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Simple Semi-Hydroponic System for Single Leaf Propagation of *Baliospermum montanum* L. - An Important Medicinal Plant

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ABSTRACT: In natural medicinal system, the usage of herbs plays a vital role in curing various ailments. *Baliospermum montanum* L., a regionally vulnerable important herb needs conservation efforts. Studies have been conducted on the propagation through stems, seeds, and micropropagation. The availability of enough plants becomes a major criterion for raising medicinal plants on a large scale. This study aimed to investigate the role of different root-inducing substances and their rooting potential in single-leaf cuttings. In the first experiment, different aged leaves (old, mature, and young) were tried, and the results indicated that the mature leaves had a positive impact on all plant growth regulator (PGRs) cuttings, especially the auxins that were tested. No response was observed for other compounds such as honey, sugar, or undiluted tender coconut water (TCW). Leaf health scores were recorded based on changes in leaf color. The second experiment targeted varying levels of PGRs on the rooting parameters of the mature leaves. In terms of root numbers, IAA (500 ppm) produces 12.5 roots per leaf cutting followed by 11.1 in IAA (1,000 ppm) and 11.1 in IAA (200 ppm). It is superior to IBA and has a minimal response in the NAA. The root length was highest in IBA (1,000 ppm) immersed leaves at 13.6 cm followed by 12.4 cm and 9.8 cm in IAA 500 ppm and IAA 200 ppm respectively. Rooted leaves were planted in wet soil for further root and shoot growth. A reasonable rooting rate in IAA alone was observed after 7 weeks in the soil. So, we minimized the starting material through this leaf propagation procedure.

Keywords: *Baliospermum montanum*, advantageous root, single-leaf cutting, water, PGRs, auxin.

INTRODUCTION

The earliest known type of medical treatment was herbal medicine (De Smet, 1997). In the majority of developing nations, 60–85% of the population depends on traditional medicine for their well-being (Sofowora, 1996). Traditional medicine is widely used in Asian countries, such as China, India, Japan, Pakistan, Sri Lanka, Thailand, and Korea (Park *et al*., 2012). Owing to their high compatibility with the human body and minimal risk of adverse effects, herbal medicines continue to be the primary source of healthcare for 75-80% of the global population (Kamboj, 2000). The Euphorbiaceae family, commonly called the *Spurge family*, is the fifth-largest family of flowering plants (Gillespie and Armbruster 1997). It is named after Mauritania's "Euphorbus" monarch and is made up of 317 genera, 49 tribes, five subfamilies, and 7,500 species, each of which has a unique set of therapeutic characteristics (Webster, 1994; Chudasama *et al*., 2018). The English names of *Baliospermum montanum* include red physic nuts, wild castors, and wild crotons.

In India, '*Danti'* is a common name for *B. montanum* (*B. solanifolium*). When herbaceous branches grow from the roots, the plant can reach a height of 10–8 m. Simple, toothed, and undulating leaves were observed. Lower leaves can be huge and occasionally 3–30 cm in length 1.5-15 cm wide, and the upper leaves are smaller.

Traditional Thai remedies use *B. montanum* roots as a component in the treatment of several ailments, including cancer, itch-causing dermatitis, and muscular and joint inflammation (Pipatrattanaseree *et al.*, 2019). The root component is used to treat piles, calculus, general anasarca, helminthic infections, scabies, dermic diseases, and suppurative ulcers. Asthma was treated with leaves, whereas snakebites were treated with seeds (Radcliffe-Smith, 2001). The plant has been reported to have hepatoprotective activity (Kumar and Mishra 2009; 2012; 2014), anthelmintic activity (Mali and Wadekar 2008), immunomodulatory activity (Patil *et al*., 2009), free radical scavenging potential (Prajakta *et al.*, 2014), toxicity against prostate cancer (Cherian *et al*., 2015), ulcer healing effects (Srivastava *et al.*, 2016), and central nervous system depressant activity (Syeda *et al*., 2015).

