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Editorial

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Importance of Indicator Plants in Mineral Investigations: An Overview

खनिज जांच में संकेतक पौधों का महत्व: एक सिंहावलोकन

Pankaj Saini

Director, Geological Survey of India, CHQ, Kolkata

Corresponding author Email: drsainipankaj8@gmail.com

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Abstract

The application of indicator plants in mineral exploration is gaining traction as a sustainable and cost-efficient approach. By analyzing the distribution and biochemical properties of specific plant species, geologists can infer the presence of subsurface mineral deposits. Although promising, this technique yields optimal results when integrated with conventional exploration methods, including geochemical analysis, remote sensing, and geophysical surveys. With continuous advancements in biogeochemical and remote sensing technologies, geobotanical prospecting is poised to become a key component of environmentally responsible mineral exploration strategies.

Keywords: Indicator plants, mineral exploration, geobotanical prospecting, biogeochemical analysis, remote sensing.

1. Introduction

The use of indicator plants is a significant scientific method in the field of mineral exploration. Vegetation adapts to its environment and can act as a marker for the presence of specific mineral elements in the soil. This branch of science, known as Geobotany, helps in identifying and understanding the role of plants that indicate the presence of various underground minerals. Plants act as natural sensors, absorbing and accumulating elements from the soil, which makes them valuable tools in geochemical prospecting[1, 2].

Over the decades, extensive research has been conducted on plant indicators and their applications in geology. The findings of these studies suggest that "each plant is a measure of the conditions under which it grows... an index of soil and climate... and also an indicator of the ecosystem of that place." The use of plant indicators in geology is based on the relationship between specific vegetation properties and various climatic, geomorphic, and geological factors (Fig. 1). The presence of certain plant species can directly or indirectly indicate the existence of mineralization in an area. Plants that signify a specific type of mineralization, rock type, or particular substrate conditions are known as indicator plants and are classified as either universal or local indicators. Local indicator plants are specific to a particular region, whereas universal indicator plants can be used wherever they are found[3].

Role of Indicator Plants in Mineral Exploration

The distribution of certain plant species is often linked to the presence of specific elements in the soil. Such plants, known as indicator plants, are used primarily for monitoring water pollution, prospecting for ore deposits, and assessing photosynthetic efficiency and nutrient uptake levels. Some plants also indicate soil characteristics such as salinity, acidity, and nutrient content. In areas where traditional mineral exploration techniques are expensive and time-consuming, the use of indicator plants provides an efficient and cost-effective alternative (Fig. 1).

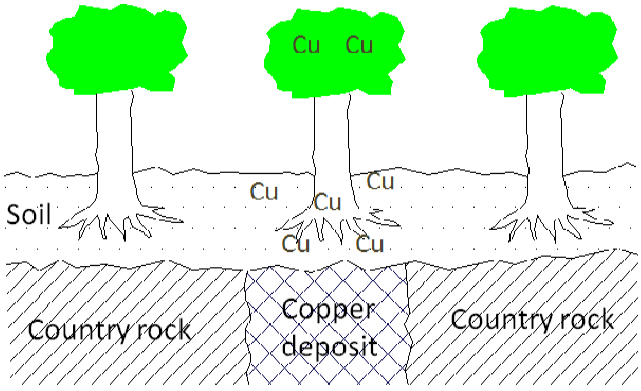

Figure 1. The use of plant indicators in geology is based on the relationship between certain measured properties of vegetation and various climatic, geomorphic, and geological factors.

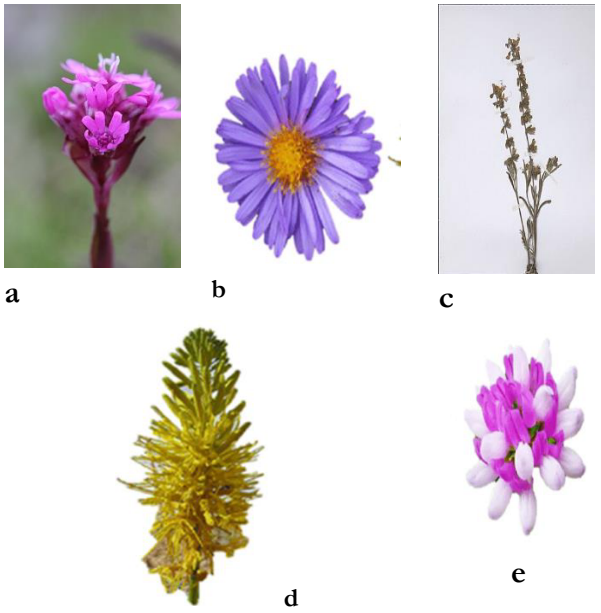

Figure 2. Indicator plants: **a**. *Silene suecica* (Lodd.) Greuter & Burdet, **b**. *Olearia ciliata* (Benth.) Benth., **c**. *Ocimum centraliafricanum* R.E.Fr or "copper plant", **d**. *Stanleya pinnata* (Pursh) Britton, **e**. *Astragalus lentiginosus* Douglas ex Hook.[4]

Geobotanical Surveys

The use of indicator plants in geological exploration has been practiced for decades. This technique has proven useful in identifying areas where minerals are not visible on the surface but can be inferred indirectly through vegetation patterns. Geobotanical surveys involve mapping the distribution of indicator plant species or identifying plant disease symptoms associated with high-metal-bearing soils (Fig. 2). Since different plant species exhibit varied responses to soil mineral composition, their presence and health condition can provide crucial insights into subsurface geological formations.

Table 1. Major Metal Indicator Plants by Element[5]

Gold (Au)	*Eucalyptus* spp. – Australia; gold particles found in leaves and bark *Acacia* spp. – Accumulates gold from deep regolith *Helichrysum* spp. – Africa; associated with auriferous soils *Pandanus candelabrum* – West Africa; often found in gold- and kimberlite-rich soils
Copper (Cu)	*Haumaniastrum katangense* – DRC; classic Cu hyperaccumulator *Silene cucubalus* – Europe; tolerates copper-rich soils *Elsholtzia splendens* – China; Cu accumulator, used in phytoremediation
Cobalt (Co)	*Haumaniastrum robertii* – DRC; cobalt hyperaccumulator *Crotalaria cobalticola* – Central Africa; thrives on Co-rich soils *Alyssum* spp. – Some species accumulate Co along with Ni
Nickel (Ni)	*Alyssum murale* – Mediterranean region; Ni hyperaccumulator *Berkheya coddii* – South Africa; grows on Ni-rich serpentine *Psychotria douarrei* – New Caledonia; accumulates >2% Ni in dry matter
Zinc (Zn)	*Thlaspi caerulescens* – Europe; Zn and Cd hyperaccumulator *Viola calaminaria* – Central Europe; found in Zn/Pb-rich soils *Arabidopsis halleri* – Europe; Zn and Cd accumulator, model organism
Lead (Pb)	*Minuartia verna* – UK/Europe; common on lead mine tailings *Armeria maritima* – Coastal Europe; tolerant of Pb and Zn *Festuca ovina* – Often found on Pb-contaminated soils
Manganese (Mn)	*Gossia bidwillii* – Australia; high Mn accumulation *Virotia neurophylla* – New Caledonia; Mn hyperaccumulator
Chromium (Cr)	*Pteridium aquilinum* – Bracken fern; accumulates Cr in polluted areas *Dicoma niccolifera* – Serpentine soils (with both Cr and Ni)
Arsenic (As)	*Pteris vittata* – Chinese brake fern; arsenic hyperaccumulator *Pityrogramma calomelanos* – Also accumulates arsenic; used in phytoremediation

Key Indicator Plants and Their Mineral Associations

Aluminum: *Hydrangea macrophylla* (Thunb.) Ser. (blue flowers indicate the presence of aluminum in soil, while pink flowers indicate its absence)

Copper: *Haumaniastrum katangense* (S. Moore) P. A. Duvign. & Plancke - Zaïre, *Ocimum centraliafricanum* R.E.Fr (Fig 2c)

Gold: Found in trace amounts in various plants, with specific plants accumulating more than others, for instance, *Eucalyptus* spp.

Nickel and Cobalt: Occur in varying amounts in plants, soil, and living organisms; *Alyssum* spp. are known hyperaccumulators of Nickel and Cobalt.

Potassium: An essential nutrient for agriculture, influencing soil fertility, e.g. *Amaranthus* spp

Phosphorus: A key component of fertilizers, essential for plant growth, e.g. *Lupinus* spp.

Coal Indicators: *Olea europaea* subsp. *cuspidata* (Wall. & G.Don) Cif., *Gymnosporia royleana* Wall. ex M.A.Lawson, among others, often grow in coal-rich areas.

Cation exchange capacity (CEC) is a measure of the soil's ability to hold positively charged ions. It is a very important soil property influencing soil structure stability, nutrient availability, soil pH and the soil's reaction to fertilizers and other ameliorants (Fig. 3.)

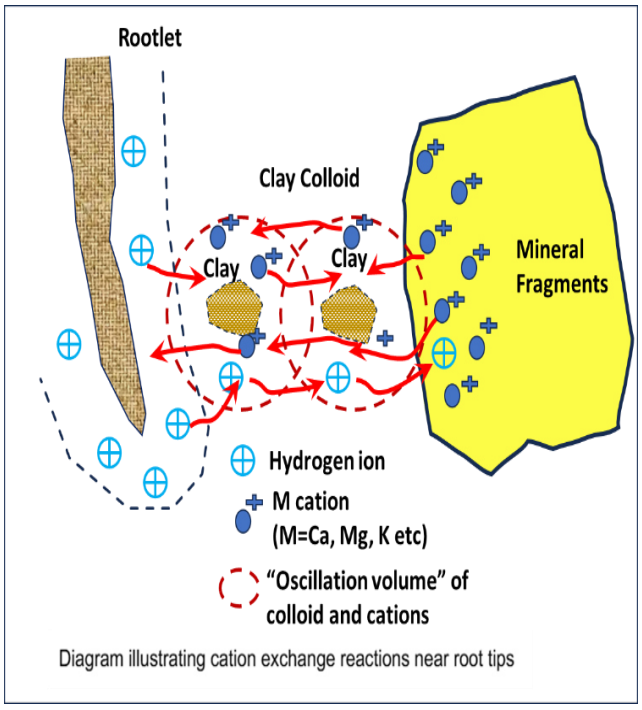

Figure 3. Diagram illustrating cation exchange reaction near root tips

Mechanism of Mineral Absorption in Plants

Plants absorb minerals from the soil through their root systems. The mineral uptake process depends on factors such as soil pH, organic matter content, and the presence of competing elements. Some plants have developed unique mechanisms to tolerate and accumulate high concentrations of metals, making them valuable indicators of underground mineral deposits.

Biogeochemical analysis of plant tissues can reveal the concentration of metals absorbed from the soil. This method, known as biogeochemical prospecting, involves collecting plant samples and analyzing their elemental composition to detect potential mineralized zones. Such studies have shown that plant species like *Thlaspi caerulescens* accumulate zinc and cadmium, while *Pteris vittata* is known for its ability to hyperaccumulate arsenic

Utility of Indicator Plants

Indicator plants not only assist in mineral prospecting but also help in understanding environmental changes. They provide insights into land-use patterns, groundwater depth, pollution levels, climate conditions, and ecosystem health. Additionally, some plants act as bio-monitors for atmospheric pollution, absorbing pollutants such as sulfur dioxide and heavy metals from the air.

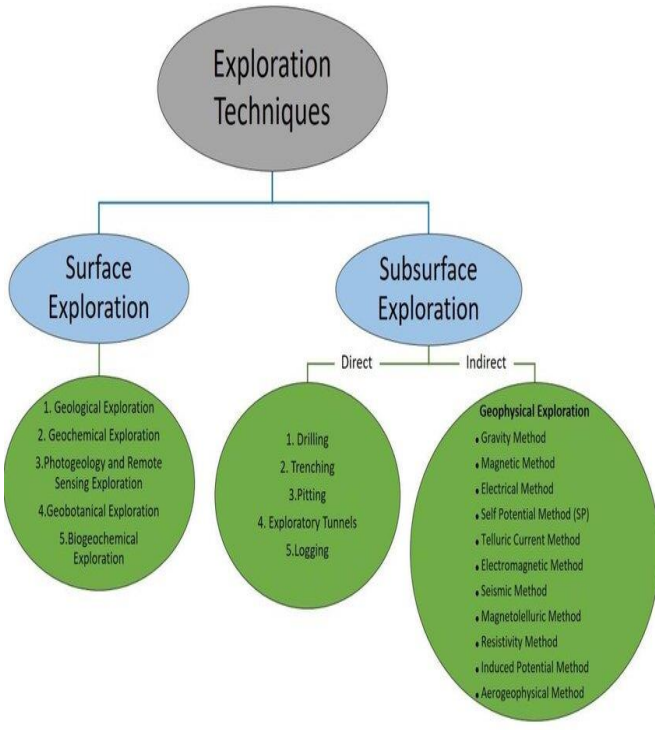

Figure 4. Different Exploration techniques[6]

Challenges and Limitations

Despite the advantages of using indicator plants in mineral exploration, there are some challenges associated with this method:

Species-Specific Responses: Not all plant species respond uniformly to mineral-rich soils, making interpretation complex.

Environmental Factors: Climatic conditions, soil moisture, and microbial activity can influence plant mineral uptake.

Regional Variability: Indicator plants that work in one geographical area may not be reliable in another due to differences in ecosystem composition.

Conclusion

The use of indicator plants in mineral exploration is emerging as an environmentally friendly and cost-effective method. By carefully studying plant species distribution and their biochemical composition, geologists can gain valuable insights into subsurface mineral deposits. However, this technique is most effective when combined with other geological exploration methods such as geochemical analysis, remote sensing, and geophysical surveys. In the coming years, advancements in biogeochemical analysis and remote sensing technologies will further enhance the precision of geobotanical prospecting, making it a vital tool in sustainable mineral exploration.

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Conflict of Interest

Not Applicable

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Research Article

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The bones of Livingston Island – history of plant succession in Antarctica

Ossos da Ilha Livingston – história da sucessão em plantas na Antártica

Fernando Augusto Bertazzo-Silva[1], Jair Putzke[1*], Lilian Pedroso Maggio[1], Angela Silva Miasaki[2], José João Lelis Leal de Souza[2], Marisa Terezinha Lopes Putzke[3], Carlos Ernesto Gonçalves Reynaud Schaefer[2]

[1]Universidade Federal do Pampa – São Gabriel – RS, Brasil
[2]Universidade Federal de Viçosa, Brazil
[3]Universidade de Santa Cruz do Sul
*Corresponding author Email: jrputzkebr@yahoo.com

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Abstract

The bones found in the beaches of Byers Peninsula, Livingston Island - Antarctica are testimonies of almost two hundred years ago when the area was full of seals and whales hunters. The remaining´s of that period are composed of complete skeletons and dispersed bones, most of them around the area called Southern Beach, which was surveyed in 2020 and 2023. The plant coverage of 33 whale bones was evaluated using a square of 20 x 20 cm, and species found were collected for identification. The soil surrounding five complete seal skeletons was studied, and its plant community evaluated. The whale bones were found colonized by 16 plant species, being *Pertusaria* sp. (lichen) the most frequent and *Deschampsia antarctica* (the Antarctic grass), *Brachythecium austrosalebrosum* and *Ditrichum* sp. (both mosses) are reported for the first time on this substrate. There were found 5 mosses, 12 lichens, and one flowering plant associated directly to seal bones and other associated with the soil in the surroundings of the skeletons. Plant succession on bones in Antarctica is also occurring and any movement of them caused by anthropic or other interferences can change the community entirely.

Keywords: Plant succession; Ecology; Pinnipedia; Skeletons.

1. Introduction

With the discovery of the South Shetland Islands in the Maritime Antarctic around 1819, trips to hunt marine mammals began. About 1000 people were involved in hunting in the South Shetland archipelago beaches and as many also embarked. Precarious camps were set up on land, the main point being in the Byers Peninsula on Livingston Island, but along other islands and even on the Antarctic Peninsula other groups of hunters were also established[1].

The hunting period ranged from ca. 1820 to 1960, and abandoned carcasses and bones that have not been carried into the sea by erosion or winds currently remain at the seashore[2]. Vegetal communities develop in bones and in their surroundings, exploring what they can offer in terms of nutrients for the environment[3]. Calcium, phosphorus, carbon, nitrogen, and sulfur are nutrients found in bones and they are essential for land plant communities[4].

Plant composition on old mammalian bones were scarcely studied in Antarctica. Olech[5] reported 23 lichens and two mosses on whale bones. Albuquerque et al.[6] cited 14 lichens and two mosses. Øvstedal and Smith[7] make reference to only two species on whale bones and three on seal bones on the revision of all lichens reported to Antarctica. Ochyra et al.[8] cited only 3 species of mosses on bones in their revision of Antarctic bryophyta. Duckett[9] refers about 7 moss species associated to bones from South Georgia and South Shetland Islands. Putzke et al.[10] described modifications around a whale skeleton assembled in King George Island by Jacques Cousteau team in 1972, indicating a *Synchitria* species associated with the nutrients offered by the skeleton. Putzke et al.[3] studied the whale bones vegetal association in Keller Peninsula – King George Island, reporting 4 mosses and 19 lichens associated.

The bones can be essential substrates for vegetation and/or be mere springboards for plants to conquer other areas, and the purpose of this work is to try to give some highlights to this question studying the plant communities associated with them in Byers Peninsula, Livingston Island - Antarctica.

2. Materials and Methods

2.1. Study area:

The whale and fur seal bones were studied in the South Beaches of Byers Peninsula on Livingston Island, one of the main islands of the South Shetland Archipelago – Antarctica during the 2019/2020 and 2021/2022 austral summers (Figure 1).

There were located in this work 33 whale bones that presented some vegetal covering and then chosen to do this study (Figure 2). In the flattened part of each whale vertebra having plant communities, a wooden square of 20 x 20 cm was laid on to calculate coverage and frequency of each species using the Braun-Blanquet11 (1932) method. In the laboratory, the data observed in the field and the photographs taken were used to hand-color the figures to study its phytosociology.

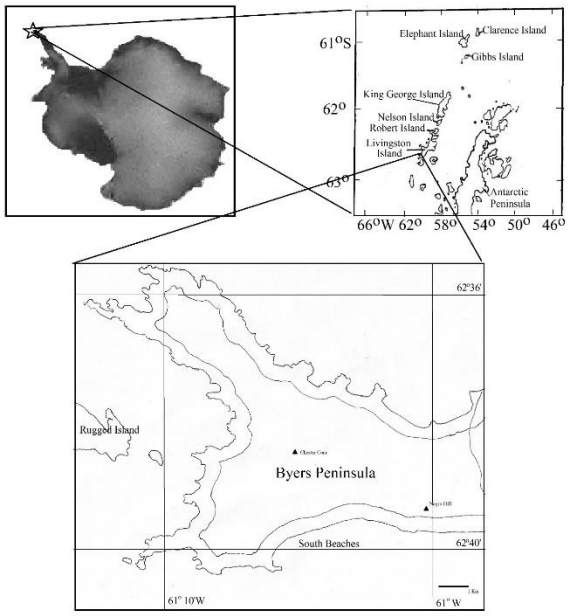

Figure 1. Schematic map of Livingston Island location and the Byers Peninsula studied, indicating the South Beaches.

