside A, a neuroprotective compound widely studied for its therapeutic effects.The manipulation of these abiotic factors, combined with the precise nutrient control provided by MS media, offers an efficient system for producing Bacopa monnieri plantlets with high levels of bacosides, thus addressing the increasing demand from the pharmaceutical industry[7].

METHODOLOGY:

Preparation of the Stock Solutions of MS media

1. A 1 L control stock solution (Stock A) was prepared, along with four separate 500 ml stock solutions, each missing one of the elements: Nitrogen, Potassium, or Phosphorus.

Table1: The concentration of the various components used while preparing stock A

Stock A-Macro Elements		
Essential Elements	**Conc in stock solution (mg/l)**	**Conc in medium (mg/l)**
NH_4NO_3	33000	1650
KNO_3	38000	1900
$CaCl_2.2H_2O$	8800	440
$MgSO_4.7H_2O$	7400	370
KH_2PO_4	3400	170

2. In addition to Stock A, three additional stock solutions were prepared: Stock B contains microelements, Stock C contains an iron source, and Stock D contains an organic supplement.

Table 2:The concentrations of the various components used in the preparation of Stock B

Stock B-Micro Elements		
Essential Elements	**Conc in stock solution (mg/l)**	**Conc in medium (mg/l)**
KI	166	0.83
H_3BO3	1240	6.2
$MnSO_4.4H_2O$	4460	22.3

$ZnSO_4.7H_2O$	1720	8.6
$NaMoO_4.2H_2O$	50	0.25
$CuSO_4.5H_2O$	5	0.025
$CoCl_2.6H_2O$	5	0.025

Table 3: The concentrations of the various components used in the preparation of Stock C

Stock C-Iron Source		
Essential Elements	**Conc in stock solution (mg/l)**	**Conc in medium (mg/l)**
$FeSO_4$	5560	27.8
$Na_2EDTA.2H_2O$	7460	37.3

Table 4: The concentrations of the various components used in the preparation of Stock D

Stock D-Organic Supplement		
Essential Elements	**Conc in stock solution (mg/l)**	**Conc in medium (mg/l)**
Myoinositol	22000	100
Nicotinic Acid	100	0.5
Pyridoxine-HCl	100	0.5
Thiamine-HCl	100	0.5
Glycine	400	2.0

Preparation of MS media

1. A control medium was prepared by using 5 ml of Stock A that contains all the macroelements.

2. A medium lacking Nitrogen was prepared using 5 ml of Stock A that does not contain nitrogen.
3. A medium lacking Phosphorus was prepared using 5 ml of Stock A that does not contain phosphorus.
4. A medium lacking Potassium was prepared using 5 ml of Stock A that does not contain potassium.
5. For each of these media, 1 ml each of Stock B, Stock C, and Stock D, along with 192 ml of distilled water was added .
6. 11.2 microlitre of 6-Benzylaminopurine (BAP cytokinin growth regulator) was added to each of these media along with 6g of sucrose and 1.6g of agar. The agar was further dissolved by heating the media in a microwave.
7. The media was then cooled to room temperature and autoclaved[8]

Preparation of sample for inoculation

1. Leaves and stems of Bacopa monnieri were taken and washed with Tween20 detergent.
2. 5g of Bavistin was added to 20 ml of distilled water and the leaves and stems were washed with distilled water.[9]
3. A final wash with 60% ethanol was done to sterilize the leaves and stems.
4. In a laminar flow chamber, under sterile conditions, the leaves and stems were transferred to the MS media in which the growth and yield can be observed.[10]

PSEUDOCODE FOR COMPARING GROWTH YIELD

1. Create Bacopa Growth Model:
   Inputs: biomass and nitrogen (`y`), time (`t`), growth parameters (`params`)
   -Compute nitrogen absorption rate
   -Modify growth rate based on nitrogen uptake
   - Determine changes in biomass and nitrogen levels
   - Output: rate of change for biomass and nitrogen

2. Initialize Starting Conditions:
   -Set initial biomass
   - Define initial nitrogen levels for control and deficient scenarios
   -Specify growth parameters