As the world is currently experiencing a serious planetary crisis that contributes to climate change and water shortages, alternative techniques for agricultural growth must be developed (Chauhan *et al*., 2023). In general, vegetative reproduction is favored over seed-based reproduction because of the seasonal seeds. The need for the hour is mass propagation and multiplication of plants in a short time to meet the growing demands. Cutting is a widely practiced method of vegetative plant multiplication used in horticulture. Every stem cutting has the potential to develop into a plant. It has numerous benefits, including being affordable, requiring little area, and spreading quickly to the chosen clones or new types produced by breeding programs (Jaleta and Sulaiman 2019). Stem cuttings are the easiest and most popular method of cultivating *B. montanum*. However, a healthy plant can produce a high-quality number of leaves than cutting. In some plants, leaves have the potential to develop into plants with the same optimal genetic traits as the parent plant (Hartmann *et al.*, 2002). Earlier Gregory and Samarantai (1950) conducted the first studies on the rooting of leaves isolated from a variety of species, including *Hedera helix*, *Phaseolus vulgaris*, *Ipomoea batatas*, *Helianthus annus*, *Chenopodium album*, *Amaranthus gangeticus*, *Cephalandra indica* and *Boerhaavia diffuse.* A study on the effect of varied nutrient concentrations on the production, and quality of Sweet Basil in a hydroponics system has been reported (Kundu *et al*., 2021).

The metabolites and plant growth regulators' interaction promotes root production over time. The primary photosynthetic organs of plants are their leaves (Wright *et al*., 2004). Although plants produce essential auxins in their shoots and leaves, artificial auxins are used to promote AR and maintain the *in vitro* survival of cuttings (Kasim and Rayya 2009). The role of endogenous and exogenous hormones in promoting plant propagation (Pijut *et al*., 2011) has been studied. Auxins are the primary inducers of root propagation in leaves (Goldsmith, 1974). In addition to auxins, phenolic substances also exert an effect. IAA and cytokinins are crucial hormones that regulate lateral root initiation, growth, vascular differentiation, and gravitropism. These two hormones work in conjunction with ethylene (Aloni *et al*., 2006). At appropriate concentrations, these chemicals act as endogenous growth agents. There is a desire to propagate cuttings using alternative rooting hormones that encourage rooting, as synthetic rooting hormones, such as IAA and IBA, are becoming increasingly expensive and difficult to obtain (Dunsin *et al*., 2016).

Currently, the two most popular techniques for *B. montanum* are seedling and field stem cutting, both of which are based on the soil. Other methods include micropropagation by Johnson and Manickam (2003) and George *et al*. (2008). In the present investigation, the selected plant sample, a novel method of propagation, and its success rate were examined. As a result, our goal was to create a semi-hydroponic leaf-cutting propagation technique and determine the role of PGR, mainly auxins (IAA, IBA, and NAA). We investigated the development of adventitious roots in leaf cuttings of *B. montanum*. Through the investigation of root length and number under various component influences, adventitious root (AR) development was examined because the phytoconstituents mainly reside in very useful root regions.

MATERIALS AND METHODS

B. montanum is grown and maintained in the Nandha Arts and Science College (NASC) Medicinal Garden, Erode, Tamil Nadu, India. The Botanical Survey of India (BSI), Southern Regional Centre, TNAU Campus, Coimbatore, Tamil Nadu, India (BSI/SRC/5/23/2021/Tech/191) verified and authenticated the plant sample. Healthy leaves were defoliated and used in the present study.

Old leaves from 0 to 20 cm above ground level (lowest), mature leaves from 21 to 40 cm (middle), and young leaves above 40 cm (upper) were collected from three separate points on the mother plant. In a preliminary study, 500 ppm PGRs were used to screen for their rooting potential. Other components, such as honey (5%), glucose (10%), sucrose (10%), and undiluted TCW were also analyzed. Leaves from the plants were collected and washed under running tap water to remove suspended soil particles. Further, the leaves were immersed in the chosen components such as PGRs and growth inducers, for 15 min and then transferred to a 200 ml beaker containing distilled water. The response of the leaf to growth from the petioles was observed on days 15^{th} and 30^{th} days. Every week, the water was changed in the leaf culture to avoid contamination by algal growth or water-borne microbes. Leaves dipped in growth inducers were carefully implanted in the soil without any damage to the petioles. The control leaves were maintained by dipping the leaves with petioles in distilled water alone, and root growth was observed in the water. Based on the output, in the second experimental study, all auxins alone were further deciphered by varied (200ppm, 500ppm, and 1,000ppm) concentrations in mature leaves.