Figure 2. A whale bone chosen and under analysis in the field work in Byers Peninsula.

2.2. Study of Pinnipedia bones:

The vegetation surrounding five seal skeletons selected (two with skin remains, and three only bones alone) was also analyzed (Figure 3). A map of the surrounding vegetation was assembled, and the species identified.

The mosses and lichenized fungi were identified in situ, or small samples were collected to do laboratory studies. The species identification was done basically following Putzke and Pereira[12] and Ochyra et al.[8] for mosses, and Redon[13] and Øvstedal and Smith[7] for lichens.

Figure 3. The seal skeleton area 1 studied and with soil samples analyzed

A complete fur seal skeleton, probably one of the oldest found in the South Beach (since the leather was almost completely decomposed) was also studied for soil chemical composition, collecting samples and studying the vegetation associated (Figure 3).

An undisturbed soil sample was collected below the fur seal skeleton between 0 and 20 cm depth. The field-oriented and preserved block collected in field was oven dried at 40°C for one week and vacuum impregnated (-5 kPa) with polyester resin diluted in 30% (volume) styrene monomer. The micromorphological study was done in a Transmission Electron Microscope. The description of the thin sections followed the propositions of Stoops[14]. A micro x-ray fluorescence spectrometer Shimadzu determined the contents of Ca, Fe, K, P, and Si in the thin section. The chemical elements were quantified by the Fundamental Parameter method (Quantitative - FP). Calibration consisted of adjusting the sensitivity coefficients of each element analyzed. The sensitivity coefficients of the Quantitative were achieved by FP method, based on four reference samples: Montana Soil II - NIST 2711a, BHVO - 2 - Basalt - USGS, COQ - 1 - Carbonatite - USGS, and SDC - 1 - Mica Schist – USGS.

One soil profile was dug, taken, and described in the site to represent the soils without the influence of bones. Diagnostic horizons, attributes, and properties were identified according to descriptions of color, texture, consistence, and thickness. The soil profile was classified according to the World Reference Base for soil resources (IUSS Working Group WRB, 2015)[15]. Soil samples were collected in each horizon, from the surface down to the lithic contact, at each pedon.

Samples were air-dried and sieved through a 2 mm sieve before texture and chemical analyzes[16]. Coarse sand (CS), fine sand (FS), silt, and clay were determined by the pipette method after dispersion with 0.1 M NaOH. Soil pH was measured with a glass electrode in a 1:2.5 suspension v/v soil and deionized water. The potential acidity (H+Al) was extracted by 1 M ammonium acetate solution at pH 7. The content of exchangeable Ca^{2+}, Mg^{2+}, and Al^{3+} was determined in a 1 M KCl extract. Exchangeable K^{+} and Na^{+} were determined after Melhich-1 extraction. From these results, the sum of bases (SB), base saturation (V), equivalent cation exchange capacity (ECEC), and total cation exchange capacity (CEC) was calculated.

The available phosphorus content (PM) was determined by a Mehlich-1 extraction solution. The total organic carbon (C) was determined by wet combustion[17]. The P adsorption capacity of the soil was determined after stirring it for 1 hour with 2.5 g of soil in 0.01 M CaCl2 containing 60 mg of P L-1. The suspension was filtered, and the remaining P in the solution (PREM) was determined by photocolorimetry[18]. Therefore, the lower the value of PREM, the higher the affinity of soils for the P in the solution.

RESULTS AND DISCUSSION

Soil background

The soil profile was dug in the upper marine terrace at 22 m.a.s.l. The soil is derived from marine sediments. Pedon was classified as Pantohypereutric Protic Akrofluvic Arenosol (Ochric, Pantonechic, Endoraptic). Lithic contact is at 200 cm depth. Epipedon is classified as ochric. The single grain is the structure of all horizons. The horizons are abruptly differentiated by texture and color (Table 1).

Table 1. Micromorphological description of the surface horizon of soil influenced by bones

Microstructure		**Single grain**
Porosity		Simple packing voids Vesicular voids
Groundmass	c/f Related distribution 2μm	Chitonic
	Coarse fraction (size, sphericity, roundness, mineralogy)	Fine sand smooth subangular quartz grains Silt smooth subangular biotite grains
	Fine fraction (size, limpidity, birefringence, color)	Clay dirty undifferentiated b-fabric 7.5YR 5/8 Clay dirty crystallitic b-fabric 7.5YR 8/3
	Organic residues	Absent
Pedofeatures		Typic Ca-rich coating associated with the coarse fraction Link capping clay coating associated with the coarse fraction

The texture is dominantly sand, and fine sand (FS) dominates fine particles. The horizons are neutral and have base saturation (V) above 80% in all horizons. $Ca^{2+}>Mg^{2+}>Na^{+}>K^{+}$ is the base dominance in the exchange complex. The contents of bases, soil organic carbon (SOC), total nitrogen (N), and extractable P by Mehlich-1 (PM) increase irregularly with depth. This pattern suggests that parent material is the main source of these elements. High values of remaining P (PREM) indicate a low affinity between minerals and P.

Soil influenced by bones

The single grain is the microstructure of thin section (Figure 4). Quartz, biotite, and plagioclase are present as silt and fine sand. Simple packing voids are between the coarse grains. Vesicular voids indicate the exclusion of gases during freezing of active layer. Coarse grains are generally coated by: a) neoformed brown clay minerals of undifferentiated birefringence; b) pink clay of crystallitic birefringence.

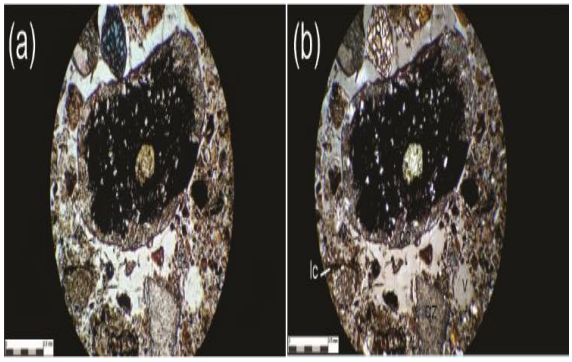

Figure 4. Thin section of soil under bones in plane-polarized light (a) and cross-polarized light (b); bi = biotite grain; cc = $CaCO_3$ coating; lc = link capping clay coating; qz = quartz grain; v = vesicular voids.

The XRF analysis indicates that the pink clay crystallitic birefringent coating is Ca-richer than the surrounding (Figure 5). The low spatial affinity between the Ca, P, and Si suggests that $CaCO_3$ composes the coating. Weathering of bones is an additional source of Ca and PO_4 ions, but they have different chemical behaviors. Water percolation promotes a limited translocation of dissolved Ca^{2+} ions because clay minerals strongly adsorb bivalent cations. The roots and microbiological respiration yield CO_2 in the atmosphere of soil. During freezing of the activity layer in winter, slowly percolating water is trapped by clasts. The residual solution becomes supersaturated and $CaCO_3$ precipitates as laminar caps in the bottom of coarse grains[19, 20]. Cryoturbation moves the grains, and, eventually, there is an alteration of $CaCO_3$ coating[21]. On the other hand, the high PREM values indicate a low affinity between P and clay minerals. Consequently, P percolates from the surface to deeper horizons. The lower P input in soils influenced by bones compared to ornithogenic soils did not guarantee apatite formation[22].

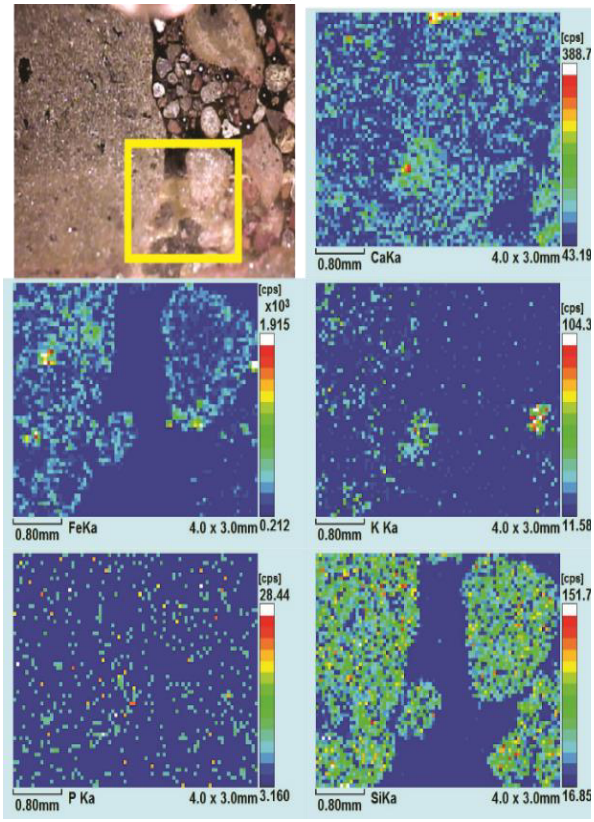

Figure 5. Spatial distribution of elements determined by XRF in thin sections

Vegetation composition

In the 33 whale bones studied (Figure 6) there were found 10 lichenized fungi, 5 moss species and the Angiosperm, *Deschampsia antarctica* Desv. (Table 2 and 3). The Antarctic grass was found for the first time in this substrate but with some sand sediments already deposited on them (Figure 8). This was also the case of *Brachythecium austrosalebrosum* and *Ditrichum* sp., both moss species found for the first time on bones. Among the mosses, *Pohlia nutans* (Hedw.) Kinb., *Brachythecium subpilosum* (Hook.f. and Wilson) A. Jaeger and *Syntrichia magellanica* (Mont.) R. I-I. Zander are cited to whale bones[8]. *Ceratodon* sp., *Bryum* sp., *Pohlia nutans*, various *Syntrichia* spp., *Brachythecium subpilosum*, *Drepanocladus* sp. and *Sanionia georgico-uncinata* were found on bones of the British Antarctic Survey and Natural History Museum collections, sampled on South Georgia and South Shetland Islands[9].

Pertusaria sp. was the species most frequently found (8 squares – 24.2%), what is not according other works published (Table 4) [23, 24]. This species had also the higher Ecological Value index (121.2).

Table 2 - Species list of plants found in the 33 whale bones studied

Group/Family	Species
Lichen/Caliciaceae	*Buellia* 1
Lichen /Caliciaceae	*Buellia*2
Lichen /Teloschistaceae	*Caloplaca sublobulata* (Nyl.) Zahlbr
Lichen	*Muscicolous lichen*
Lichen / Pertusariaceae	*Pertusaria* sp.
Lichen	*Gray sterile lichen*
Lichen	*Placoid sterile lichen*
Lichen / Lecanoraceae	*Rhizoplaca aspidophora* Vain.
Lichen	*White sterile lichen*
Lichen /Verrucariaceae	*Verrucaria* sp.
Moss/ Ditrichaceae	*Ditrichum* sp.
Moss/Pottiaceae	*Syntrichia filaris* (Müll. Hal.) R.H. Zander
Moss/ Pottiaceae	*Hennediella heimii* (Hedw.) Zand
Moss/Brachytheciaceae	*Brachythecium austrosalebrosum* (Müll. Hal.) Paris
Moss/ Amblystegiaceae	*Sanionia uncinata* (Hedw.) Loeske
Angiosperm/Poaceae	*Deschampsia antarctica* Desv.

Verrucaria sp. had the highest coverage, what can be justified by the disposition of bones too close to the sea shore, since *Caloplaca sublobulata* was also found in the community (three on the same square) and both are associated to high salt availability[13].

In one square (bone 32) a giant thallus of *Lecidea* sp. was found, with 9.5 cm diam., in a very old and fragmentary bone. This is an indication that if bones are stabilized, lichens can grow at considerable diameters and that bones are a suitable substrate for very old growing lichens and need to be protected.

Sanionia uncinata was found on three bones only (9.1%), sometimes greatly covered by muscicolous lichens, differently from what was found in other islands[23]. *Sanionia* species had the highest coverage of all species in cryptogamic communities of Antarctica[25, 26], but this is not the case of Whale bones in Byers Peninsula.

Table 3. Species coverage (%) on each sampled area of the whale bones in Byers Peninsula.

	B1	B2	CS	ML	PR	GL	PL	RA	WL	VR	DS	SF	HH	BA	DA	SU
01			2.2							0.9						
02			0.1		2.9	24.5										
03										25.7						
04			0.5							19.3						
05										11.6						
06				12.8							18.9					
07	3.3				8.1	1.1										
08	2.2				4.3	2.5										
09					6.6											
10					9											
11					10.5											
12					13.3											
13			3.7		0.9											
14												6.7				
15			5.7									6.6				
16															16.2	
17												1.6				
18													0.6			
19														31.9		
20		0.5				7.2										
21							3.7	3.1					0.4			
22													0.5			
23								8.2								
24		3.3														
25		4.2														
26		0.5				26.6			12.7	8.3						
27								9.1								
28								9.6								
29				48,9									7.6			
30				39.3									4.4			4
31				9.8							1.4					3.8
32								0.4	16.6				0.1			
33				37.5									1.1			5
Total	5.5	8.5		148.3	55.6	39.9	3.7	30.4	20.3	65.8	20.3	14.9	14.7	31.9	16.2	12.8

B1 - *Buellia* sp. 1 (greenish); B2- *Buellia* sp. 2 (yellowish); CS - *Caloplaca sublobulata*; ML - Muscicolous lichen; PR - *Pertusaria* sp.; GL - Gray lichen; PL - Placoid lichen; RA - *Rhizoplaca aspidophora*; WL - White lichen; VR - *Verrucaria* sp.; DS – *Ditrichujm* sp.; SF - *Synchitria filaris*; HH - *Hennediella heimii*; BA - *Brachythecium austrosalebrosum*; DA - *Deschampsia antarctica*; SU – *Sanionia uncinata*.

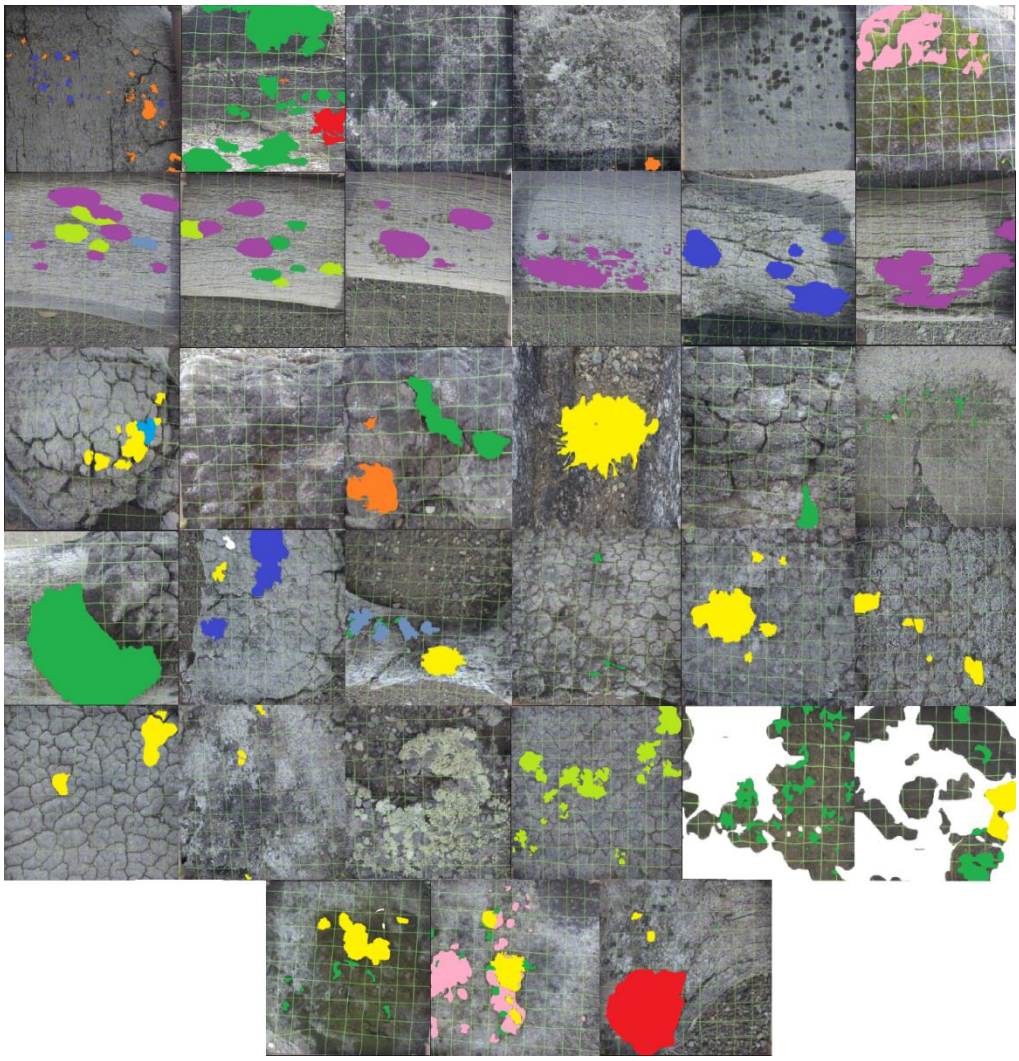

Figure 6. Images of the 33 whale bones quadrats studied and hand colored plant coverage for phytosociological evaluation

Table 4. Species with higher frequencies found on whale bones in Southern Beach, Byers Peninsula, Livingston Island – Antarctica.

Species	**Nº of squares**	**F (%)**	**IES**
Muscicolous lichens	5	15.15%	90.9
Caloplaca sublobulata (Nyl.) Zahlbr	5	15.15%	45.45
Gray sterile lichen	5	15.15%	60.6
Rhizoplaca aspidophora Vain.	5	15.15%	60.6
Verrucaria sp.	5	15.15%	75.75
Pertusaria sp	8	24.24%	121.2
Hennediella heimii (Hedw.) Zand	7	21.21%	63.63
Deschampsia antarctica Desv.	1	3.03%	9.09

Nº = number of squares in which the species was observed; F = (%) frequency of the species in 33 squares studied; IES = Ecological value Index.

This is probably because the bones are very old and muscicolous lichens are already colonizing the moss formations on this substrate (15 % of frequency and coverage of 148.3, the highest among all species found). This is an observation that allows us to conclude that plant succession on bones in Antarctica is also occurring and that any movement of the bones caused by Anthropic interference can change completely the community as already demonstrated[25].

From the five seal skeletons studied (Figure 5), three of them were represented only by pure bones and two also presented skin remains. In one skeleton without skin only *Deschampsia antarctica* was present forming tufts up to 10 cm and in the another two only *Polytrichum piliferum* was present. When skin is still among the remains, the vegetation is dense, with Sanionia uncinata forming small carpets and *Polytrichum piliferum* and/or *Ditrichum* sp. forming tufts. In one of those skeletons the alga *Prasiola crispa* was also present. The muscicolous *Ochrolechia frigida* was constant in one skeleton with skin remains, indicating the old condition of this piece. Probably the skin remains contribute highly to plant establishment while pure skeletons usually have only poor vegetation directly associated.

