INCREDIBLE RESEARCH WITH MURASHIGE AND SKOOG MEDIUM (MS) IN PLANT TISSUE CULTURE ON *SELAGINELLA BRYOPTERIS* (SANJEEVANI BOOTI)

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ABSTRACT
Since ancient times, medicinal plants have been integral in treating a variety of human health issues, representing a valuable natural resource that offers crucial medical support to diverse populations. They play a dual role as essential therapeutic remedies and fundamental ingredients for traditional and contemporary medicine production. *Selaginella bryopteris*, known as "sanjeevani", is a lithophytic plant indigenous to India. It holds medicinal significance in Indian traditional medicine and is among the plants believed to be the potential sanjeevani or "sanjeevani booti". Different components of medicinal plants, such as seeds, flowers, roots, leaves, fruits, peels, and whole plants themselves, are utilized for their medicinal benefits. These plants contain a variety of metabolites including carbohydrates, tannins, flavonoids, alkaloids, terpenoids, and steroids, known for their therapeutic properties in treating a wide range of diseases. Recent, advancements in modern techniques have led to the development of specialized protocols for large-scale production of various secondary plant metabolites. Plant tissue culture, in particular, has emerged as a crucial tool contributing significantly to the production of specific secondary metabolites on a large-scale by using whole plants or using specific tissues of these plants in laboratory conditions. Various treatments can induce shoot and leaf development, with the most effective being the application of 1.5 mg/L BAP. In vitro-raised *Selaginella bryopteris* were planted in pots and grown for approximately 2-3 months in polyhouse conditions for further study. The explants exhibited proliferation within 5-8 days, with shoot regeneration observed by 15- 20 days. Shoots measuring approximately 8.5 cm and containing 9 nodes were developed within 15-20 days. The outcome of this study revealed that MS medium containing 1.5 mg/L BAP produced 8.5±1.01 shoot length, demonstrating a closely comparable outcome.

Keywords: Medicinal plants, Flavonoids, Alkaloids, Indigenous, *Selaginella bryopteris*, etc.

1. INTRODUCTION

Selaginella bryopteris, also known as Sanjeevni booti and belonging to the family *Selaginaceae*, is a lithophytic fern recognized for its exceptional capacity for regeneration. (Ganeshaiah et al., 2009). In India, Sanjeevni is a prominent component in indigenous pills aimed at addressing conditions such as spermatorrhoea, venereal diseases, constipation, colitis, indigestion, and urinary issues (acting as a diuretic).

Additionally, it is utilized for treating unconscious patients and reducing body temperature in individuals suffering from fever. (Singh et al; 2012 and Shweta et al; 2013). Certain species of *Selaginella*, such as *S. tamariscina* (Wang et al., 2010; Xu et al., 2018), *S. lepidophylla* (Pampurova et al., 2014; Rafsanjani et al., 2015; Yobi et al., 2013), *S. bryopteris* (Deeba et al., 2016), *S. arizonica*, *S. eremophila*, and *S. rupincola* from the southwestern deserts of North America (Yu et al., 2017a), are known as resurrection plants. These plants exhibit remarkable tolerance to desiccation, enabling them to survive near-complete dehydration (<10% relative water content) during extended drought periods and resume normal growth upon rehydration (VanBuren et al., 2018).

The phenomenon where dry and seemingly "dead" plants revive upon rehydration is a source of fascination for both plant biologists and the general public (Xiao et al., 2015), making resurrection plants a unique category of ornamental plants. Notably, the nuclear genomes of Selaginella are among the smallest in green plants (Baniaga et al., 2016; Little et al., 2007; Obermayer et al., 2002). As a result, resurrection species of Selaginella present excellent opportunities for investigating the mechanisms of desiccation tolerance using genomic-based approaches (VanBuren et al., 2018).

S. *bryopteris* is highly valued for its medicinal properties, but a systematic propagation method has yet to be established. Currently, most *S. bryopteris* is harvested indiscriminately from its natural habitats, raising concerns about habitat disruption and potential population decline. Conventional leaf cutting, a common propagation technique, often yields

insufficient quantities due to low efficiency and susceptibility to environmental fluctuations. Therefore, ensuring a stable supply to meet growing demand is crucial, necessitating the development of effective propagation methods.
In vitro culture enables consistent year-round production of uniform plants under controlled conditions, facilitating rapid propagation (Fernández et al,1993 and Park et al., 2020). Moreover, it supports species conservation and the propagation of plants that are challenging to reproduce naturally (Barnicoat et al., 2011). Despite these advantages, the application of in vitro culture for reproducing *S. bryopteris* has been limited, highlighting the need for further research in this area.