All data were recorded based on the number of roots per leaf cutting and root length (cm). ANOVA was performed on the recorded data, and Duncan's Multiple Range Test (DMRT) was applied to determine the mean separation ($P > 0.05$).

RESULTS AND DISCUSSION

Plants are essential for both the survival and existence of the earth (Fernando, 2012). The ability of plants to regenerate sexually and asexually is the most crucial component for maintaining it. Although many synthetic compounds replace other plant-derived components made from plants, there is no alternative for plant-derived food made from plants. To produce nutritious food plants, crops, or vegetables in the current environment, soilless agriculture can be effectively implemented and considered as a substitute (Butler and Oebker 2006). Dornelas Jr *et al*. (2018) reported immersing whole leaves at various rooting hormone concentrations while studying the *in vitro* rooting of leaves.Indoor plants that thrive in water include philodendron, aglaonema, English ivy, wandering jews, coleus, syngonium, tradescantia, purple passion, lucky bamboo, begonias, pelargoniums, pothos, African violet, *Monstera adansonii*. Herbs that grow rapidly in water include mint, sage, oregano, basil, rosemary, and lavender. Simple cutting from the base of a plant and growing in fresh spring water towards root formation or the entire plant is a low-maintenance approach.

Hydroponic systems have been studied in *Arabidopsis thaliana* as models for growth determination (Kopittke *et al*., 2010; Berezin *et al*., 2012; Conn *et al*., 2013; Alatorre-Cobos *et al*., 2014) and Spinach (Maneejantra *et al*., 2016). It has been successfully used to produce rice (Gregorio *et al*., 1997; Kim *et al*., 2005), maize (Gibbs *et al*., 1998), lettuce (Kratky, 2009; Gent, 2012; Barbosa *et al*., 2015), potato (Chang *et al*., 2012), cowpea (Aliyu *et al*., 2016), tomato (Rosa-Rodríguez *et al*., 2020; Verdoliva *et al*., 2021), tomato and tobacco (Berezin *et al*., 2012; Conn *et al*., 2013; Alatorre-Cobos *et al*., 2014), and strawberries (Treftz and Omaye, 2015).Dore (1965) noted that plants belonging to the Solanaceae, Acanthaceae, Begoniaceae, Crassulaceae, and Piperaceae families were more likely to develop roots in detached leaves.The stabilization of the solution and effective utilization of resources depends on a balanced supply of nutrients. In hydroponic systems, pH, electrical conductivity, oxygen level, and temperature are crucial for optimum crop yield (Conn *et al*., 2013; Jones Jr, 2016; Baiyin *et al*., 2021; Ali Al Meselmani, 2022).

The current research focused on the *in vitro* root propagation of *B. montanum* leaves (Fig. 1) in water with the assistance of various growth compounds (Fig. 2).In our study, leaves of different ages were exposed to growth inducers, such as honey, glucose, sucrose, and undiluted TCW, along with PGRs (Fig. 3).The leaves responded positively to PGRs immersion, and no significant root formation was observed in other components (Graphs 1a and 1b) based on rooting numbers and percentages.Graph 1 depicts that 500ppm concentration IAA-exposed leaves responded positively, with a maximum survival and rooting rate of 83.33% in mature leaves, followed by mature leaves with IBA (75%) and moderate with NAA (16.66%).Forster *et al*. (1998) measured the general development of phosphite-fertilized plants, such as the leaf area and dry weights of leaves, stems, and roots. Guan *et al.*, (2015) demonstrated that auxin (IAA) controls AR production during three stages and practically every developmental stage. Auxins, cytokinins, and gibberellins, which are significant and frequently used rooting hormones, are artificial hormones that can promote the germination of cuttings. Alternative hormones are natural substances that enhance the germination of cuttings, including saliva, honey, willow tea, coconut water, and honey have been reported by Shield (2012).The removal of the leaves is regarded as a wound that triggers the formation of disorganized cell masses or calluses (Ikeuchi *et al*., 2013).When *Dracaena purple-compacta* ornamental plant canes are propagated vegetatively, Agampodi and Jayawardena (2009) showed that coconut water extracts containing natural IAA promote the growth of adventitious roots. However, in our preliminary work on *B. montanum*, we observed the production of very small roots with a survival and rooting rate of 8.33%.Our analysis of honey also resulted in a survival and rooting rate of 8.33%, but it was not reliable because of complex and varied sources.The other compounds (glucose and sucrose) did not induce root- or callus-like production.