The skeletons without skin are probably remains of the hunting period when this part of the seals was collected to be sold in the around the world markets. So, based on our results, probably the huge amount of skeletons without skins in South Beach of Byers Peninsula contributed scarcely to plant establishment.

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Conflicts of interest

The authors declare no conflicts of interest.

Authors Contribution

All authors contributed equally to the conception, design, data collection, analysis, and writing of the manuscript. Fernando Augusto Bertazzo-Silva, Jair Putzke, Lilian Pedroso Maggio, Angela Silva Miasaki, José João Lelis Leal de Souza, Marisa Terezinha Lopes Putzke, and Carlos Ernesto Gonçalves Reynaud Schaefer have read and approved the final version of the manuscript.

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Research Article

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Plagiomnium succulentum (Mitt.) T. J. Kop. (Family Mniaceae): New Moss for Nilgiri hills, Tamil Nadu, South India

Sonu Yadav

Shaheed Smarak Government College, Yusufpur, Ghazipur-233227

Uttar Pradesh, India

Corresponding author Email: sonubryo@gmail.com

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Abstract

Plagiomnium succulentum (Mitt.) T. J. Kop. is a corticolous taxa, growing on the bark of higher plants. The species is characterized by glossy, yellowish green, pleurocarpous, epiphytic, creeping, leaves distantly arranged on stem, oblong-ovate, margin throughout minutely dentate with distinct border; costa single, beglieter cells present; leaf-cells rounded-hexagonal to quadrate 2-3 row of narrow elongated, thick walled cells forming a distinct border throughout the leaf. During the present study taxon has been critically investigated and identified from Nilgiri Hills. It is reported for the first time from Nilgiri Hills.

Keywords: Corticolous, Moss, Mniaceae, Nilgiri hills, South India

1. Introduction

The Nilgiri Hills, located in the southern state of Tamil Nadu, represent a prominent hilly district and stand as one of India's most significant treasure troves of flora and fauna, second only to the Eastern Himalayas in terms of biodiversity richness[1-3]. Also known as the "Nilgiris"—which translates to "Blue Mountains"—the region derives its name from the striking bluish-purple bloom of *Strobilanthus kunthianus* (Acanthaceae), which blankets the hillsides in a vibrant hue during its flowering season. Nestled within these hills is the popular hill station of Ootacamund, more commonly known as Ooty and often referred to as the "Queen of Hill Stations." The Nilgiri Hills also form the heart of India's first and oldest biosphere reserve—the Nilgiri Biosphere Reserve—which plays a critical role in the conservation and diversification of the region's unique ecological heritage..

Plagiomnium is represented by four species in India[4, 5] out of which three species have been earlier reported from south India[4]. In Nilgiri hills only *Plagiomnium rhynchophorum* (Harv.) T. J. Kop. has been earlier reported[4]. However, in the course of the present study, *Plagiomnium succulentum* has been collected and documented for the first time from this region, marking a significant addition to the known bryoflora of the area. This new record highlights the unexplored richness of bryophyte diversity in the Nilgiri Hills and underscores the need for continued bryological exploration in the region. Such findings contribute valuable insights into the distribution patterns and ecological preferences of moss species in South India.

2. Materials and Methods

The plant specimens were collected carefully with the help of sharp edged knife from different localities of Nilgiri hills, Tamil Nadu, South India. The collected materials were kept in brown paper packets in case of dried specimens and blotting papers used in case of highly wet specimens. The collected materials were air dried at room temperature. After drying the specimens were kept in brown paper packets (Size '6 x 4' Inch) which were labeled with complete details like collection numbers, localities, altitude, habitat, plant's name, date of collection and name of collectors. After this process, all specimen packets were deposited in herbarium boxes (Size '15 x 6' Inch). The collected plants have been successfully preserved in the Lucknow University Bryophyte herbarium (LWU). The observations were under stereoscopic binocular and Leica microscope.

1.1. Taxonomic description

Plagiomnium succulentum (Mitt.) T. J. Kop.

Plagiomnium succulentum (Mitt.)T. J. Kop., *Ann. Bot. Fennici* 5: 145. 1968.

Synonym: *Mnium succulentum* Mitt. *J. Linn. Soc. Bot. Suppl.* 1: 143. 1859.5

(Plate 1, Figs. 1-10)

Plants glossy, yellowish green, pleurocarpous, epiphytic, lax, creeping, 3-4.5 cm long and 2-3 mm wide with leaves; cross-section of stem circular, 0.43-0.50 mm in diameter, two rows of outer cortical cells slightly thick walled, small, brown in colour, inner cortical cells thin walled, large, 22-45 x 19-26 µm, central strand well developed with small, hyaline and thin walled cells; leaves distantly arranged on stem, oblong-ovate, 4.1-6.1 x 1.5-2.5 mm; margin throughout minutely dentate with distinct border; costa single, percurrent, beglieter cells present; leaf-cells rounded-hexagonal to quadrate, apical cells 11-19 x 8-15 µm, middle cells 15-22 x 11-22 µm, basal cells rectangular, 19-38 x 12-20 µm, 2-3 row of narrow elongated, thick walled cells forming a distinct border throughout the leaf. Plants are vegetative.

1.2. Habitat:

Plants are epiphytic, growing on bark as pure population.

1.3. Range

India, East Nepal, Tonkin, Sumatra, Java, China, New Guinea, Philippines, Taiwan, Vietnam and Japan[7, 8].

1.4. Distribution in India

Eastern Himalaya: Assam, West Bengal: Darjeeling, Meghalaya: Khasia Hills & Arunachal Pradesh. Western Himalaya: Uttarakhand: Mussoori. South India: Tamil Nadu: Palni Hills[5, 7, 9].

1.5. Specimens examined

South India: Tamil Nadu, Nilgiri Hills, Upper Bhawani: Avalanche, alt. ca. 2100 m, S. C. Srivastava & Party, 9 October, 2000, 12530A/00, 12560/00, 12561/00, 12562/00 (LWU).

2. Discussion

The taxa *Plagiomnium succulentum* was described earlier as *Mnium succulentum* Mitt. by Gangulee[7]. Kopenon5 described Mnium succulentum Mitt. as *Plagiomnium succulentum*. Daniels[5] reported *Mnium succulentum* as *Plagiomnium succulentum* which is a valid name till now. It is widely distributed taxa within country[7]. In Tamil Nadu it has been earlier reported from Palni hills by Daniels[5]. During present study it has been collected from Avalanche locality in Nilgiri hills. The species is characterized by creeping plant habit, oblong-ovate leaves with distinct border, costa and a group of beglieter cells (Plate 1, Figs. 1, 2, 5-8, 10).

3. Conclusion

Plagiomnium succulentum (Mitt.) T. J. Kop. is being recorded as new record for Nilgiri hills, South India based on the results of the current study.

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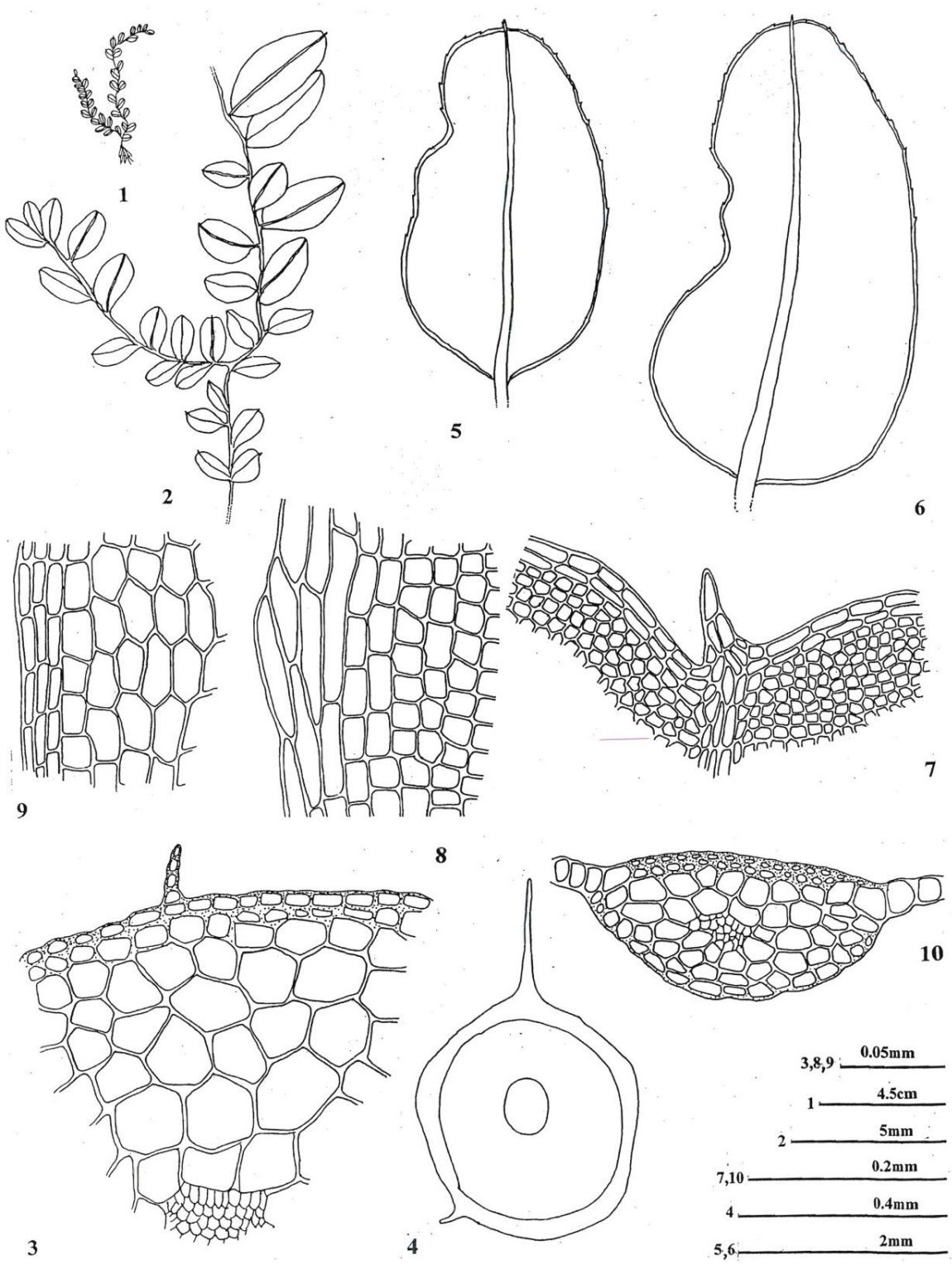

Plate 1, Figs. 1-10: *Plagiomnium succulentum* (Mitt.) T. J. Kop.: 1, 2. Habit of plants. 3, 4. Cross-sections of stem. 5, 6. Leaves. 7. Apical leaf-cells. 8. Median leaf-cells. 9. Basal leaf-cells. 10. Cross-section of leaf. All figures drawn from 12560/2000 (LWU).

Conflicts of interest

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Review Article

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Impact of Environmental Stress on the Physiology of Plants

Pallavi Dixit

Department of Botany, Mahila Vidyalaya Degree College, Lucknow

Corresponding author Email: drpallavidixit80@gmail.com

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Abstract

Stress causes a plant to develop in a suboptimal or terrible state, compromising its capacity to grow, produce crops, develop, or even die if the stress level exceeds the plant's tolerance limitations. It is made up of a diverse set of variables that may be divided into two categories: environmental stress factors (abiotic stress factors) and biotic stress factors (biological stress factors). While biotic stress factors are biological threats (pathogens and pests) that a plant faces during its life, abiotic stress factors include any number of environmental issues that impede plant growth, such as light, waterlogging, temperature, salt, drought, and heavy metal toxicity. Due to continued climate change and deteriorating circumstances, human activity has caused an imbalance in the level of food security. This research looks at the broad views of the various types of plant stress, their effects, and how plants react to these different types of stress. To cope with the stresses they face, plants usually exhibit a range of defensive mechanisms in response to stress.

Keywords: Plants stress, abiotic and biotic, plant response, Heavy metal stress, Physiology

1. Introduction

A plant may experience stress from any external factor that affects its growth, yield, or any other part of its life cycle. Any changed physiological condition caused by environmental factors might disturb homeostasis. Environmental perturbations in this homeostatic condition are referred to as biological stress. The rapid change from certain normal environmental circumstances upsets this initial at-home feeling, and the plant suffers as a result. A system deviates from its usual state due to a recognized situation termed stress, which results in strain—a physical or chemical alteration. Numerous causes contribute to decreased agricultural output, including these environmental stresses. Along with their negative impacts on the existing crop, they also create obstacles to the entry of new species into the ecosystem, which prevents the use of such species for agriculture.

Plants are subject to environmental and unfavorable limitations, which have been described using the stress concept that Hans Selye first introduced in 1936. However, the meaning of stress in plants differs greatly from that of stress in humans and animals.

The quantity of research articles on plant stress and plant stress detection that can be found in journals of botany, plant physiology, ecophysiology, and plant biochemistry has increased dramatically during the last ten years. This process is still ongoing and might pick up even more speed in the future. The state of the art in stress research is demonstrated by several recent books, including Stress and Stress Coping in Cultivated Plants by McKersie and Leshem, Plant Adaptation to Environmental Stress by Fowden et al., Proceedings of Symposia or Plant Stress Reviews by Larcher and Lichtenthaler, and the recently published Vegetation Stress, edited by Lichtenthaler. The latter contains more than 90 original contributions on stress detection and stress effects in plants. Stress bei Pflanzen, an enticing new textbook written in German, has also been edited[1–9].

2. Methodology

The overall design of this study was exploratory. The research paper is an effort that is based on secondary data that was gathered from credible publications, the internet, articles, textbooks, and newspapers. The study's research design is primarily descriptive.

3. Literature Review

3.1 Stress Factors

Under normal conditions, plants can only develop and reproduce more efficiently, yielding high-quality products, as we have previously discussed. However, plants are subject to different levels of biotic and abiotic stresses, which change the environment in which they thrive. Another name for these components is stressors. Plants respond to these pressures by activating defence mechanisms and adaptation processes, which we will discuss later in this chapter. This section discusses several important biotic and abiotic stresses and how they affect plant development (Fig. 1).

Principal types of Abiotic Stress have been mentioned below:

1. Drought or Water Stress
2. Salt-induced stress
3. Thermal stress
4. Light stress.
5. Stress due to heavy metal

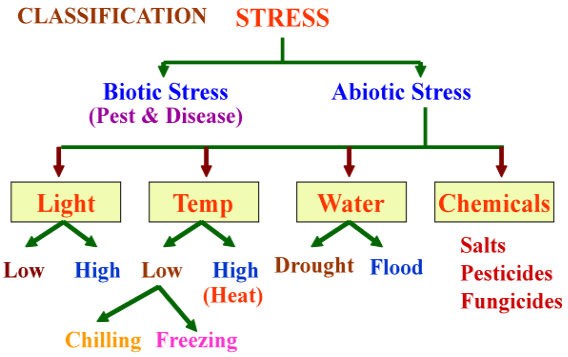

Figure 1. Various Stress factors

3.2. Drought or water stress

Drought is defined as a lack of water that is severe enough to prevent plants from growing. Drought is classified into two primary categories: soil drought and atmospheric drought. An air-dry spell is caused by dry soil. The combination of high temperatures, strong winds, and low atmospheric humidity causes a plant to lose the majority of its water during an atmospheric drought. Abiotic stressors that affect a plant's overall health and growth include flooding, too much salt, insufficient rainfall, too much light, and temperature fluctuations. Plants suffer from a water deficit when transpiration rates rise or when root water absorption is impeded. Wilting is the first response to drought stress because of the decrease of turgor pressure, which makes plant cells swell and stay rigid[10].

Compared to non-resistance cultivars, drought-tolerant sesame cultivars produce more seeds, have higher levels of carotenoid content in their leaves, and have higher levels of proline in their roots. Furthermore, by generating more of these metabolites, the plant was able to withstand the drought stress condition. The height, number of leaves, leaf area, number of pods, pod dry matter, and total plant weight of two bean cultivars all significantly decreased under drought stress. Under drought stress, proline level rises but protein content may fall[11].

3.3. Salt Stress

The accumulation of too much salt in the soil is referred to as "salt stress" and can sometimes lead to plant mortality by impeding plant development. In dry regions, it is one of the primary abiotic factors that reduces agricultural productivity. Salinity has several detrimental effects on agricultural output, seed germination, and plant productivity, to name a few. Ion toxicity, membrane disruption, oxidative

stress, water potential, and decreased cell development and division are other ways that high salt levels can damage plants. Each of these effects harms plant growth and crop yield. Senescence, growth inhibition, and fast growth were among the adverse consequences that plants under salt stress encountered. It may lead to death because of the extended exposure to the salt. Salt stress affects a wide range of plant characteristics, such as physiology, morphology, anatomy, chemical composition, and water content of plant tissues.

Under salinity stress, mineral ion absorption may be diminished. The phenomenon known as decreased ion transit rate under salt stress circumstances is caused by poor root absorption rate or low xylem sap efficiency. Under stressful circumstances, either too much NaCl soaked into the spaces between cells or a poor nutritional supplement caused a reduction in cell division or growth. When plants experience osmotic stress, they are forced to relocate away from salt-accumulating regions and grow roots that are adapted to the saline environment. Salt stress for an extended period decreases the quantity of water and nutrients that the roots can absorb[12].

3.4. Temperature stress

Heat limits a plant's ability to grow and function. Cool-season plant growth is restricted by the hot summers in many places of the world. According to several studies, the optimal temperature range for C_3 plant development is 15–25°C. During the warm season, high temperatures prevent photosynthesis and the accumulation of carbohydrates. Additionally, C_3 plants experienced increased cell membrane damage, which led to protein folding and even cell death. Warm-season plants, or C_4 plant species, have been shown to sustain winter harm. Furthermore, because the C_4 species absorbed less water, they needed to self-modify to be able to absorb nutritional components with poor solubility[13].

C_3 plants can slow down their growth and survive the high-temperature stress situation by using less nitrogen fertilizer throughout the summer. The plants would develop faster and use all of their stored carbohydrates if there were more nutrients available in the root zone during the high-temperature stress, leaving them with no reserves to resist the winter's low temperatures[14].

Mesophyts need a relatively narrow temperature range of about 10°C for optimal growth and development. Outside of this range, the extent of damage is determined by the duration and intensity of the temperature shift. The tissues of higher plants that are actively developing can tolerate both short-term exposure to temperatures of 55°C or more and long-term exposure to temperatures over 45°C. However, nongrowing cells or dried tissues (such as seeds and pollen) continue to function at extremely high temperatures. Certain species' pollen grains can withstand temperatures as high as 70°C, while some dry seeds can withstand as high as 120°C.

3.5. Light Stress

Light stress is another stressor that negatively impacts plants and their growth. Light is a vital environmental factor for plant growth and development and one of the most important components of photosynthesis as an energy source. However, photo-destruction and photo-inhibition, which are harmful to plant functions, can also result from the changed quantity and quality of light. Variations in light intensity, whether high or low, impair a plant's capacity to maintain equilibrium and perform its normal metabolic functions.