Our study involved *in vitro* propagation of new whole plants by using the leaf and shoot of *S. bryopteris*. By modifying the several components that make up the medium, we have attempted to find the ideal circumstances for the proliferation.

2. MATERIALS AND METHODS

2.1 Plant material

S. bryopteris plants were collected from Chamoli, Uttarakhand. The plants were grown in plastic pots filled with humus soil sourced from their native habitat and watered with tap water every 5 days. They were cultivated in a polyhouse under natural light conditions for 2-3 months at 25 ± 5 °C.

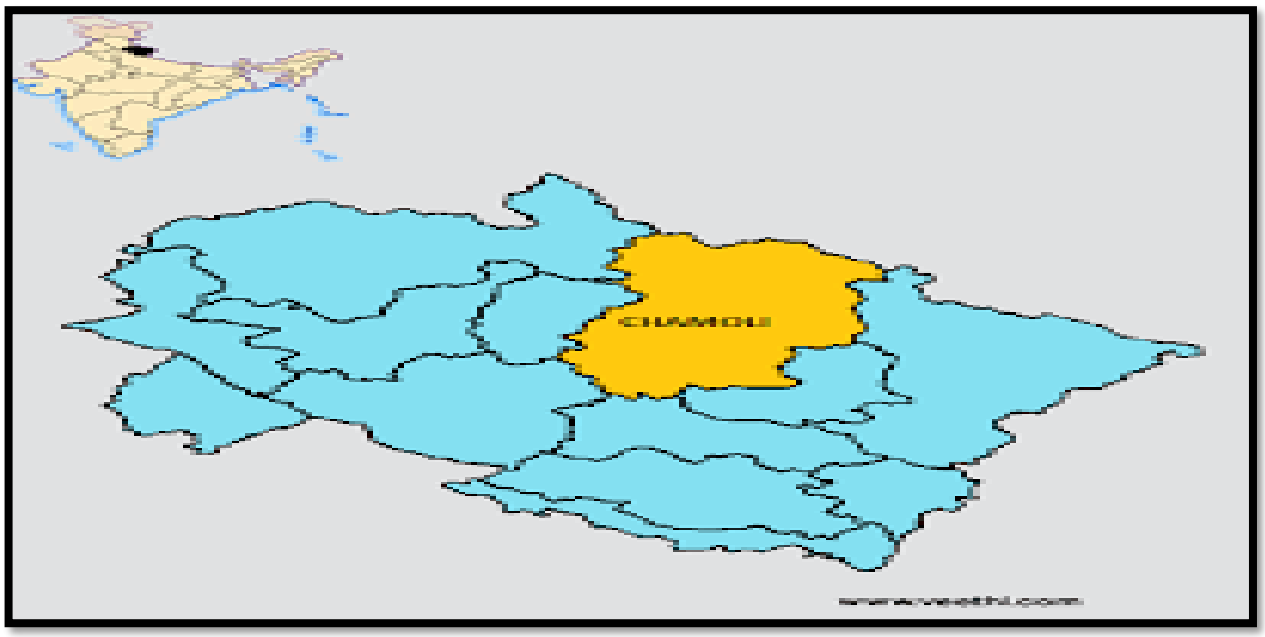

Fig. 1: Figure showing the map of the plant material collection site.
(**Source**:https://www.google.com/search?q=chamoli+map&rlz=1C1VSNG_enIN701IN703&oq=chamoli+map&aqs=chrome..69i57j0i512j0i22i30j0i10i15i22i30j0i22i30l4j0i390i512i650l2.5263j0j15&sourceid=chrome&ie=UTF8#imgrc=Yg_FqbRfH4QNfM&imgdii=x5LcHRNmRyVOzM)

2.2 Identification of collected sample

The collected plant sample was identified from the Himalayan Forest Research Institute for further study. The authenticated plant samples were used for *in vitro* micropropagation**.**