The old leaves also produced a moderate response to IAA, with 25% survival and a 6.66% rooting rate, but the health score insists on the deterioration of leaves from green to pale and yellow within 2 weeks. When a leaf is pulled away from a plant, senescence accelerates. The protein content may drop to less than half of its initial level within a few days (Osborne, 1962). The roots develop on the petiole, leading to the persistence of greenness and continued photosynthesis for an extended time, increasing the leaf's dry weight and protein content. Because of auxin synthesis in leaves and stem tips, there are increased opportunities for root formation in leaves (Ljung *et al*., 2001). In IBA and honey, the survival rates were 16.66% and 8.33%, respectively, however, no rooting was observed, which questioned their survival. Nullified responses to glucose and sucrose immersion.There were no responses in younger leaves (Graphs 1a and 1b).The leaf health in auxin-exposed leaves is green, and exposure to other components turns the leaves pale green or yellow.

Overall, the observed results in Graph 1 demonstrate that the mature leaves are effectively rooted. The top stem part, however, had a lower rate of clonal development and rooting rate than the bottom and mid segments, which is explained by Hackett (1988); Haissing & Riemenschneider (1992), backed by the plant's little rooting. The leaves from the upper section of the plant are still at an extremely early stage of growth, and rooting is difficult because of limited nutritional storage. Juvenile plant leaves may react differently to mature plant leaves because they may have varying amounts of stored resources and morphological changes may be limited (Zhang *et al.*, 2015).

According to Kinsman (1990), AR helps to maintain plant population dynamics and individual plant survival. Although interactions with other hormones and general hormone homeostasis are necessary for the establishment of lateral roots, auxin has long been known to govern AR formation and is routinely used to induce root appearance in cuttings (Ivanchenko *et al*., 2008; Fukaki and Tasaka 2009; Stoeckle *et al*., 2018). Druege *et al*. (2019) demonstrated that other hormones may influence root growth by altering the homeostasis, transport, or signaling pathways governed by this hormone. In tobacco explants, hormone concentrations affect the formation of adventitious roots formed or not (Eberhard *et al*., 1989). MicroRNAs (miRNAs) involved in hormone-controlled plant growth (Curaba *et al*., 2014) have been studied.

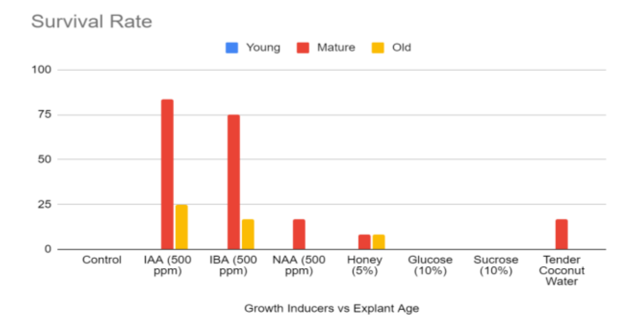
Based on the results of the preliminary experiment, further studies were performed using only mature leaves. The auxins (IAA, IBA, and NAA) were used at different concentrations (200, 500, and 1,000 ppm) as immersion solutions for 15 min. Onthe 15th day of observation, in terms of root percentage IAA (500ppm) was superior (83.33%)followed by 75% each in 1,000ppm IAA, 500ppm IBA and 1,000ppm IBA. IAA (200ppm) treatment resulted in a 66.66% rooting rate. The root numbers were the highest in IAA (500ppm) at 5.7 followed by 4.9 in 1,000ppm IAA. The root length parameter shows high in IBA (1,000ppm) with 8.3cm followed by 7.5cm and 6.65cm in IBA 500ppm and IBA 200ppm respectively. Leaf health scores were better for IAA and IBA than for NAA exposure.