Being photoautotrophs, plants benefit greatly from visible light because they use photosynthesis to maintain a positive carbon balance. Higher wavelengths of electromagnetic radiation, especially those in the UV range, can damage membranes, proteins, and nucleic acids, which can impair biological processes. However, even in the visible spectrum, exposures considerably over the light saturation threshold for photosynthesis cause a great deal of light stress, which can damage chloroplast structure and reduce photosynthetic rates (a process known as photoinhibition)[15].

3.6. Heavy Metal Stress

Heavy metals interfere with morphological, physiological, biochemical, and molecular processes in plants. Environmental Pb and Cd have a major effect on plant development and production. Conversely, plants need zinc from the soil for vital functions. Zn aids in the transport and accumulation

of Pb and Cd in the aerial parts of maize plants. Moreover, Zn, Pb, and Cd interaction hinders the absorption and translocation of additional divalent metals. This study shows how histone acetylation and DNA methylation affect metal stress tolerance through Zn transporters and caution against overusing zinc fertilizers in metal-polluted soil. Cerium dioxide (CeO2) nanoparticles are pollutants of increasing concern since they are seldom immobilized in the environment[16].

4. Critical Analysis

The review by Hasan et al.[17] explores the effect of O_3 on stomatal regulation via phytohormone-mediated guard cell communication. By considering several physiological systems, the authors of this review updated our existing knowledge of ozone-induced stomatal regulation. The insights presented will improve our understanding of the molecular mechanisms associated with the O^3 stress response, especially as they affect stomatal regulation, MAPK activity, and phytohormone signaling. Abiotic stresses disrupt K^+ transport and homeostasis.

A review by Viudes et al.[18] suggests that the production of a polysaccharide mucilage upon water imbibition of myxodiasporous species' seeds (myxospermy) or fruits (myxocarpy) may play a part in how plants react to environmental stressors. The understudied topic of myxodiaspory development was the main focus of this review. A detailed characterization of the molecular actors led to the discovery of the mucilage secretory cell (MSC) toolbox, which aids in the production of seed mucilage in *Arabidopsis thaliana*[19].

Ahmadi et al.[20] found that mild stress increased the activity of the enzyme catalase (CAT) whereas severe stress lowered it when maize was grown under different nitrogen levels and drought stress. Additionally, they discovered that CAT activity was significantly increased when nitrogen fertilizer was administered at its highest dosage. Furthermore, drought stress significantly increased the activity of Superoxide Dismutase (SOD). At the mild water stress level, peroxidase (POD) activity rose; however, at the severe water stress level, it decreased and even fell below the control level.

The review by Hasan et al.[21] explores the effect of O_3 on stomatal regulation via phytohormone-mediated guard cell communication. By considering several physiological systems, the authors of this review updated our existing knowledge of ozone-induced stomatal regulation. The insights presented will improve our understanding of the molecular mechanisms associated with the O_3 stress response, especially as they affect stomatal regulation, MAPK activity, and phytohormone signaling. The review by Monder and colleagues emphasizes the main negative impacts of the contemporary environment on grape quality and, by extension, wine quality. Abiotic stresses disrupt K^+ translocation and homeostasis. The main electrical and osmotic activities of K^+ are presented, emphasizing their intimate connection to transport networks, membrane energetics, and cellular K^+ homeostasis. The application of stress-sensitive determinants and the creation of plants with greater stress tolerance will benefit from the new information.

Hasanuzzaman et al.[22] discussed the physicochemical foundation of ROS generation, processes unique to each cellular compartment where they are created, and possible unsettling consequences. The authors further stress the importance of the antioxidant defense system for ROS homeostasis and detoxification in light of the most recent discoveries. Furthermore, identifying stress signals is a crucial first step toward a proper response and plant survival. Essential signaling modules in eukaryotes, plant mitogen-activated protein kinase (MAPK) cascades control how the body reacts to environmental stresses such as excessive salt, drought, high temperatures, and pest and pathogen infestations.

Sekmen et al.[23] claim that the APX activities were influenced by the species and salt content of the plants. The APX activity in sea plantain and hoary plantain leaves increased and was constant under 100 and 200 mM NaCl. H_2O_2 is converted to oxygen and water by the oxidoreductase enzyme catalase. Because this enzyme has a low affinity for H_2O_2 and doesn't need a reducing power, it can only remove large volumes of H_2O_2. It also has a rapid rate of reactivity.

5. Discussion

Abiotic stress can adversely affect a plant's relationships with other species in several secondary ways. This is why an abiotic stress that was

previously present in a community environment is often transformed into a biotic component that induces plant stress, leading to a disrupted or altered relationship between the stressed plant and other interacting species. This phenomenon is shown figure 2:

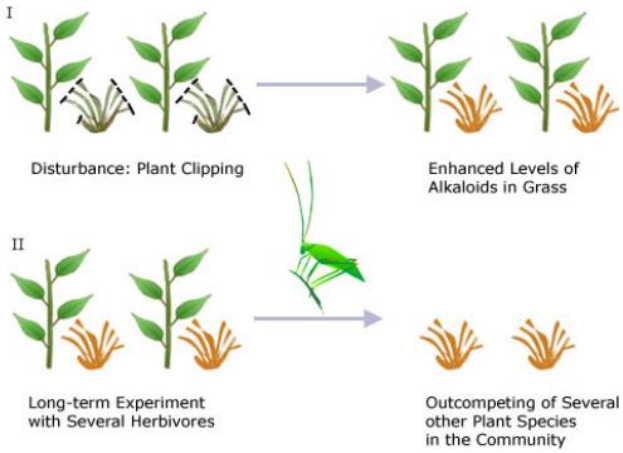

Figure 2. Impact of Biotic Interactions: (I) Grass resistance to herbivores is increased by clipping (resistant grass is highlighted in red). (II) Communities shift when a species has the chance to outcompete rival species.

Physically removing endophytic symbiont-containing grasses can increase the levels of alkaloids in the plant tissue, enhancing the plant's resistance to herbivores (Figure 2). Long-term studies have shown that plants that are better able to fend off herbivores are at a competitive advantage and are thus indirectly outcompeting other plant species. As a result, the community's organization is changed[24].

Extreme temperatures can cause damage to membranes and enzymes. Any abiotic substance that adds proteins and sterols and alters the lipid bilayer that makes up plant membranes has the potential to disrupt cellular processes. The physical properties of the lipids have a major impact on the roles of the integral membrane proteins, which include carriers, H+-pumping ATPases, and channel-forming proteins that regulate the flow of ions and other solutes. High temperatures cause the fluidity of membrane lipids to rise while the strength of hydrogen bonds and electrostatic interactions between polar protein groups in the membrane's aqueous phase decreases. As a result, high temperatures change the structure and makeup of membranes and can cause ion leakage. The three-dimensional structure required for structural cellular components or enzymes to function correctly can also be lost by extreme heat, which can lead to an incorrect structure and activity of the enzymes. Misfolded proteins often precipitate and cluster together, which can lead to serious problems for the cell[25].

Examples of Reactive Oxygen Species (ROS) that can result from salt stress include superoxide, hydrogen peroxide, and hydroxyl radicals. These ROS damage membrane lipids, proteins, and nucleic acids. Plants have developed enzyme systems for scavenging reactive oxygen species to protect themselves from oxidative harm. Catalase (CAT), peroxidase (POX), and ascorbate peroxidase (APX) are some of the enzymes that are involved in the metabolism of ROS. Antioxidant enzymes have been shown to help the body resist the harm caused by salt stress. It has been shown that the breakdown of membrane polyunsaturated fatty acids results in the buildup of malondialdehyde during serum stress. Changes in APX, POX, and CAT activity under salt stress have been examined in studies on several plant species. Increased POX and APX activities in cowpea (*Vigna unguiculata* (L.) Walp), CAT activity in tobacco (*Nicotiana tabacum* L.), and decreased CAT activity in cowpea have all been reported under salt stress. Malondialdehyde (MDA) is produced when the polyunsaturated fatty acids in plant membranes degrade under salt stress[26–30].

6. Conclusion

The effects of biotic and abiotic stress on plant physiological systems. The objective is to enhance plant performance under difficult growth conditions, when plants must deal with elemental stress, biotic stress, water stress, and sub-optimal or supra-optimal temperature. It is feasible for producers and breeders to boost nutritional element efficiency if they completely know the impacts of various stressors on plant cells, their mobility within the cells, and their influence on physiological processes. This will boost crop yield and help producers become more resilient to global stresses.

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Conflict of Interest

Not Applicable

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Research Article

Physiological Responses of *Boerhavia diffusa* L. to the Heavy Metal Stress: Reflections on Chlorophyll, Proline and Nitrate Reductase Activity

Abdussalam A.K.*[1], Ratheesh Chandra P.[2] and Prajith P. K.[3]

[1]Department of Post Graduate Studies and Research in Botany, Sir Syed College (Affiliated to Kannur University), Talipramaba, Kannur, Kerala, India.

[2]Department of Botany, Kannur University, Mananthavady Campus, Wayanad, Kerala, India.

[3]Department of Botany, Nehru Arts and Science College (Affiliated to Kannur University), Kanhangad, Kasaragod, Kerala, India.

Corresponding author Email: salam@sirsyedcollege.ac.in

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Abstract

The present study investigates the physiological responses of Boerhavia diffusa L., a traditionally valued medicinal plant, to heavy metal stress induced by Cadmium (Cd), Chromium (Cr), Mercury (Hg) and Lead (Pb). The study focuses on the accumulation of proline, alterations in chlorophyll content and the activity of nitrate reductase across different plant tissues (root, stem, and leaf) during developmental stages. Results revealed that proline content significantly increased in all tissues of B. diffusa exposed to heavy metal treatments compared to their respective controls. Notably, on the 20th day, root tissues under cadmium treatment exhibited more than a twofold increase in proline content. Similar trends were observed in mercury and chromium treatments, whereas lead induced a comparatively lower accumulation. Stem tissues showed maximal proline levels in chromium- and lead-treated plants. Leaf tissues displayed the highest proline accumulation under lead exposure over eight times that of the control followed by mercury and cadmium. Interestingly, chromium treated leaves recorded relatively lower proline levels than other metal treatments. Chlorophyll analysis indicated a marked decline in chlorophyll a and b content in cadmium, chromium and mercury treated plants, with the most significant reduction observed under chromium stress. The chlorophyll a/b ratio remained largely unaffected across treatments. Lead exposure showed only a marginal effect on chlorophyll content. Nitrate reductase activity, essential for nitrogen assimilation, was significantly reduced in all heavy metal treatments, particularly in leaf tissues. Cadmium had the most pronounced inhibitory effect, followed by mercury, chromium and lead. The activity was consistently higher in control leaves, with root and stem tissues showing notably lower levels. The findings underscore the differential tolerance and physiological adaptability of B. diffusa under heavy metal stress and highlight its potential as a bioindicator or candidate for phytoremediation in contaminated environments.

Keywords: *Boerhavia diffusa,* Heavy Metals, Chlorophyll Content, Proline Accumulation, Nitrate Reductase Activity.

1. Introduction

Boerhavia diffusa L. (Hogweed), known as "Thazhuthama" in Malayalam and "Punarnava" in Sanskrit, is a widely distributed herbaceous plant recognized for its medicinal significance in traditional Ayurvedic systems[1]. Its bioactive compounds such as punarnavine, glycoproteins, and phenolics confer diverse therapeutic properties including hepatoprotective, antidiabetic, anti-inflammatory and antioxidant effects[2,3].

Frequently found in disturbed habitats and polluted environments. *B. diffusa* exhibits a remarkable tolerance to ecological stress and strain, including heavy metal exposure[4]. However, there is a lack of comprehensive studies detailing its Physiological and biochemical reactions in response to heavy metal exposure, particularly concerning essential metabolic indicators[5] (Reddy et al., 2018). This study was conducted to explore the comparative physiological responses of *B. diffusa* when exposed to Cadmium (Cd), Chromium (Cr), Mercury (Hg) and Lead (Pb). Using a hydroponic setup with Hoagland nutrient medium artificially contaminated with these metals, key physiological parameters were monitored, including chlorophyll content, proline accumulation, and nitrate reductase activity each a critical marker for assessing stress response, metabolic alterations and detoxification strategies in plants[6,7]. By analyzing these specific physiological markers over a 20-day period, the study aims to understand the plant's tolerance mechanisms and adaptive strategies under varying concentrations of heavy metals. These insights not only help clarify the plant's resilience in contaminated environments but also raise important considerations regarding the safety of using such medicinal plants when grown in polluted conditions[8, 9]. Ultimately, the study contributes to a broader understanding of the ecological and pharmacological implications of heavy metal stress on *B. diffusa*.

2. Materials and Methods

Boerhavia diffusa plants were grown in a Hoagland nutrient solution augmented with defined concentrations of heavy metal contaminants. The nutrient solution was deliberately contaminated with the following compounds: cadmium chloride (30 µM), potassium dichromate (400 µM), mercuric chloride (10 µM), and lead acetate (600 µM). Growth conditions, treatment protocols, and sampling techniques adhered to the procedures established by Abdussalam et al.[10]. The proline levels in various plant tissues, including roots, stems, and leaves, were quantified using the methodology proposed by Bates et al.[11]. Proline was extracted from the tissues, and its concentration was measured via a colorimetric assay. Chlorophyll content was determined following the procedure set forth by Arnon[12]; fresh leaf samples were harvested and processed for chlorophyll extraction, which was subsequently quantified using spectrophotometry. Nitrate reductase activity was evaluated utilizing the method recommended by Hageman and Reed[13], as detailed by Sadasivam and Manikam[14], through the measurement of nitrite production under suitable biochemical conditions with the appropriate substrate. The concentrations of cadmium, chromium, mercury, and lead in the root, stem, and leaf tissues were determined using Atomic Absorption Spectrophotometry (AAS). Sample preparation was conducted according to the approach described by Allan[15], ensuring precise quantification of metal concentration in the plant tissues.

Fig. 1. *Boerhavia diffusa* in its natural habitat

Fig. 2. *Boerhavia diffusa* is growing in Hoagland solution containing cadmium (Cd), chromium (Cr), mercury (Hg), and lead (Pb).

2. Results

2.1. Proline: The proline content in the roots exhibited a gradual increase in response to heavy metal treatment throughout all developmental stages when compared to the respective control groups. Notably, by the 20th day, proline levels in plants treated with cadmium were more than double those of the controls (Table 1). A similar increase was observed in plants exposed to chromium and mercury; however, the proline increases in plants treated with lead was comparatively lower (Table 1). In stem tissues, proline levels also demonstrated a significant increase across all treatments, with the highest concentrations found in plants subjected to chromium and lead. The leaf tissues of plants treated with lead showed a proline content exceeding eight times that of the control. Furthermore, proline levels in the leaves of plants treated with mercury and cadmium were significantly elevated compared to controls, while proline content in leaves treated with chromium was comparatively lower than in the other treatment groups (Table 1). The increase in proline content during the developmental stages of the leaves was statistically significant across all treatments relative to control plants.

2.2. Chlorophyll: In comparison to control plants, the levels of chlorophyll a and b were diminished in plants treated with cadmium, with the chlorophyll a/b ratio remaining stable (Table 2). A significant decrease in both chlorophyll a and b was recorded under chromium toxicity ($P<0.01$). Similar results were noted in plants treated with mercury. Conversely, plants exposed to lead displayed minimal changes in chlorophyll a and b levels compared to controls, maintaining the same proportion of chlorophyll a/b. Overall, total chlorophyll content was reduced in response to all heavy metal treatments (Table 2).

2.3. Nitrate Reductase: Control plants exhibited a notably high level of nitrate reductase activity in leaf tissues, with root and stem tissues displaying less than half of this activity (Table 3). Treatment with cadmium resulted in a significant decrease in nitrate reductase activity across all tissue types, particularly in leaf tissues. Similarly, plants subjected to the other three heavy metals demonstrated a significant reduction in nitrate reductase activity compared to both control and cadmium-treated plants.

3. Discussion

The accumulation of proline in *B. diffusa* under heavy metal stress provides valuable insights into the plant's adaptive responses to cadmium, chromium, mercury, and lead toxicity. Proline, a versatile amino acid, plays crucial roles in maintaining osmotic balance, scavenging free radicals, and stabilizing cellular structures. Despite exposure to heavy metals, proline accumulation remained unchanged in the root tissues, indicating a potential saturation point in its biosynthesis, or possibly a localized protective mechanism unique to the roots. In contrast, significant increases in proline were noted in the stem tissues, peaking around the 20th day of treatment. This correlation suggests that the stem may be compensating for osmotic stress primarily arising from metal transport and accumulation, especially as osmotic conditions are altered by increased metal ion concentration. The parallel rise in both proline levels and metal bioaccumulation specifically in the stem reflects a strategic physiological adjustment by *B. diffusa* to endure heavy metal stress. It aligns with findings by Hopkins[16] and Rai et al.[17], who documented similar proline responses in other plant species subjected to heavy metal toxicity.

Such adaptations are crucial for mitigating oxidative stress and disturbances caused by toxic metal uptake. Interestingly, under Cadmium, Chromium, Mercury, and Lead treatment, *B. diffusa* displayed significant variations of proline accumulation, particularly pronounced when subjected to chromium and lead, which exhibited higher bioaccumulation potentials.

This is crucial as the mechanism by which proline alleviates osmotic stress could suggest a plant strategy for physiological adaptability to unfavorable growth conditions. Conversely, the relationship between proline levels and chlorophyll content in *B. diffusa* exposes a compelling intersection between stress physiology and photosynthetic efficiency.

The progressive decline in chlorophyll a, b, and the overall chlorophyll a/b ratio under heavy metal treatments denotes a disruption in photosynthetic

processes, with potential implications for plant growth and productivity. Chlorophylls are critical for photosynthesis, and their reduction tends to correlate with decreased photosynthetic rates, as supported by numerous studies[18, 19]. While proline accumulates in response to osmotic stress from heavy metals, the reduced chlorophyll content signals a compromised photosynthetic apparatus, leading to diminished energy production vital for plant growth and development.

Although chlorophyll loss due to cadmium, chromium, mercury, and lead is documented in various studies[20], the effects of lead reported by De Agostini et al.[21], it is vital to consider that chlorophyll reduction may not directly hinder proline accumulation but could impede overall growth due to diminished photosynthetic performance. This duality of function raises intriguing questions regarding the balance between stress adaptation via proline accumulation and the plant's ability to sustain growth and metabolic functions under heavy metal-induced stress. In the present study, we observed that the nitrate reductase activity of control plants was markedly higher in leaf tissue compared to root and stem tissues, which exhibited less than half the activity (Table 2).

Nevertheless, exposure to cadmium resulted in a marked decrease in nitrate reductase activity throughout all plant tissues, with the most significant impact observed specifically in the leaf tissues. This trend was consistent in plants exposed to other heavy metals, including Chromium, Mercury, and Lead, which also demonstrated significant decreases in nitrate reductase activity when compared to both the control and cadmium-treated plants.

These results corroborate findings from previous studies that have shown heavy metal stress adversely affects enzymatic functions, including nitrate reductase, across a range of plant species[22, 23, 24].

The reduction in nitrate reductase activity observed in our study indicates that heavy metal toxicity may disrupt nitrogen metabolism, a notion supported by Nascimento et al.[25], who documented similar detrimental impacts on nitrate assimilation in plants subjected to heavy metal exposure. Thus, our findings contribute to the growing body of evidence that heavy metals can severely impair vital physiological processes, thereby affecting overall plant health and productivity.