3. Run Growth Simulation:
   - Use ODE solver for both control and deficient conditions across a time range

4. Determine Yield:
 -Calculate growth yield percentage by comparing final biomass in deficient and control conditions

5. Visualize Results:
 - Generate a graph showing biomass changes over time for both scenarios

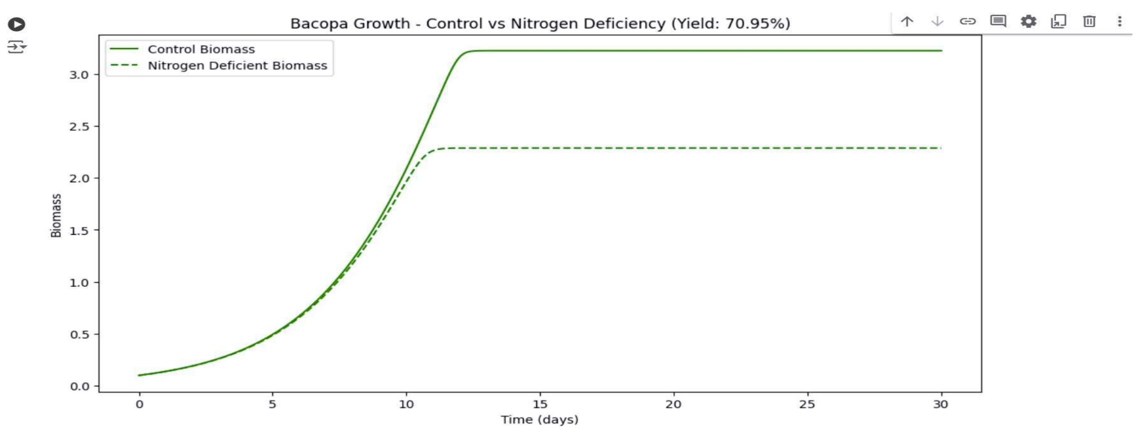
RESULTS:

The media prepared without nitrogen showed positive results with growth of the stem from the leaf explant. The growth period was over 2 weeks. The stem growth was compared with the growth of the stem in the control media, and its growth yield was calculated. Despite the observed growth, the yield was notably lower compared to the control, underscoring the critical role of nitrogen in optimizing plant development.

Stem length in control = 2.96 cm

Stem length in media without N = 2.1 cm

Growth yield = Stem length in media without N/stem length in control media = 2.1/ 2.96*100=70.95%.



INTERPRETATION OF GRAPH:

The graph depicts Bacopa growth under two conditions: adequate nitrogen (Control) and limited nitrogen (Nitrogen Deficiency). The biomass (y-axis) is plotted against time in days (x-axis). The growth patterns can be described in three distinct phases:

- Initial Growth Phase: Both conditions exhibit slow biomass increase, modeled by the exponential growth equation:

  $$B(t) = B_0 \cdot e^{rt},$$

  where B(t) is biomass at time t, B_0 is initial biomass, and r is the growth rate.

- Exponential Growth Phase: From day 5, rapid biomass increase is observed, represented by a modified logistic growth equation:

  $$B(t) = B_{ax} / (1 + ((B_{ax} - B_0)/B_0) \cdot e^{-rt})$$

  where B_{ax} is the maximum attainable biomass (Control: ≈3.0, Nitrogen Deficient: ≈2.2).

- Plateau Phase: Around day 15, growth stabilizes, modeled as

  $$B(t) = B_{ax} \text{ for } t \geq t_{a\ eau},$$

  where $t_{a\ eau}$ is the time when growth ceases.

Nitrogen deficiency significantly impacts Bacopa growth, with the nitrogen-deficient biomass yield approximately 70.95% of the control (calculated as (2.2/3.0) × 100). This 29.05% reduction in growth efficiency underscores nitrogen's crucial role in Bacopa development

CONCLUSION:

Stem length decreases in the Bacopa monnieri study under nitrogen-deficient conditions indicate a significant effect on stem growth. More specifically, in nitrogen-deficient media, the average stem length was 2.1 cm, while in nitrogen-sufficient control media, it was 2.96 cm. This translates to a growth yield of roughly 70.95% in settings where nitrogen is scarce.The reduction of 29.05% in stem length observed in the presence of nitrogen deficit underscores the crucial function of nitrogen in promoting vigorous vegetative growth. This decrease highlights the importance of nitrogen for optimizing biomass and general plant health. According to the research, Bacopa monnieri's growth capacity is severely hindered by a nitrogen shortage, which may have an impact on the plant's overall output and medicinal efficacy. This results in a reduction in stem elongation.

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