2.3 Initiation of primary shoots and propagation of adventitious shoots

Primary frond tips (0.8–1.2 cm in length) were used as explants. They underwent a cleaning process with running tap water for 2 hours, followed by surface sterilization for 15 minutes using a 0.1% (w/v) HgCl2 solution. Afterwards, the frond tips were rinsed five times with sterile distilled water. Subsequently, the surface-sterilized tips were inoculated onto 1/2 MS medium (Murashige and Skoog, 1962) supplemented with cytokinin, 3% (w/v) sucrose, and 0.7% (w/v) plant agar, and adjusted to pH 5.8 before autoclaving. A PGR-free 1/2 MS medium was used as a control to assess the impact of cytokinin on the induction of original shoots, with varying concentrations of BAP (1.0, 1.5, or 2.0 $mg \cdot L^{-1}$). Cultures were incubated in darkness at 25 ± 2 °C. After 15-20 days, the rate of original shoot induction was recorded. Original shoots referred to those originating from the apical and lateral bud primordia. A successful induction was confirmed when the explant produced at least one original shoot.

Fig 2: Showing the methodology of plant tissue culture of *S. bryopteris*

RESULTS AND DISCUSSION

In the present investigation**,** we successfully cultured *S. bryopteris* shoot tips *in vitro*, leading to the mass propagation of sporophytes under carefully optimized conditions for growth and proliferation. The selection of nutrient medium plays a crucial role in the effective cultivation of plants (Gamborg et al., 1976), influencing plant growth and propagation through its diverse components (Morini et al., 2000, Fernández et al., 2003, Jung et al., 2006 and Shin et al., 2009). MS medium, specifically designed for fern culture, incorporates varying concentrations of components essential for optimal growth (Fernández et al., 2003 and Rybczyński et al., 2010). We found that Inter-nodal segments cultured in an MS-based medium exhibited superior regeneration responses. In this study, it was found concentration of BAP proved most effective in stimulating shoot regeneration and hence subsequently utilized in additional media assessments. All treatments can induce shoot and leaf, But the most effective treatment we observed was the concentration of 1.5 mg/L BAP (Table 1 and Fig 3).

Table 1: Effect of Culture Media on Shoot Regeneration and Production of Multiple Shoots from Nodal Explants of *Selaginella bryopteris*.

Medium Code	Media Content	No. of Shoots	Shoot Length (cm)
MS	MS + 0.0 mg/L (control)	2	5.6
SM1	MS + 1.0 mg/L (BAP)	8	7.1
SM2	MS + 1.5 mg/L (BAP)	9	8.5
SM3	MS + 2.0 mg/L (BAP)	5	7.7

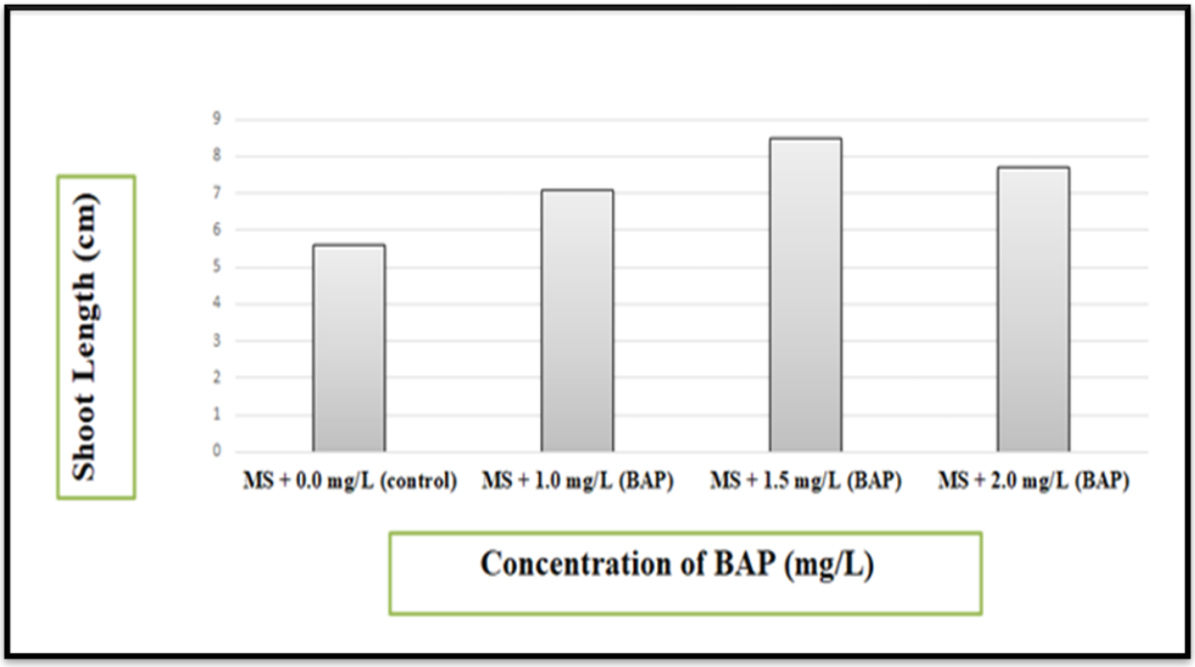

Fig 3: Effect of different concentration of BAP on shoots length formed from the *S. bryopteris*.