5. Conclusion

The study demonstrates that while proline accumulation serves as an effective adaptive response to heavy metal stress in *B. diffusa*, it also highlights the associated challenges regarding chlorophyll content. The significant reduction in nitrate reductase activity across all examined tissues due to cadmium, chromium, mercury, and lead exposure underscores the detrimental impact of heavy metals on nitrogen metabolism and overall plant health[26].

These findings align with existing literature, reinforcing the notion that heavy metals compromise essential physiological processes in plants. Future research should focus on further elucidating these interactions to optimize phytoremediation strategies, particularly by enhancing both chlorophyll synthesis and proline accumulation to improve the resilience and productivity of *B. diffusa* in contaminated environments.

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Authors Contributions

Abdussalam AK conceptualized and conducted the major research work. All authors contributed equally to the plant collection from various regions of Kerala, participated in overall design, data collection, analysis, and writing of the manuscript. Abdussalam AK, Ratheesh C and Prajith PK have read and approved the final version of the manuscript.

Conflicts of Interest

Authors declare no conflict of interest.

Table 1: Effect of Heavy Metals on Proline content in *Boerhavia diffusa* (mg g-1 dry weight)

Treatments	Tissues	Interval (Days)					
		0	4	8	12	16	20
Control	Root	0.351±0.07	0.476±0.01	0.517±0.02	0.620±0.05	0.673±0.03	0.714±0.01
	Stem	0.247±0.01	0.280±0.01	0.299±0.01	0.325±0.04	0.351±0.01	0.373±0.02
	Leaf	0.106±0.06	0.127±0.01	0.139±0.01	0.144±0.01	0.159±0.02	0.166±0.01
Cadmium	Root	0.351±0.02	0.561±0.01	0.770±0.02	0.858±0.01	1.521±0.12	1.891±0.10
	Stem	0.247±0.01	0.297±0.01	0.341±0.03	0.395±0.02	1.324±0.18	1.643±0.10
	Leaf	0.106±0.05	0.144±0.01	0.185±0.01	0.213±0.03	0.263±0.12	0.296±0.02
Chromium	Root	0.351±0.03	0.570±0.01	0.785±0.03	0.930±0.01	1.450±0.031	1.860±0.08
	Stem	0.247±0.02	0.355±0.02	0.452±0.01	1.520±0.01	1.850±0.031	2.530±0.20
	Leaf	0.106±0.01	0.251±0.03	0.363±0.02	0.427±0.04	0.591±0.02	0.914±0.04
Mercury	Root	0.351±0.03	0.623±0.02	0.959±0.01	1.259±0.01	1.537±0.20	1.875±0.02
	Stem	0.247±.0.10	0.452±0.07	0.732±0.02	0.917±0.01	1.253±0.10	1.289±0.02
	Leaf	0.106±0.02	0.315±0.01	0.406±0.01	0.538±0.07	0.686±0.02	0.905±0.01
Lead	Root	0.351±0.01	0.734±0.05	0.858±0.01	0.921±0.02	1.280±0.11	1.530±0.31
	Stem	0.247±0.04	0.625±0.01	0.936±0.01	1.531±0.02	2.425±0.02	2.870±0.82
	Leaf	0.106±0.01	0.381±0.01	0.562±0.01	0.685±0.01	0.934±0.02	1.216±0.08

Values are mean of 5 replicates ±standard error

Table 2: Effect of Heavy Metals on Chlorophyll content in *Boerhavia diffusa* (mg g^{-1} dry weight)

Treatments	Tissues	Interval (Days)					
		0	4	8	12	16	20
Control	Chl.a	0.98±0.02	1.13±0.06	1.37±0.06	1.57±0.05	1.78±0.08	2.17±0.08
	Chl.b	0.57±0.01	0.63±0.04	0.78±0.03	0.86±0.08	0.92±0.04	1.07±0.05
	a/b	1.71±0.03	1.79±0.10	1.75±0.05	1.82±0.04	1.93±0.08	2.02±0.11
	Total	1.55±0.04	1.76±0.05	2.15±0.12	2.43±0.12	2.70±0.05	3.24±0.16
Cadmium	Chl.a	0.98±0.03	0.91±0.02	1.23±0.07	1.42±0.08	1.55±0.08	1.84±0.10
	Chl.b	0.57±0.01	0.42±0.02	0.53±0.02	0.66±0.03	0.78±0.01	0.93±0.05
	a/b	1.71±0.02	2.16±0.09	2.32±0.12	2.15±0.11	1.98±0.06	1.97±0.05
	Total	1.55±0.04	1.33±0.08	1.76±0.07	2.08±0.02	2.33±0.05	2.77±0.16
Chromium	Chl.a	0.98±0.02	0.63±0.02	0.75±0.03	0.93±0.04	1.23±0.09	1.34±0.08
	Chl.b	0.57±0.06	0.39±0.01	0.41±0.02	0.58±0.02	0.68±0.02	0.84±0.05
	a/b	1.71±0.03	1.61±0.06	1.82±0.08	1.60±0.09	1.80±0.08	1.65±0.06
	Total	1.55±0.06	1.02±0.05	1.16±0.04	1.51±0.06	1.91±0.05	2.23±0.08
Mercury	Chl.a	0.98±0.02	0.72±0.02	0.89±0.03	1.13±0.07	1.37±0.08	1.49±0.07
	Chl.b	0.57±0.02	0.43±0.01	0.59±0.01	0.67±0.01	0.73±0.03	0.86±0.02
	a/b	1.71±0.07	1.67±0.04	1.50±0.04	1.68±0.04	1.87±0.05	1.63±0.07
	Total	1.55±0.03	1.15±0.07	1.48±0.05	1.80±0.04	2.10±0.09	2.27±0.13
Lead	Chl.a	0.98±0.02	0.83±0.03	1.12±0.07	1.37±0.05	1.48±0.04	1.93±0.10
	Chl.b	0.57±0.01	0.45±0.02	0.63±0.01	0.73±0.02	0.83±0.03	0.94±0.13
	a/b	1.71±0.08	1.84±0.08	1.77±0.04	1.87±0.08	1.78±0.09	2.05±0.10
	Total	1.55±0.08	1.28±0.07	1.75±0.05	2.10±0.02	2.31±0.11	2.87±0.10

Values are mean of 5 replicates ±standard error

Table 3 : Effect of Heavy Metals on Nitrate reductase activity in *Boerhavia diffusa* (μ moles NO_2 g^{-1} dry weight)

Treatments	Tissues	Interval (Days)					
		0	4	8	12	16	20
Control	Root	9.55±0.16	11.3±0.90	13.2±0.12	13.9±0.22	14.6±0.11	15.2±0.24
	Stem	16.4±0.13	17.2±0.86	17.9±0.15	18.1±0.62	19.6±0.11	21.7±0.32
	Leaf	33.5±1.01	39.2±1.11	46.8±1.24	53.1±0.45	52.6±0.47	53.8±0.21
Cadmium	Root	9.55±0.26	6.78±0.14	7.13±0.21	8.98±0.19	9.96±0.11	10.2±0.14
	Stem	16.4±0.13	12.7±0.12	14.2±0.26	16.1±0.23	17.5±0.15	17.9±0.11
	Leaf	33.5±1.01	30.4±0.05	32.1±1.02	35.6±1.20	37.6±0.41	37.8±1.31
Chromium	Root	9.55±0.36	8.42±0.45	9.21±0.17	10.5±0.74	10.9±0.67	11.4±0.26
	Stem	16.4±0.23	10.5±0.11	11.4±0.88	12.6±0.21	13.3±0.19	15.7±0.01
	Leaf	33.5±1.01	25.7±0.45	26.8±0.56	28.6±0.43	29.6±0.44	31.8±0.41
Mercury	Root	9.55±0.16	5.62±0.14	6.98±0.32	7.54±0.14	9.70±0.12	10.6±0.19
	Stem	16.4±0.23	13.1±0.13	14.7±0.98	15.8±0.51	17.9±0.32	18.0±0.55
	Leaf	33.5±1.01	26.0±0.45	27.8±0.82	28.5±0.56	29.4±0.49	31.7±0.41
Lead	Root	9.55±0.16	9.84±0.21	10.8±0.42	11.6±0.13	12.8±0.52	14.6±0.21
	Stem	16.4±0.23	12.6±0.32	13.7±0.11	13.9±0.31	12.0±0.41	11.9±0.30
	Leaf	33.5±0.31	25.7±0.79	27.7±0.41	28.9±0.43	29.3±0.28	29.7±1.43

Values are mean of 5 replicates ±standard error

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Research Article

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Morphological Report on *Gymnostomiella vernicosa* (Hook. ex Harv.) M.Fleisch. (Pottiaceae) from Ranthambhore National Park, Rajasthan

Shiv Charan Sharma and Tripti Sharma

Department of Bioscience and Biotechnology, Banasthali Vidyapith (Rajasthan)

Corresponding author Email: stripti@banasthali.in

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Abstract

This morphological report provides a detailed description of *Gymnostomiella vernicosa* (Hook. ex Harv.) M. Fleisch. (Pottiaceae), a rare moss species collected from Ranthambhore National Park, Rajasthan. The species is characterized by small, dioicous, light-green plants that grow in mats or tufts. The stems are reddish-brown, with leaves that are spathulate to oblong, having a broadly rounded apex and flat, plain margins. The moss exhibits distinct cell patterns, with upper lamina cells being papillose and basal cells being smooth and long-rectangular. *Gymnostomiella vernicosa* thrives in terrestrial habitats, particularly on moist rocks, and has a wide geographical distribution, including regions in India, East Nepal, Bhutan, China, and beyond. The species' occurrence in Rajasthan is significant, as it represents one of the few bryophytes adapted to the state's xerothermic climate. This report adds to the knowledge of bryophyte diversity in Rajasthan, particularly in the Ranthambhore region.

Keywords: *Gymnostomiella vernicosa*, Pottiaceae, moss, Ranthambhore National Park, Rajasthan, morphological description.

1. Introduction

Rajasthan, the second-largest state of India, offers a diverse and extreme climate that significantly impacts the distribution of vegetation, including bryophytes. With its fluctuating temperature, limited precipitation, and arid conditions, the state's climatic conditions are not conducive to the flourishing of most plant species[1]. These environmental stresses present challenges for the survival of bryophytes, especially in the northern and north-western regions. However, some bryophytes have adapted to these conditions and have been documented in areas like Ranthambhore National Park, which is situated in the south-eastern region of Rajasthan[2].

Gymnostomiella vernicosa is a somewhat rare moss species in this region, with only a few scattered and unverified reports documented by earlier researchers[3, 4, 5]. Intriguingly, this elusive moss was discovered growing on a moist rock surface along the route to Ranthambhore National Park. At first glance, it bore a resemblance to a liverwort, but detailed microscopic examination confirmed its true identity as a moss species. The detailed morphological and anatomical description of this species is provided in this paper, likely for the first time from this region.

2. Materials and Methods

A detailed field survey was conducted between 2011 and 2018 across various regions of Rajasthan in different seasons to collect bryophytes, with particular emphasis on Ranthambhore National Park, where the climate is relatively more favorable for bryophyte growth. Specimens were collected, labeled, and processed for further morphological and ecological study. Voucher specimens were deposited in the Banasthali University Rajasthan India (BURI) Herbarium, for future reference.

1.1. Taxonomic description

Gymnostomiella vernicosa (Hook. ex Harv.) M. Fleisch. Musci Buitenzorg 1: 310 (1904)

(Plate 1, Fig. 1-11; BURI 7860237)

Dioicous, very small, delicate, light-green plants growing in mats or tufts. The stem is forked or simple, reddish-brown, and about 0.8 cm in height. Leaves are up to 0.2 mm long, with a narrower base than apex, spathulate to oblong in shape, erect to spreading when moist, and somewhat erect and in-rolled when dry, with a broadly rounded apex and plain, flat margins. The plant has a percurrent, slender costa. Upper lamina cells are irregularly hexagonal to short-rectangular and papillose, while the basal cells are smooth, long-rectangular, and shorter towards the margin. Sporophyte not seen.

Habitat:

1.2. Habitat: Plants are terrestrial, growing on moist rocks as a pure population.

1.3. Range: India, East Nepal, Bhutan, China, Sri Lanka, Taiwan, Philippines, Africa and Tonkin[7, 8].

1.4. Distribution in India: Eastern Himalaya[7, 8]; Western Himalaya[9]; Western Ghats[10, 11]; Kerala[11]; Eastern Ghats[12], Rajasthan: Ranthambhore National Park Area.

In Rajasthan, *Gymnostomiella vernicosa* has been specifically documented in the Ranthambhore National Park, which represents a unique distribution for this species within the state. Other bryophytes found in Rajasthan are generally more resilient to arid conditions, with only a few species being able to survive the xerothermic climate[6].

1.5. Specimens examined: India: Rajasthan- Legit: Shiv Charan Sharma and Afroz Alam; alt. ca. 450 m; Det. Afroz Alam; BURI-7860237/2011; date: 9/8/2011

2. Discussion

Gymnostomiella vernicosa is a rare and delicate species of moss found in moist habitats, primarily on rocks. The climatic conditions of Rajasthan present a significant challenge for bryophyte populations, as most regions experience extended dry periods. However, species such as *G. vernicosa* are resilient enough to withstand these harsh conditions, surviving in areas with the right microhabitats, such as moist rock faces in Ranthambhore National Park. The plant is a small, dioicous moss forming mats or tufts, with light green stems up to 0.8 cm tall. Its tiny leaves (up to 0.2 mm) are spathulate to oblong, erect when moist, and inrolled when dry. Microscopically, the upper lamina cells are papillose and irregularly shaped, while the basal cells are smooth and rectangular. A slender, percurrent costa runs through the center of each leaf.

3. Conclusion

Gymnostomiella vernicosa represents a rare and ecologically significant species in Rajasthan's bryophyte flora. Its occurrence in the Ranthambhore National Park highlights the ability of certain bryophytes to adapt to dry, xerothermic conditions, provided they can find appropriate microhabitats. This species contributes to the biodiversity of the park and serves as an indicator of the delicate balance of life in arid ecosystems. Further studies and surveys across different regions of Rajasthan will be valuable to understand the full distribution and ecological significance of this moss.

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Authors Contributions

SCS collected and draw the Camera Lucida drawings. TS compiled the data and wrote the first draft of the manuscript. Both the authors have finalized the manuscript.

Conflicts of Interest

Authors declare no conflict of interest.

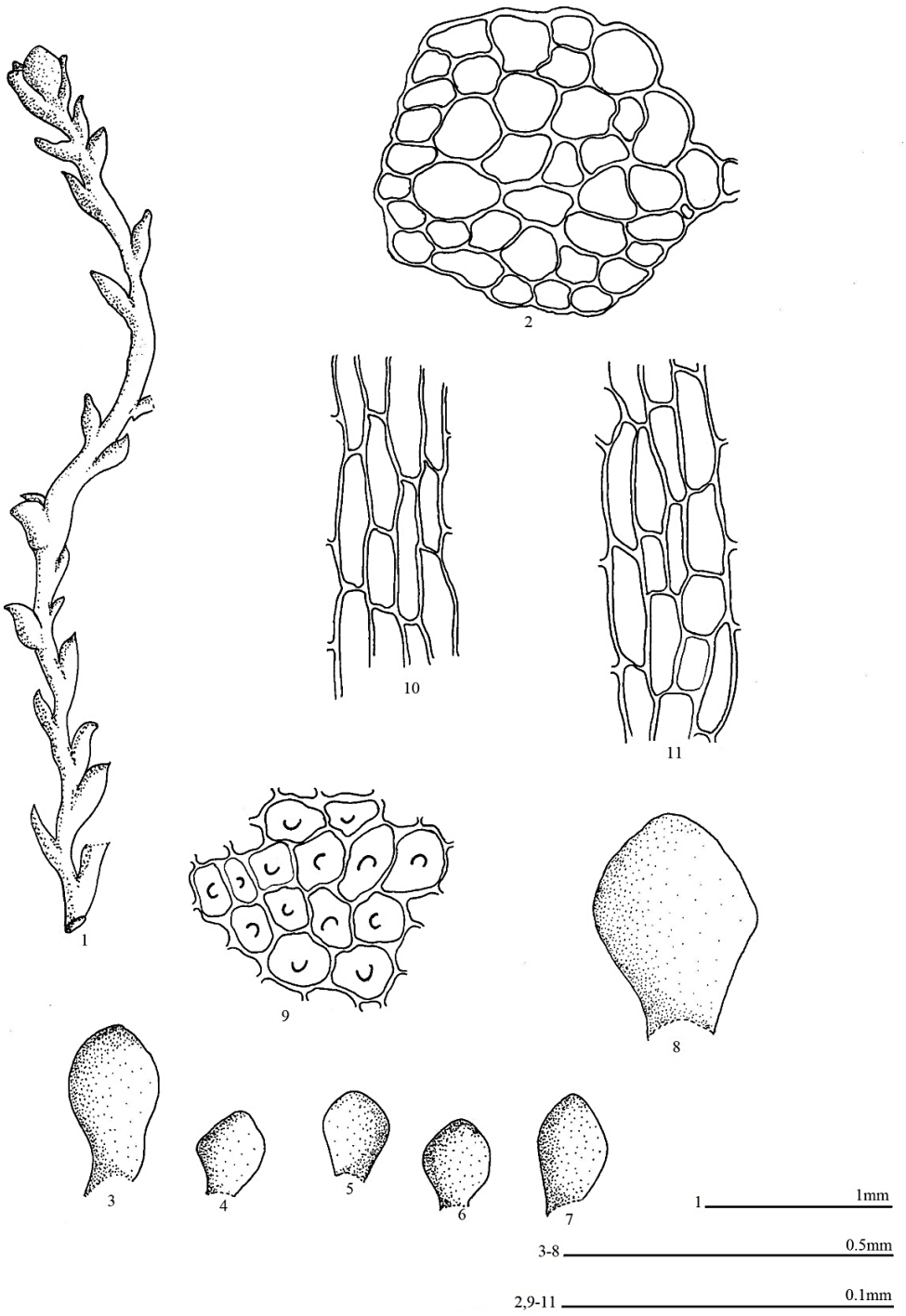

Plate 1: Figs. 1.11: *Gymnostomiella vernicosa* (Hook. ex Harv.) M.Fleisch.: 1. A portion of plant, 2. Cross section of axis, 3-8. Leaves, 9. Leaf cells with papillae, 10-11. Marginal cells of leaf (BURI 7860237/2011)

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Nano-Biofortification of Millets: A Review

Priya Kushwaha[1], Rahul Verma[1], Amit Kumar Singh*[1] and Pallavi Dixit[2]

1 Plant Nutrition and Stress Physiology Lab, Department of Botany, University of Lucknow, Lucknow, U.P, India

2 Department of Botany, Mahila Vidyalaya Degree College, Lucknow, U.P, India

Corresponding author Email: amitunpg@gmail.com

Abstract

The world's population faces nutritional insecurity due to a reliance on grain-heavy diets low in micronutrients. In drought-prone regions of Asia and Africa, millets are a crucial energy source, rich in proteins, vital amino acids, minerals, and vitamins. Biofortifying staple crops is a cost-effective strategy to combat micronutrient deficiencies, particularly in impoverished areas where access to supplements is limited. While the green revolution boosted agricultural output, it often overlooked the nutritional quality of food. Biofortification using nanoparticles and nanomaterials can enhance nutrient delivery and improve crop health without significantly harming the environment. Micronutrient-enriched nano fertilizers can increase yields and nutritional quality, especially for zinc and iron. Studies indicate that nano-fertilizers improve nutrient utilization, reduce soil toxicity, and decrease application frequency, making nanotechnology a promising solution for sustainable crop production, especially in developing nations.

Keywords: Low Micronutrients, Millets, Biofortification, Nanoparticles, Nutritional quality, Nano-fertilizers.

1. Introduction

World Health Organization (WHO) defines micronutrients as compounds required in < 100 mg/d, which includes vitamins (vitamin A, B_9) and minerals such as iron (Fe), zinc (Zn), and iodine (I). Disease caused by the deficiency of these micronutrients in humans is known as 'Micronutrient Malnutrition' or 'Hidden Hunger', which causes early death, impaired health, vision effects, mental disorders, learning impairment, improper growth, and lassitude. According to the World Health Organization (WHO), 45% of deaths in children below 5 years of age are due to malnutrition[1].

In developing countries, hidden hunger or micronutrient deficiency is of grave concern because it contributes to about 0.5% of deaths in India in 2016. About 42% of children <5 yrs. and 40% of pregnant women are anemic due to iron deficiency, referred to as iron deficiency anemia (IDA) [2]. About 24.8% of the world's population suffers from iron deficiency[3]. National Family Health Survey- 4 showed that India has the highest percentage of anemia worldwide. Iron deficiency in children is about 58.6%, in non-pregnant women is about 53.2%, and in pregnant women about 50.4%[4]. Zinc deficiency can lead to death from various diseases. In humans, it affects multiple systems, including the immune, skeletal, reproductive, gastrointestinal, neurological, and intestinal systems.

Consequently, zinc deficiency can present itself in several ways, with the most common being increased rates of pneumonia, malaria, diarrhea, and other illnesses.

The government has taken many initiatives against micronutrient deficiency, but the problem still exists in a large population. Food fortification, dietary diversification, nutritional education, micronutrient supplements, and maintenance of environmental sanitation and hygiene are various ways to overcome the problem.

Food fortification is one of the best ways to tackle the problem in all age groups. Enrichment of food with micronutrients can be achieved by biofortification of staple food crops.

Biofortification is a way to increase the micronutrient content in food crops. It can be achieved by various methods, such as conventional plant breeding, transgenic approaches, agronomic approaches, and Nanobiofortification.

Conventional breeding: utilizes the genetic variation present in different gene pools of the target crop.

Transgenic approach: A genetically engineered plant is made by altering the gene segment.

Agronomic approach: by the application of the nutrient-containing fertilizers either by foliar spray or by soil application.

Nanobiofortification: Application of the Nano-fertilizers to ensure target-bound slow delivery of nutrients to plants, reduce nutrient volatilization, reduce nutrient leaching, and increase bioavailability of nutrients.

India is the leading producer of millet, accounting for about 80% of the global millet production[5]. Millets are also known as 'small seeded plants' which include Pearl millet (*Pennisetum glaucum* L.), Finger millet (*Eleusine coracana* L.), Foxtail millet (*Setaria italica* L.), Proso millet (*Panicum miliaceum* L.), Barnyard millet (*Echinochloa* spp.), Kodo millet (*Paspalum scrobiculatum* L.), and Little millet (*Panicum sumatrense* L.). Among all millets, about 95% are pearl millets (*Pennisetum glaucum* L.) [6, 7, 8]. The sixth largest production is finger millets (*Eleusine coracana* L.), which serve as the primary food for the rural population of east and central Africa and southern India[9]. Millets are dominant over rice and wheat due to their high nutritional quality, they contain high amounts of protein, dietary fibers, iron, zinc, calcium, phosphorus, potassium, and vitamins. B and some essential amino acids[10].

Little millet (*Panicum sumatrense* L.)

Barnyard millet (*Echinochloa* spp.) Kodo millet (*Paspalum scrobiculatum* L.)

Foxtail Millet (*Setaria italic* L.) Proso millet (*Panicum miliaceum* L.)

Pearl millet (*Pennisetum glaucum* L.) Finger millet (*Eleusine coracana* L.)

Millets also have some antinutritional compounds like phytates, polyphenols, and tannins that reduce the absorption of multivalent cations like Fe^{2+}, Zn^{2+}, Ca^{2+}, Mg^{2+}, and K^{+}[11]. The anti-nutritional compound can easily be removed by decortication, malting, fermentation, roasting, flaking, and grinding. 80% of millet is used as food, and the rest is used as fodder and in the brewing industry[12]. Millets are a super food for infants, lactating mothers, senior citizens, and convalescents. Millets are considered 'gluten-free' as they release sugar very

slowly into the bloodstream[13]. As millets are rich in protein and dietary fibers, they are recommended for people suffering from diabetes and cardiovascular disease[14]. They are rich in flavonoids and phenolic acids, which help fight against free radicals generated by oxidative stress and lower blood sugar levels[15].

Among all millets, pearl millets have the highest content of iron, zinc, and lysine (17-65 mg/g of protein). Finger millets have high levels of Ca that help to strengthen bones, K that helps to prevent diabetes, renal and cardiovascular diseases, Mg, and some essential amino acids like Met, Lys, Trp, and polyphenols[10].

The emergence of green nanotechnology has led to significant interest in this field among global scientific researchers. Nano-materials harmful effects can be minimized by implementing green nanotechnology, which is an effective means of reducing these risks[16]. Nanotechnology has emerged as one of the most active areas of study[17].

Green chemistry employs chemical principles to minimize or eliminate the use of hazardous substances, leading to a significant decrease in toxic residues that are harmful to both humans and the environment. Green synthesis is considered a feasible method for nanoparticle synthesis because it is biocompatible, unrestricted, and environmentally friendly.

To prepare nanoparticles, the most efficient method is green synthesis, which minimizes toxic substances and enhances stability while being eco-friendly and cost-effective. Both environmental and biomedical contexts favor green synthesis methods as a more effective approach[18]. The phytochemical compounds found in plants include phenols, terpenoids, polysaccharides, and flavonoids that possess redox properties. Thus, they are advantageously used for the green synthesis of nanoparticles.

Compared to chemical and physical methods, green synthesis has several advantages, such as being non-toxic[19], environmentally friendly[20], eco-friendly, cost-effective[21], and more sustainable[22]. The process of biogenic reduction of metal precursors to corresponding NPs is advantageous for its environmental impact[23], durability[24], chemical safety[25], affordability[26], and mass production[27].

2. Significance and Nutrient Value of Millets

2.1. Staple Food:

Millions of rural poor people in developing countries rely on millets as their primary food source. Millets are among the oldest food crops known to humankind and were the first cereal crops used for domestic purposes. Instead of belonging to a specific taxonomic group, they are categorized by their functional or agronomic characteristics. Millets are exceptionally hardy and thrive in arid regions, making them increasingly popular as staple foods, particularly in large areas of India and sub-Saharan Africa. Their high productivity and short growing season in dry and hot conditions contribute to their desirability. Due to their nutritious content, millets are now often referred to as "Nutri-cereals."

A class of cereal crops is represented by millets. Major and minor millets are the two categories into which they are separated. Pearl millet and sorghum are the two main millets. Finger millet, Proso millet, Foxtail millet, Kodo millet, Small millet, and Barnyard millet are the minor millets. Millets are often referred to as "Small-seated grasses" and include various types such as pearl millet, finger millet, foxtail millet, proso millet, barnyard millet, kodo millet, and tiny millet. A substantial portion of millet production is made up of pearl millet. As a result, millets are commonly consumed in multigrain form to take advantage of their combined nutritional benefits.

Millets are native to many regions around the world, but they are believed to have evolved in tropical western Africa, where the highest diversity of both wild and cultivated varieties can be found. In East Asia, millets have been cultivated for over 10,000 years and have played a significant role as staple food crops throughout human history, particularly in Asia and Africa.

Millets are an important food source in resource-limited countries in Asia and Africa, with an average yearly production of 14.2 million tons and 12.4 million tons, respectively. They rank second in calorie content after cereal grains. As illustrated in Figure 1, India is the world's largest producer of millets, contributing approximately 49l% of global output, as shown in Figure 2.

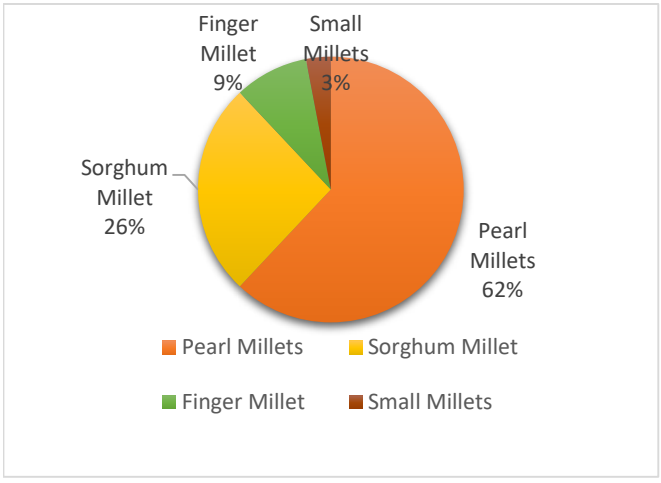

Figure 1. Production of Millet in India (2023-24) [Source: APDEA].

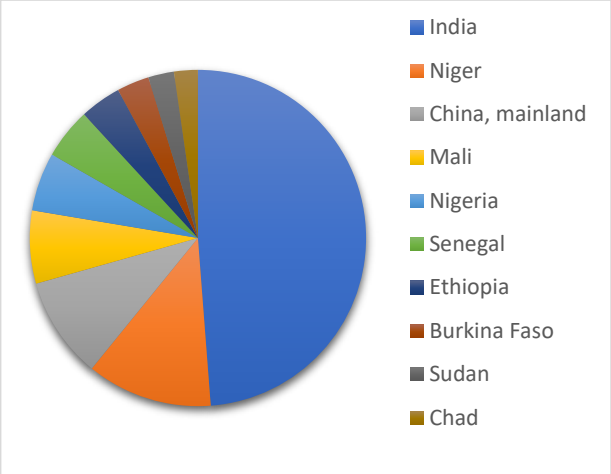

Figure 2. Global Production of Millets (Source: FAOSTAT-2023)

2.2. Nutritional Value:

Millets are an excellent source of protein, vitamins, and minerals, making them highly nutrient-dense. Approximately 80% of millet grains are used for food, while the remainder is used for animal feed and alcoholic beverages[10, 12]. Millets are recommended for the health of newborns, nursing mothers, the elderly, and individuals recovering from illness. These grains are considered gluten-free because they release sugar into the bloodstream gradually. Additionally, millets contain health-promoting flavonoids and phenolic acids, which play a crucial role in lowering blood glucose levels and preventing oxidative stress caused by free radicals. When compared to other millets, pearl millet has higher levels of iron (Fe), zinc (Zn), and lysine, ranging from 17 to 65 mg per gram of protein. Foxtail millet contains significant amounts of fat (4%) and protein (11%), with its protein composed of prolamins (39.4%), glutelins (9.9%), and albumins and globulins (13%). For these reasons, foxtail millet is recommended as an ideal diet for diabetics. Additionally, it boasts high concentrations of carotenoids, phenols, and phenolic acids, which may act as antioxidants[28]. Finger millet grains are particularly rich in minerals such as calcium, magnesium, and potassium[29]. According to Pettifor[30], adequate calcium intake is vital for maintaining healthy bones, while potassium may help delay the onset of diabetes, kidney issues, and heart problems. Barnyard millet is notable for its high concentrations of polyphenols[29] and essential amino acids such as methionine, lysine, and tryptophan[31]. According to Saleh et al.[10], barnyard millet is recognized as the best source of crude fiber (13.6%) and iron (186 mg/kg dry matter), while Proso millet boasts the highest protein content at 12.5%. Additionally, barnyard millet grains contain beneficial components like glucan and gamma-aminobutyric acid (GABA), which serve as antioxidants and help reduce blood lipid levels[32]. Given its low carbohydrate content compared to other millets, barnyard millet is recommended as an ideal grain for individuals with type II diabetes. The greatest advantage of Kodo millet is its high magnesium content (1.1g/kg dry matter). Therefore, millets are eaten as a multigrain to benefit from the combined health advantages.

Millets	Nutritional Value
Pearl Millets [*Pennisetum glaucum* (L.)]	Rich in iron (Fe), zinc (Zn), and lysine, containing between 17 to 65 mg of lysine per gram of protein, which is higher than that found in other millets. The total phenolic content in pearl millet has been reported to be 168 mg per 100 grams, with ferulic acid equivalents present in the soluble phenolic fraction. Additionally, the total flavonoid content in pearl millet is 49 mg per 100 grams, expressed in catechin equivalents within the soluble phenolic fraction[33].
Foxtail Millet [*Setaria italica* (L.)]	High amount of protein (11%) and fat (4%). The protein fractions are represented by albumins and globulins (13%), prolamins (39.4%), and glutelins (9.9%). It is thus recommended as an ideal food for diabetics. It also contains significant amounts of potential antioxidants like phenols, phenolic acids, and carotenoids[28].
Finger Millet [*Eleusine coracana* (L.)]	Rich in Fe, Zn, and lysine (17–65 mg/g of protein) compared to other millets. Total phenolic contents reported are 168 mg/100 g (pearl millet) and ferulic acid equivalents in the soluble phenolic fraction. Total flavonoid contents have been reported as 203–228 mg/100 g (finger millet), catechin equivalents in the soluble phenolic fraction[29].
Proso Millet [*Panicum miliaceum* (L.)]	Rich in Fe, Zn, and lysine (17–65 mg/g of protein) compared to other millets. Total phenolic contents reported are 168 mg/100 g (pearl millet) and ferulic acid equivalents in the soluble phenolic fraction. Total flavonoid contents have been reported as 140 mg/100 g (proso millet) catechin equivalents in the soluble phenolic fraction[10].
Barnyard Millet [*Echinochloa esculenta* (L.)]	Functional constituents, viz. g-amino γ-aminobutyric acid (GABA) and β-glucan, are used as anti-oxidants and in reducing blood lipid levels[32].
Kodo Millet [*Paspalum scrobiculatum* (L.)]	High magnesium content (1.1 g/kg dry matter)[24].

3. Nano-Fertilizer:

Fertilizers play a crucial role in the production of agricultural products, accounting for 35-40% of agricultural output. Some fertilizers have a direct impact on plant development. According to Ombedi and Saigusa[35], a significant amount of fertilizers applied in the environment is lost, with 40-70% of nitrogen (N), 80-90% of phosphorus (P), and 50-90% of potassium (K) not reaching plants. This inefficiency can lead to long-term financial losses for farmers.

To encourage the use of fertilizers, particularly urea, and to boost agricultural productivity, the government provides subsidies to reduce costs. However, unbalanced fertilization, nitrate contamination in the soil, and repeated application of fertilizers have negatively affected the natural balance of the soil. A promising solution to these issues is the development

of fertilizers based on nanotechnology, which focuses on creating more effective fertilizer products. In addition to providing stress tolerance, the nano fertilizer triples the efficiency of nutrient usage (NUE).

The gradual and targeted efficient release will also take place since these nanofertilizers contain growth promoters, nutrients, and nanoscale polymers. When weighed against the prices and needs of chemical fertilizers, nano fertilizers are more cost-effective and may be used in lower quantities.

The U.S. Environmental Protection Agency defines nanomaterials (NMs) as substances containing particles with at least one dimension between 1 and 100 nanometers (nm). Nanofertilizers (NFs) are a specific type of NM that can function as carriers for traditional chemical fertilizers, enhancing nutrient efficiency and acting as sources of macro- or micronutrients for crop plants.

Thanks to their unique mechanisms of action, NFs are generally more effective than conventional fertilizers. They improve nutrient utilization, reduce nitrogen loss, and have a minimal environmental impact.

Nanotechnology employs nanoscale or nano-structured materials that have been researched as controlled-release vectors or fertilizer carriers. This has resulted in the creation of "intelligent fertilizers," which improve nutrient uptake and lower emissions[36]. Due to their small size and significantly increased surface area, NFs can be easily absorbed by plants, and their general mechanism of uptake by plants is shown in Figure 3.

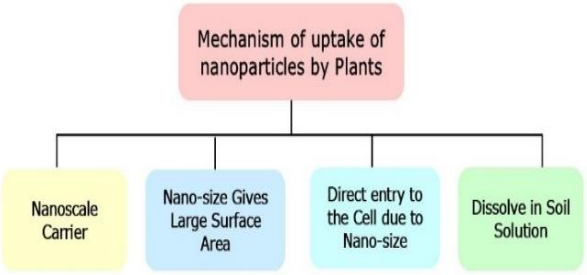

Figure 3. Generalized Mechanism of Nanoparticle Uptake

4. Agronomic Biofortification:

The agronomic approach to plant micronutrient biofortification aims to quickly and effectively address the deficiencies of essential elements in soil and plant life. This method focuses on producing a variety of vitamins and minerals. Fertilization is a key component of this technique, as it enhances the micronutrient content of crops such as legumes and grains. It's important to note that in developing countries, the agronomic biofortification strategy can be particularly beneficial[37]. To enhance micronutrient content in crops through agronomic biofortification, White and Broadley[38] recommended using phytoavailable micronutrient fertilizers, regularly correcting soil alkalinity, practicing crop rotation, and introducing beneficial soil microbes deliberately. Additionally, Graham et al.[39] discussed agricultural tools designed to improve crop nutrient levels, including crop systems, soil amendments, and fertilizers. Research indicates that micronutrient fertilization not only increases agricultural yields but also improves the nutritional quality of crops, thus addressing the related issues of human micronutrient deficiencies and health problems[30].

About one-third of the world's population consumes foods that are low in zinc[41]. Zinc is vital for the hormonal regulation of carbohydrate metabolism and serves as an essential micronutrient in various enzymes. The human body absorbs zinc primarily in the form of zinc gluconate. A deficiency of zinc in soil leads to low uptake of this mineral by plants. Two common sources of zinc are zinc sulfates ($ZnSO_4$) and zinc oxide (ZnO). ZnO nanoparticles, because of their high surface area to volume ratio and low volatility, are effectively absorbed, stored, and metabolized by plants to address zinc deficiencies[42]. Research has shown that applying ZnO nanoparticles at a rate of 5 mg per kg of soil increases the zinc concentration in sorghum (*Sorghum bicolor* (L.) Moench) and finger millet (*Eleusine coracana* (L.) Gaertn. ssp. coracana) grains by 94%. Additionally, seed priming with ZnO nanoparticles at a concentration of 5 ppm increases the zinc content of the grains by 13.96% compared to control plants[43]. Significantly, ZnO nanoparticles have not been associated with any unique toxicity or hazards at the nanoscale.