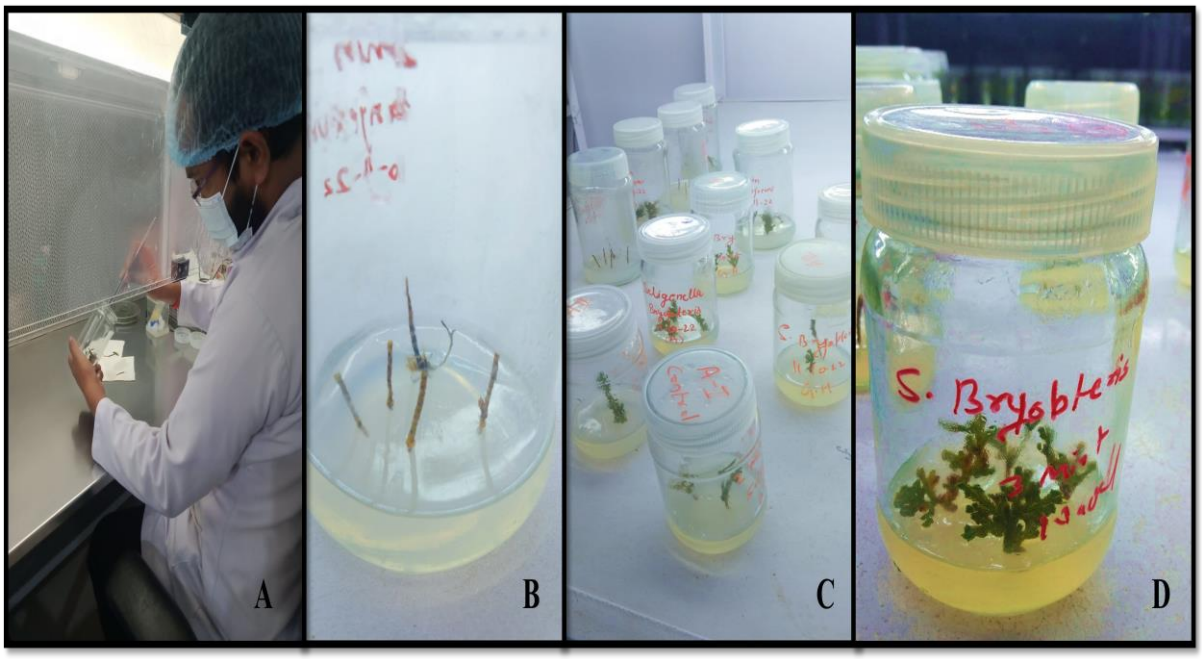

Fig 4: Showing the *in vitro* micro propagation of S. *bryopteris*. **A)** Transferred shoot on Media **B)** Initiation of Shoot induction **C)** Shoot Induction on Different BAP Concentration **D)** Showing the Proliferation of induced shoot

CONCLUSION

In the present investigation, it was found that the optimal conditions for shoot regeneration and multiple shoot production were observed with MS medium supplemented with 1.5 mg/L BAP, resulting in the highest response. Following this, MS medium containing 1.5 mg/L BAP produced 8.5±1.01 shoots, demonstrating a closely comparable outcome. The explants exhibited proliferation within 5-8 days, with shoot regeneration observed by 15- 20 days. Shoots measuring approximately 8.5cm and containing 9 nodes were developed. The regenerated plants were transferred to a greenhouse for acclimatization, affirming the practical viability of the developed protocol for ongoing research and large-scale propagation efforts.

DATA AVAILABILITY

No data were used to support this study.

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AUTHOR'S CONTRIBUTION
Ashish Jaiswal - Writing original draft, Experimental work, Data curation, writing, review and editing. **Jyoti Kumari-** Data curation, Writing, review and editing. **Shikha Rangra Chandel -** Conceptualization, formal analysis editing and reviewing.

CONFLICTS OF INTEREST
The authors declare that they have no conflicts of interest.

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