Foliar spraying of nano-chelated fertilizer in paddy fields is an effective method for enhancing rice biofortification. The application of nano chelated iron fertilizer significantly increases the nitrogen, phosphorus, and potassium content in white rice. Additionally, it promotes greater plant height, longer panicle length, increased grain weight, and higher paddy production compared to untreated controls. After using nano chelated iron fertilizer, rice crops exhibit elevated levels of protein and macronutrients. This suggests that the fertilizer enhances macronutrient absorption while reducing the reliance on chemical fertilizers. The most significant improvements in the quality and quantity of rice occurred during the nursery and booting phases when 2.5 g/L of nano chelated iron fertilizer was applied. This method proved to be cost-effective while requiring only a tiny amount of fertilizer. Iron in the soil can be present as maghemite (γ-Fe_2O_3), hematite

(α-Fe_2O_3), or magnetite (Fe_3O_4). However, due to various transformation processes in the soil, iron can become difficult for plants to absorb. Sufficient iron levels promote increased chlorophyll content, enhanced root development, and better seed germination. According to Kumar et al. (2021), priming finger millet (*E. coracana*) seeds with 100 ppm of Fe_3O_4 nanoparticles resulted in a 12.3% increase in the grain's iron content compared to treatment with $FeSO_4$.

5. Conclusion

Both plant and human health are greatly affected by deficiencies in micronutrients. One of the most effective strategies to ensure global food security is the development of nutrient-enriched crops through sustainable agricultural practices. This text outlines the most common methods of biofortification using nanomaterials. Nanotechnology-based techniques can contribute to the production of nutrient-rich foods by minimizing losses from soil leaching and volatilization. Additionally, these techniques can aid in genetic transformation processes that enhance the absorption, translocation, and accumulation of micronutrients. As a result, these methods can effectively biofortify food crops, helping to sustainably reduce human micronutrient deficiencies. When applied in appropriate quantities, micronutrient-enriched nanofertilizers pose minimal environmental risks while generally offering agronomic benefits, such as improved crop health and increased soil fertility. Agronomic biofortification can significantly enhance both yields and the nutritional quality of certain crop-micronutrient combinations, particularly those involving zinc and iron. Furthermore, nanoparticles, due to their smaller size and larger surface area, can replace conventional fertilizers. Numerous studies indicate that nanoparticles enhance the digestion and absorption of nutrients in grains, which, in turn, can help alleviate human nutritional deficiencies.

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Authors Contributions

Kushwaha P did the resources, writing the original draft of the paper and performing writing this review article. Verma R did the editing and correction of the paper. Kushwaha P and Verma R did the process of investigation and conceptualization. Singh A. K and Dixit P supervised the paper and had close supervision in the process of preparing the paper. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

Authors declare no conflict of interest.

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Bioinformatics' usefulness in relation to the Bryophytes

Khushbu Anand and Afroz Alam*
Department of Bioscience and Biotechnology, Banasthali Vidyapith (Rajasthan)
Corresponding author Email: afrozalamsafvi@gmail.com

Abstract

Bryophytes, such as mosses, liverworts, and hornworts, are vital species for studying plant evolution because they serve as a bridge between aquatic algae and terrestrial vascular plants. Their haploid-dominant life cycle, basic shape, and absence of intricate vascular systems offer special insights into the evolution of plants. By making it possible to analyze massive genomic, transcriptomic, and proteomic datasets, the development of bioinformatics has significantly advanced the study of bryophytes. This study examines the use of bioinformatics techniques and tools, with a particular emphasis on transcriptome analysis, comparative genomics, genome sequencing, and molecular clock research, to comprehend the evolution of bryophytes. Important new information about the evolution of bryophytes, the dynamics of gene families, and adaptations to terrestrial life has been made possible by these methods. We also discuss the challenges in bryophyte research and outline future directions, emphasizing the need for expanding genomic datasets, integrating multi-omics approaches, and leveraging advanced computational tools.

Keywords: Bryophytes, bioinformatics, plant evolution, genomic analysis, transcriptomics, comparative genomics, adaptation.

1. Introduction

Because of their distinct biological characteristics and evolutionary status as early land plants, bryophytes—which include mosses, liverworts, and hornworts—are becoming more and more important in the field of bioinformatics. Utilizing computational tools to examine their genomic, transcriptomic, and metabolomic data, their application in bioinformatics encompasses multiple important domains. They offer crucial details regarding the transition from aquatic algae to terrestrial plants. These tiny plants differ from vascular plants in that they have a haploid-dominant gametophytic phase, a basic shape, and no vascular supply. Their ability to maintain ancestral characteristics while displaying notable alterations that made the shift to land easier explains their evolutionary relevance. Our knowledge of bryophyte evolution has been completely transformed by bioinformatics, especially in the fields of transcriptomics, comparative genomics, and genome sequencing. By making it possible to analyze large biological datasets, these techniques have assisted scientists in understanding the molecular mechanisms behind their evolutionary history and adaptations. Species like *Physcomitrium patens* (moss) and *Marchantia polymorpha* (thalloid liverwort) are used as model systems for the study of plant evolution, development, and stress responses. Functional genomics is made simpler by their prominent haploid gametophytic phase, which allows simple genome editing through homologous recombination or CRISPR[1].

The expansion of bryophyte genome and transcriptome databases has led to the emergence of specialized bioinformatics resources. MarpolBase provides tools like BLAST and JBrowse to examine transcripts, proteins, and genomes across a variety of bryophyte species, including Arabidopsis comparisons. PEATmoss offers a gene expression atlas for bryophytes, which makes transcriptomic research easier[2,3].

2. Methodology

This paper's appraisal uses a qualitative methodology and secondary data sources, primarily academic literature. Using academic databases that are available to the public, such as NCBI, Google Scholar, PubMed, Scopus, and Web of Science, a thorough analysis of pertinent papers was carried out. In order to investigate important concepts, synthesize data, and interpret outcomes, the process comprised a methodical study, extensive reading, and in-depth analysis of the chosen sources. The main focus of this paper is a textual documentary analysis that provides a thorough assessment of the body of knowledge already available on the topic.

3.1. Bryophytes as Evolutionary Models

Using bioinformatics methods, phylogenomic time trees are generated to identify evolutionary connections and gene incongruences among bryophytes over a 500 million-year period. These studies make use of programs like Newick tools for phylogenetic tree processing and AliView for sequence alignment. In the plant kingdom, bryophytes play a vital evolutionary role by bridging the gap between vascular plants and green algae. The persistence of essential traits including a haploid-dominant life cycle, unbranched sporophytes, and adaptations for terrestrial living such resistance to desiccation4 underscores their evolutionary relevance[4].

Because of their small size, scant fossil record, and phenotypic flexibility, bryophytes provide special difficulties for conventional taxonomic and evolutionary research, despite their crucial importance in comprehending plant evolution. By enabling the sequencing and comparison of genomes, transcriptomes, and proteomes, bioinformatics has emerged as a crucial tool for overcoming these obstacles and obtaining insights that would be challenging to obtain from morphology alone[5].

3.2. Bioinformatics Tools and Techniques in Bryophyte Research

Large-scale transcriptome and genome datasets for bryophytes have been made possible by next-generation sequencing (NGS) and other high-throughput sequencing methods. The moss *Physcomitrium patens*, the liverwort Marchantia polymorpha, and a number of hornwort species are among the significant bryophyte species that have been employed as model organisms for genomic studies. Bioinformatics tools such as DESeq2, OrthoFinder, and BLAST have facilitated comparative genomics, gene family analysis, and differential expression studies.

3.2.1. *Coevolution Detection Using CoMap:*

Coevolutionary analysis (amino acid residue) were conducted in CoMap v1.5.2, which employs compensation and clustering approaches. Parameters included aligned sequences, a phylogenetic tree, a substitution model, and discrete rate distribution. Coevolving residues were assessed for statistical significance (p-value ≤ 0.05) using R, with bootstrap replicates ($n = 1000$) and false discovery rate (FDR) evaluation[6]. Ground-breaking findings about the genomic traits of bryophytes have been made possible by these methods, including the identification of significant regulatory networks, the loss of genes associated with vascular development, and a deeper understanding of adaptations to terrestrial environments. The availability of many bryophyte genomes facilitates comparative studies to understand gene family evolution, whole-genome duplications, and gene loss events, particularly when mosses are compared to liverworts and hornworts[5].

3.3. Phylogenomics and Comparative Genomics

By using large datasets of orthologous genes to construct phylogenetic trees, phylogenomics has shed light on the evolutionary connections between bryophytes and vascular plants. Bioinformatics has resolved long-standing disputes over bryophyte monophyly and their relationship to vascular plants. For example, recent analyses have altered our understanding of early plant diversification by showing that hornworts belong to a clade that also contains mosses and liverworts. Comparative genomics has provided valuable new insights into

the genetic advances and reductions that have occurred in bryophytes. For example, because bryophytes lack genes involved in the creation of lignin and other processes necessary for vascular growth, they have a simpler morphology than vascular plants. However, *Physcomitrium patens* and other bryophyte gene duplication occurrences suggest a history of genomic innovation that may have been crucial for adaption to terrestrial environments.

3.4. Key Insights from Bioinformatics in Bryophyte Evolution

Bioinformatics has uncovered several key insights into bryophyte evolution:

3.4.1. ***Genomic Innovation and Reduction:*** Early land plant gene duplication events served as the foundation for later terrestrial adaptations. Gene loss in bryophytes is indicative of a more simplified evolutionary path than the vascular plant's elaboration of characteristics.

3.4.2. ***Regulatory Networks:*** Although bryophyte-specific regulatory modules imply distinct adaptations, the conservation of transcription factors and non-coding RNAs between bryophytes and vascular plants suggests common developmental processes.

3.4.3. ***Adaptation to Terrestrial Life:*** The molecular mechanisms behind bryophytes' ability to withstand desiccation, a crucial adaptation for colonizing terrestrial ecosystems, have been clarified by transcriptome investigations.

3.4.4. ***Molecular Clock Studies:*** Bioinformatics has revised the chronology of the divergence of vascular plants and bryophytes by combining genetic data with fossil calibrations, placing this event in the Cambrian epoch (515–494 million years ago).

3.5. Metabolomics and Ecometabolomics:

Analysis of Secondary Metabolite: Bryophytes generate a variety of secondary metabolites that include cytotoxic, antifungal, and antibacterial qualities. ClassyFire and ChemOnt ontology are two examples of bioinformatics pipelines that automate compound classification. These pipelines use ultra-performance liquid chromatography coupled with mass spectrometry (UPLC/ESI-QTOF-MS). These techniques aid in determining the ecological functions of substances, such as sesquiterpenoids for pathogen defense or flavonoids for light protection[7, 8].

3.5.1. ***Data Processing Tools:*** To overcome obstacles such the absence of reference spectra for new chemicals, software such as xcms, metAlign, and MzMine analyzes huge metabolomic datasets from bryophytes are utilized in forensic bioinformatics to track down plant pieces. In educational labs, students learn techniques like as DNA isolation, PCR, gel electrophoresis, and genotyping (e.g., RAPD-PCR). They also sequence plastid introns and utilize bioinformatics to identify species. These techniques take advantage of bryophytes' capacity to adhere to surfaces and preserve DNA[9,10].

3.5.2. ***Educational Resources***: By combining bryophyte forensics with bioinformatics, open-inquiry laboratories let students learn how to identify species by analyzing sequence data, improving their molecular biology and computational analysis abilities[11].

3.6. Environmental and Ecological Studies:

Bioindicators: Because bryophytes are sensitive to changes in their surroundings, they are perfect for biomonitoring. In order to associate species composition with environmental variables, bioinformatics examines their distribution and life-form patterns across land cover types using techniques such as variance partitioning and canonical correspondence analysis[12].

3.6.1. ***eDNA Metabarcoding***: By processing sequencing data through computational pipelines, hybridization capture and PCR-based environmental DNA (eDNA) metabarcoding track the variety of bryophytes in environments such as rivers.

3.7. Phenotypic analysis and bioimaging:

Image processing: For bioinformatics applications, high-quality macroscopic and microscopic photographs of bryophytes—like those in the Scapaniaceae family—are annotated with machine-actionable metadata. In computational ecology, these datasets aid in picture segmentation and machine learning while evaluating phenotypic diversity[13].

4. Challenges and Future Directions

There are still a number of issues in bryophyte genomics despite tremendous progress. Studies of the molecular clock are challenging due to the poor fossil record of bryophytes. Furthermore, the haploid-dominant genomes of bryophytes necessitate specific computational algorithms since they differ from the diploid-dominant genomes of vascular plants. Because just a small portion of the estimated 20,000 bryophyte species have been sequenced, there is also a lack of taxon sampling[5].

Data Gaps: Comprehensive bioinformatics investigations are hampered by scant fossil records and the absence of constitutive reference spectra for bryophyte metabolites.

Future studies should focus on expanding genomic datasets to include more bryophyte species, particularly underrepresented hornworts and liverworts. Multi-omics approaches may provide a more complete understanding of evolutionary processes by integrating transcriptomics, metabolomics, and genomes. Additionally, advancements in machine learning and artificial intelligence may enhance our ability to spot subtle evolutionary patterns in massive datasets[14].

Emerging Technologies: New developments like as single-cell RNA sequencing, long-read transcriptome sequencing, and nanopore protein sequencing could improve bryophyte bioinformatics and provide more profound understanding of proteomics and gene regulation[15].

Multidisciplinary Methods: By combining ecological, metabolomic, and genomic data using bioinformatics, bryophyte adaptations and their ecological roles will be better understood, promoting conservation and biotechnology uses[16].

5. Discussion and Conclusion

Bryophytes are crucial for studying plant evolution, ecological adaptations, and bioactive compounds in bioinformatics. Databases, metabolomic pipelines, and sequence alignment tools are examples of computational resources that enable researchers to use bryophyte data for environmental monitoring, forensics, and evolutionary biology. As genomic and transcriptome datasets expand, bioinformatics will continue to uncover bryophytes' promise for both scientific and commercial applications other than *Physcomitrella patens*[17, 18, 19], and *Marchantia polymorpha*[20, 21]. Bioinformatics has revolutionized our understanding of bryophyte evolution by providing us with essential information about the genetic innovations and adaptations that enabled the transition from aquatic to terrestrial life. By using genomic and transcriptome data, bioinformatics has identified key regulatory networks, clarified adaptations like desiccation tolerance, and identified the evolutionary connections between bryophytes and other plant lineages. With the development of sequencing technologies and computational tools, bryophytes can be used as a model to better understand the deep evolutionary history of terrestrial plants.

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Authors Contributions

AA conceptualized the manuscript and decided the various aspects to be covered. KA collected and compiled the data and wrote the first draft of the manuscript. Both the authors have finalized the manuscript.

Conflicts of Interest

Authors declare no conflict of interest.

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Agrobacterium rhizogenes mediated transformation, initiation and multiplication of hairy roots in *Spilanthes acmella* Murr. (Asteraceae)

Hajera Sana*[1] and A. Sabitha Rani[2]
[1]Department of Botany, Veeranari Chakali Ilamma Women's University, Hyderabad- 500095, Telangana State, India
[2]Department of Botany, University College of Sciences, Osmania University, Hyderabad- 500007, Telangana State, India
*Corresponding author Email: hajsanaera@gmail.com

Abstract

Spilanthes acmella is an important medicinal plant that belongs to the family Asteraceae. Traditionally, this plant has been used for years to cure toothaches, stammering, stomatitis and many other ailments. This plant is an important source of various medicinally important secondary metabolites like phenolics, coumarin, spilanthol, scopoletin, triterpenoids etc. The present study was carried out with the objective to transform this plant with Agrobacterium rhizogenes to initiate the hairy roots formation. This study demonstrated the transformation, initiation and multiplication of hairy roots from nodal segments and leaf explants taken from field grown and in vitro developed *S. acmella* plants. The hairy roots produced from different explants were white, slender, showed negative geotropism and lateral branching. PCR analysis of hairy root was performed that confirmed the bacterial transformation. Among the field grown and in vitro grown plants, the explants from the in vitro grown plants gave a high percentage of root induction. Nodal segments from in vitro grown plant gave 90% hairy root induction and leaf segments from in vitro grown plants with petiole gave the highest rate of root induction which was 92%. Nodal segments from field grown plants gave 85% and the leaves from field grown plants gave 80% of hairy root induction. This study offers great potential to establish the protocol for hairy root induction which can be used as an alternative source for the continuous production of this plant's important secondary metabolites and active biocompounds.

Keywords: *Spilanthes acmella*, *Agrobacterium rhizogenes*, hairy roots, transformation, PCR.

1. Introduction

The toothache plant, *Spilanthes acmella* Murr., is a significant medicinal plant that is a member of the Asteraceae family. It can be found all over the world in tropical and subtropical areas. According to Nelofar et al.[1], it has been shown to possess a number of biological properties, including antipyretic, antidiuretic, anti-inflammatory, antioxidant, immunomodulatory, hepatoprotective, anticancer, and antitoothache1. Important secondary metabolites such as coumarin, phenolics, triterpenoids, myrecene, α and β amyrin, spilanthol, and scopoletin have been discovered to be produced by the plant. Spilanthol, an alkamide found in the plant's roots and all of its aerial parts, is the active chemical component.

Hairy roots, produced by the genetic transformation by *Agrobacterium rhizogenes* are gaining importance for production of secondary metabolites *in vitro*.

A. rhizogenes, which carries the root-inducing (Ri) plasmid, infects injured plant tissues to produce hairy root cultures. Like the roots of native plants, they have a similar ability for biosynthesis and can produce secondary metabolites. The benefits of hairy roots culture (HRCs) include significant biomass output, long-term preservation, genetic and biochemical stability, and rapid growth rates independent of phytohormones[2]. More significantly, compared to undifferentiated callus and cell suspension cultures, hairy root cultures frequently collect phytochemicals at higher amounts[3]. Therefore, *A. rhizogenes* may change the targeted medicinal plant into hairy root lines that can produce secondary metabolites and bioactive chemicals with pharmaceutical uses.

2. Materials and Methods

2.1. Establishment of *S. acmella* plants

The seeds of *S. acmella* were procured from Medicinal and Aromatic Plants Research Station, Professor Jayashankar Telangana Agricultural University, Rajendranagar, Hyderabad, sown in soil taken in pots and the plants were grown and maintained (Fig.1). The plant material required for the hairy root induction were collected from these plants.

Figure 1. *Spilanthes acmella*

2.2. Transformation and Initiation of Hairy Roots by *Agrobacterium rhizogenes*

The Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, provided the *Agrobacterium rhizogenes* (MTCC 532), which was then cultivated on solid nutrition agar medium to activate it. A loop full of the bacteria was taken and cultivated on liquid nutrient broth medium in order to induce hairy roots. In an orbital shaker with continuous stirring, *A. rhizogenes* was injected in nutrient broth culture media and allowed to stand at 250 rpm for 16 hours at 25^{0}C. After that, the bacterial suspension was put into a sterile centrifuge tube and spun for ten minutes at 5000 rpm. After that, the residue was suspended in liquid MS media that had been enhanced with 3% sucrose.

MS medium[4] supplemented with 0.8% agar, 3.0% (w/v) sucrose and pH of 5.7 was maintained and used as growth medium for induction of hairy roots from explants.

The concentration of *A. rhizogenes* cultures was standardized for transformation and induction of hairy roots. Different bacterial concentrations having different optical densities (0.2-0.8 OD) at 600 nm were tested for their transformation percentage. The concentration with optical density of 0.6 were found to give the highest transformation percentage and hence only these concentrations were used for hairy root induction.

Hairy roots were induced from the *S. acmella* leaf and nodal explants by transforming them with *A. rhizogenes*. Overnight grown cultures of *A. rhizogenes* were taken and they were seen to have 0.6 optical density (O.D) at 600 nm. The young leaves and nodal segments were collected from in vitro and field grown plants of *S. acmella*. Two types of leaf explants were taken, some bearing petiole and some without petiole. They were cut into small pieces and pricked with a sterile needle and soaked in the bacterial suspension for half an hour. The explants were removed and blotted dry on a sterile blotting paper. Then they were placed onto the co-cultivation medium (MS-medium) in dark for three different time intervals i.e. 24, 48 and 72 hours. After co-cultivation, the leaves were inoculated onto hormone free MS medium containing antibiotic cefotaxime (250 mg/l) to check the non-transformed bacterial growth. The cultures were maintained at 25^{0}C with 16/8 h light and dark period.

For each treatment, 20 leaf and nodal explants were inoculated with *A. rhizogenes* and the experiment was performed in triplicates. Some explants were not treated with the bacteria and maintained as control. The results were expressed in percentage transformation frequency.

2.3 PCR analysis of Hairy roots

The production of hairy roots and the genetic alteration of *A. rhizogenes* were verified by PCR analysis of hairy roots. PCR amplification was

performed using the DNA from hairy roots, plasmid DNA from the *A. rhizogenes* strain (positive control), and DNA from untransformed leaves (negative control). The alkaline lysis method[5] was used to recover plasmid DNA, while the CTAB method[6] was used to extract DNA from hairy roots and non-transformed leaves. Primers specific to rol B and rol C were used in the polymerase chain reaction. The suppliers of the rol B and rol C primers were Xcelris Genomics in Ahmedabad, India. The rol B gene's 3' primer sequence was TTAGGCTT CTTTCTTCAGGTTTACTGCAGC, while its 5' primer sequence was TGGATCCCAAATTGCTATT CCTTCCACGA. In DNA amplification, this amplified the 780 base pair (bp) DNA fragment. The rol C gene's 3' primer sequence was GATTGAAAACTT GCAC, while its 5' primer sequence was ATGGCTGAAGACGACCTGTT TTAGCC. In DNA amplification, this amplified the 540 base pair (bp) DNA fragment. As positive and negative controls, respectively, 50 ng of plasmid DNA from *A. rhizogenes* and DNA from untransformed leaf tissues were used. Hairy root DNA was used as a therapy. Table 1 lists the primers used in this experiment.

Table 1: Primer sequence used for PCR detection of transgene and length of PCR amplified fragment

Gene	DNA Sequence	Length of PCR amplified Fragment (bp)
Rol B	*Forward* TGGATCCCAAATTGCTA TTCCTTCCACGA *Reverse* TTAGGCTTCTTTCTTCA GGTTTACTGCAGC	780
Rol C	*Forward* ATGGCTGAAGACGACC TGTT *Reverse* TTAGCCGATTGAAAACT T GCAC	590

In order to purify DNA, RNA was extracted by treating the sample with DNase-free RNase, which was purchased from Pure-gene in the United States. RNase and other proteins were eliminated by treating them with chloroform:isoamyl alcohol (24:1). To verify the quality and amount of isolated genomic DNA, a Nanodrop spectrophotometer was used. The measured genomic DNA was diluted with TE buffer (10 mM Tris HCl, 1 mM EDTA) to a concentration of about 20 ng/ul, which is suitable for PCR use straight away. The PCR amplification was carried out in a final reaction volume of 30 µL with 1X PCR buffer (Bangalore Genei), 1.5 mM $MgCl_2$, 1 mM each of the four dNTPs, 1.25 U of Taq polymerase (Bangalore Genei), and 0.5 mM each of 5' and 3' primers with 3 µl of the total DNA from transformed roots in order to verify the presence of the rol B and rol C genes. Following a 3-minute initial denaturation at 94^0C, 35 cycles of PCR were conducted at 94^0C for 30 seconds, 550C for 30 seconds, and 72^0C for 1 minute, with a final extension at 72^0C for 7 minutes.

The PCR products were put onto a 1.2% Agarose gel (Himedia, molecular grade) after amplification. The gel was made with 1X TBE buffer that contained 0.5 µg/ml of ethidium bromide. The amplified products underwent 3-3.5 hours of cooling electrophoresis at 100 V. Following separation, the gel was examined using a UV transilluminator and captured on camera.

2.4. Multiplication of hairy roots

The hairy roots from various explants were placed in ½ MS liquid and solid medium that was free of hormones and antibiotics. Under constant darkness, they were kept in flasks on an orbital shaker at 25^0C and 100 rpm. Hairy roots were multiplied by sub culturing in fresh medium every 15 days. The fresh and dry weights of hairy roots were noted for different intervals of time i.e. 7, 15 and 30 days.

3. Results

3.1 Induction of Hairy roots

The hairy roots produced from different explants were white, slender (Plate 1).They showed negative geotropism and lateral branching (Plate 2). All the nodal segments induced hairy roots within 14-16 days of co-cultivation with the bacteria. For nodal segments from field grown plants, the best co-cultivation time was observed to be 72 hours which gave 85% of hairy root induction. The co-cultivation time of 24 and 48 hours gave 40 % and 70% of hairy root induction respectively (Table 2). For nodal segments from in vitro grown plants, the best co-

cultivation time was observed to be 72 hours which gave 90% of hairy root induction. The co-cultivation time of 24 and 48 hours gave 45 % and 68% of hairy root induction respectively. Of the two explants, nodal segments from the in vitro grown plants showed good response (90%) than the field grown plant (85%).

Table2: Evaluation of nodal segments of *S.acmella* for hairy root induction on MS media with 250mg/l cefotaxime

Explants	Co-cultivation time	Explants inoculated	No. of explants responded	Hairy root induction (%) Mean ± SE
Nodal segments from field grown plants	24	20	8	40±0.63
	48	20	14	70±0.52
	72	20	17	85±0.70
Nodal segments from *in vitro* grown plant	24	20	9	45±0.24
	48	20	13	68±0.43
	72	20	18	90±0.75

Table 3: Hairy root induction from leaf explants of *S. acmella* on MS media with 250mg/l cefotaxime

Explants	Co-cultivation time	Explants Inoculated	Explants Responded	Hairy root Induction (%) Mean ± SE
Leaf segments from field grown plants with petiole	24	20	10	50±0.27
	48	20	16	80±0.72
	72	20	7	35±0.63
Leaf segments from *in vitro* grown plant with petiole	24	20	12	60±0.56
	48	20	18	92±0.45
	72	20	11	55±0.66
Leaf segments from field grown plants without petiole	24	20	0	0
	48	20	0	0
	72	20	0	0
Leaf segments from *in vitro* grown plants without petiole	24	20	0	0
	48	20	0	0
	72	20	0	0

Leaf explants with and without petiole region from field grown and in vitro raised plants were tested for hairy root induction. Hairy roots were produced within 12-14 days from leaves containing petiolar region from field grown plants and in vitro grown plants.

For the leaf segments from field plants with petiole, the best co-cultivation time was observed to be 48 hours which gave 80% of hairy root induction. The co-cultivation time of 24 hours gave 50 % of hairy root induction whereas the percentage of hairy root induction reduced to 35% on increasing the co-cultivation time to 72 hours. For the leaf segments from in vitro plants with petiole, the best co-cultivation time was observed to be 48 hours which gave 92% of hairy root induction. The co-cultivation time of 24 hours gave 60 % of hairy root induction whereas the percentage of hairy root induction reduced to 55% on increasing the co-cultivation time to 72 hours (Table 3).The leaf explants without petiole region collected from field grown and also *in vitro* regenerated plants did not show any hairy root induction in any of co-cultivation periods i.e., 28, 48

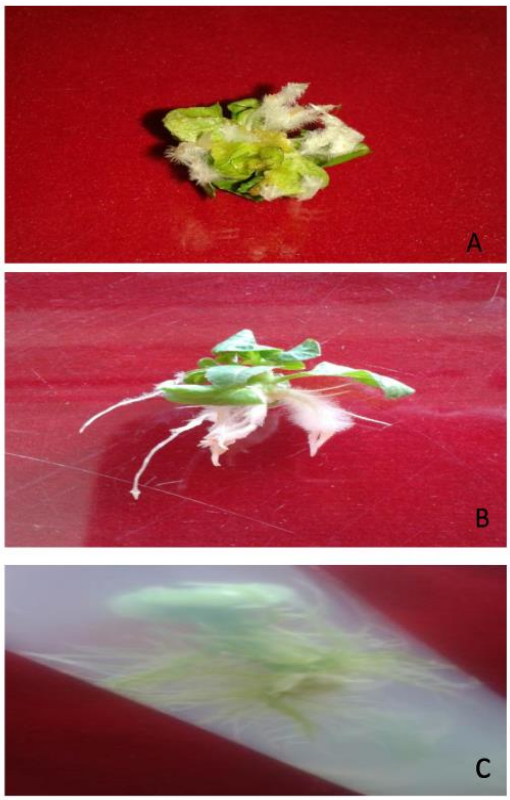


Plate 1: Induction of Hairy Roots from different explants of *Spilanthes acmella* by genetic transformation with *Agrobacterium rhizogenes* (A,B: Leaf explants; C: Nodal segment)

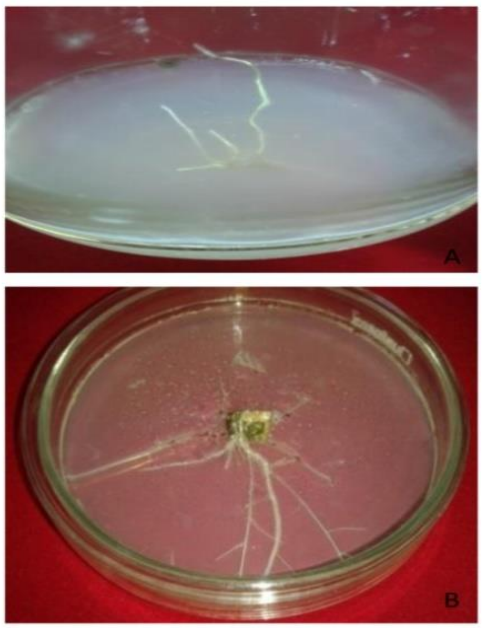


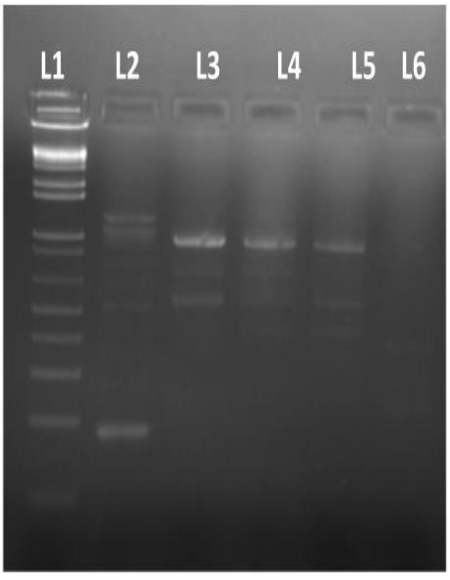
Plate 2: Hairy roots induced from Leaf explants of *S.acmella* showing negative geotropoism and lateral branching. (A: Hairy roots showing negative geotropism; B: Hairy roots showing lateral branching)

and 72 hours. This one of the important observation noticed in the present study

Among the different explants evaluated for hairy root induction, leaf explants (with petiolar region) collected from in vitro grown plants induced high percentage of hairy roots (92 %) with 48 h co-cultivation period. This was followed by nodal explants of in vitro grown plants with 90% hairy root induction at 72 h of co-cultivation.

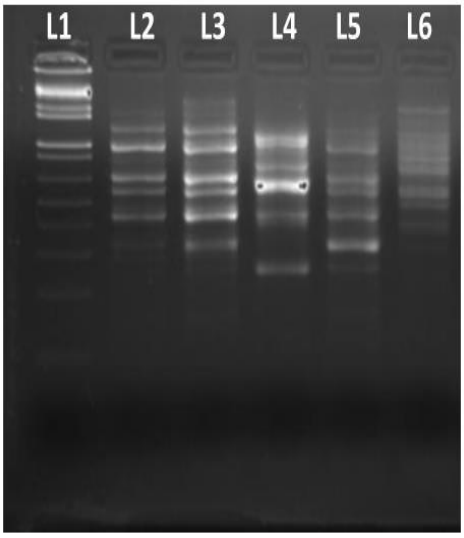
3.2. PCR analysis of Hairy Roots

The transformation event was indicated by PCR analysis of the transformed hairy roots, which revealed amplification of DNA at 540 bp and 780 bp in the hairy root samples and in the positive control (plasmid). However, there was no amplification in the negative control, which consisted of unaltered leaf tissues. This demonstrated that the integration of the rol C and rol B genes of *Agrobacterium rhizogenes* led to the development of hairy roots. As a result of the integration, the altered leaf explants' DNA was amplified at 540 bp and 780 bp, respectively (Fig. 2 and Fig. 3). Hence, the PCR analysis has confirmed that the hairy roots were produced from the leaf explants due to the genetic transformation with *A. rhizogenes*.



L1: ladder
L2: positive control (bacterial DNA)
L3: hairy root (nodal region)
L4: hairy root (leaf segment)
L5: hairy root (*in vitro* leaf)
L6: negative control (non-transformed leaf)

Fig 2: PCR Analysis of Hairy roots to confirm the genetic transformation with *A.rhizogenes* with rol B primers at 780 bp.



L1: 1kb ladder
L2: hairy root (nodal region)
L3: hairy root (leaf segment)
L4: positive control (bacterial DNA)
L5: hairy root (*in vitro* leaf)
L6: negative control (non-transformed leaves)

Fig 3: PCR Analysis of Hairy roots to confirm the genetic transformation of *A.rhizogenes* with rol C primers at 540 bp

3.3. Growth and Multiplication of hairy roots

To multiply the hairy roots, induced roots were cultured on basal media without any hormonal concentration. To observe the growth, the hairy roots induced from leaf explants were subcultured onto MS and ½ MS solid and MS and ½ MS liquid medium (Plate 3). They grew well in all the media but better growth was observed on MS liquid medium compared to MS solid medium. Hence MS and ½ MS liquid medium was selected for their multiplication.

The hairy roots were grown on MS and ½ MS solid and liquid media without any plant growth regulators. The roots were harvested and fresh and dry weights were noted at different time intervals of culture (7, 15 and 30 days). There was an increase in the fresh and dry weights of hairy roots with an increase in the number of days of incubation (Table 4).

For the hairy roots cultured on MS medium, the fresh and dry weights were 0.54 g and 0.02 g after 7 days of culture. The fresh and dry weights increased to 2.85 g and 0.25 g after 15 days of culture. A maximum of 5.04 g and 0.80 g fresh and dry weights respectively was obtained after 30 days of culture.

For the hairy roots cultured on ½ MS medium, the fresh and dry weights were 0.75 g and 0.13 g after 7 days of culture. The fresh and dry weights increased to 3.41 g and 0.30 g after 15 days of culture. A maximum of 6.05 g and 0.99 g fresh and dry weights respectively was obtained when roots were cultured on ½ MS medium after 30 days of culture.

Of the two different media, more growth of the hairy roots was observed in MS half strength liquid medium without any hormonal supplementation compared to full strength MS basal liquid media.

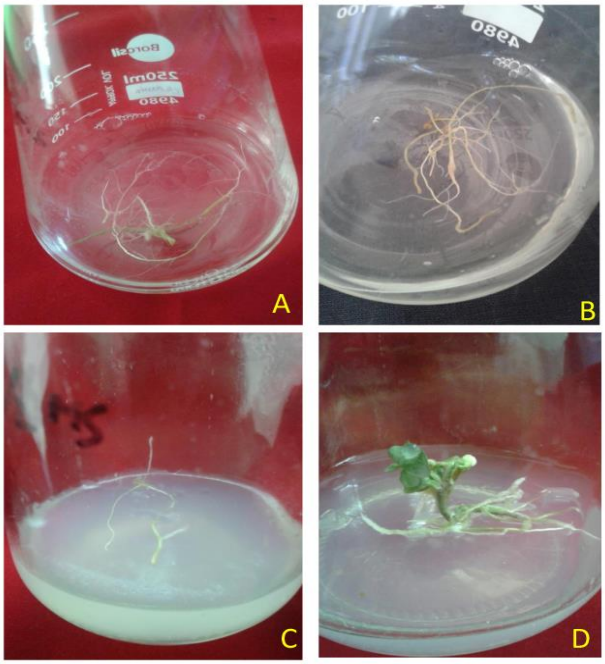


Plate 3: Multiplication of hairy roots from Leaf explants of S. *acmella.* A: MS liquid medium ; B: ½ MS liquid medium; C: MS solid medium; D: ½ MS solid medium

4. Discussion

Hairy root cultures have the potential to produce large amounts of useful secondary metabolites that are used as tastes, colors, and medications. Because of their rapid development in growth hormone-free culture media and genetic stability, genetically modified hairy roots produced by infecting plants with *Agrobacterium rhizogenes* are an excellent source for the synthesis of bioactive compounds[7]. The genetic stability of transformed root cultures is explained by the stable integration of the plasmid into the host plant genome[8].

The study of the genus *Spilanthes*' production of hairy roots is still in its early stages. *A. rhizogenes* strains MTCC 2364 and MTCC 5329 have been reported to produce hairy roots in *Spilanthes paniculata* by infecting the cotyledons and hypocotyl segments[9].

Agrobacterium rhizogenes strain MTCC 532 was used in this investigation to infect *S. acmella* nodal segments and leaf explants in vitro in order to create cultures of altered roots. Compared to other strains 10–13, strain 532 is also successful in inducing hairy roots in cultures of *Plumbago rosea*[10], *Rubia tinctorum*[11], *Arachis hypogaea*[12], and *Withania somnifera*[13].

This work is the first to document the formation of hairy roots from *S. acmella* leaf and nodal segments. Likewise, prior research has documented the induction of hairy roots from nodal segments in plants such as *Arnebia hispidissima*[14], *Berberis aristata*[15], and *Withania somnifera*[16]. According to this study, S. acmella's production of hairy roots is influenced by the kind of explants and the length of co-cultivation. This study is in line with the *Berberis aristata*[15] hairy root induction investigation.

5. Conclusion

This investigation is helpful in establishing *S. acmella*'s hairy roots. In order to produce hairy roots that may be used economically to produce significant secondary metabolites of this plant, these can be multiplied in bioreactors on a big scale. To improve the concentration and secretion of high-value metabolites like spilanthol, hairy roots would be the ideal candidate for metabolic engineering of the secondary metabolite pathways.

6. Authors Contribution

Hajera Sana is responsible for conducting the research work and drafting the manuscript. Prof. A. Sabitha Rani, as the supervisor, provided guidance throughout the study, reviewed the results, and contributed to the finalization of the manuscript.

7. Acknowledgements

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8. Data Availability

Available upon written request from the corresponding author

9. Conflicts of interest

Authors declare no conflicts of interest.

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