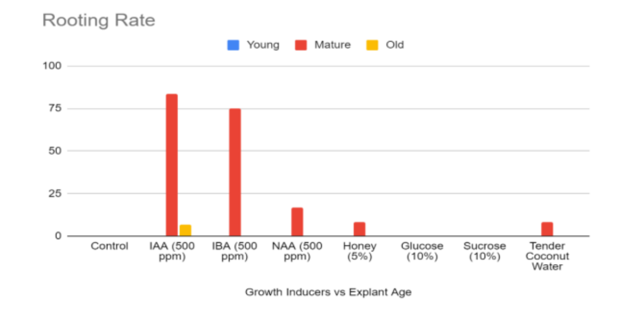
When observing the results on the 30th day, IAA (500 ppm) was superior in terms of the number of roots produced with an average of 12.5 roots per cutting followed by 11.1 in 1,000 ppm IAA and 8.8 in 200 ppm IAA. At 1,000 ppm, 500 ppm, and 200 ppm IBA, the responses were 3.2, 2.9, and 2.6 respectively. Druege *et al*., (2016) proposed that IAA buildup, wound-induced jasmonic acid (JA) and ethylene (ET) production, and self-regulatory canalization and maximization of responsive target cells would cause AR formation. An accurate understanding of the mechanisms governing excision-induced AR creation sheds light on the processes that underlie plant regeneration capacity and creates new avenues for the effective and sustainable use of plant genetic resources. But Alves Dos Santos et al., (2019) propagate by both petiole down cutting without immersion in hormone and petiole up cutting with immersion in IBA can be utilized to grow *P. carniconnectivum*.

Alternatively, root lengths were higher in IBA (1,000 ppm) with 13.6 cm followed by 12.4 cm at 500 ppm and 9.8 cm at 200 ppm. IAA shows moderate length (7.3 cm in 500 ppm, 6.2 cm in 1,000 ppm, and 5.6 cm in 200 ppm) whereas root production and length were less significant with NAA exposure. Vikram Choudhary *et al*., (2022) reported on the leaves of the Lucknow-49 guava variety rooted with rooting percentages of 70% and 72%, respectively, when exposed to 1,500 ppm IBA dipping for 1 and 2 min. However, our results are not satisfactory because IAA is superior with 66.66% (500 ppm IAA), 58.33% (1000 ppm IAA), and 50% (200 ppm IAA) compared to IBA (58.33%-1,000 IBA; 41.66%-500 ppm; 33.33%-200 ppm) in the leaves. Callus-like development was observed at the edge of leaf petioles with NAA exposure during the early days, and after 20 days, minimal roots were produced (8.33%) at all concentrations (Fig. 5; Fig. 6; Fig. 7 & Table 1). Overall, IBA surpassed the IAA output and the minimum response in the NAA.

Most plant cuttings are unable to establish independent plants; instead, they develop roots and decay (Evans and Blazich, 1999; Gorelick, 2015). According to these findings, the cuttings were well-rooted after 14 days of culture and deteriorated after 4 weeks, which is implicated in the number of stable roots and poor quality of roots, which is consistent with previous findings. Therefore, rooted leaves were transferred to moist soil for further growth development and analysis. From the observed results, rooted sample leaves were found to be difficult to establish, with the exception of IAA-treated leaves(Fig. 8). Plants are subjected to hydroponic conditions thatcannot be considered physiological. Consequently, when plants are grown in other systems, phenotypes or plant reactions discovered using hydroponic systems may change in intensity. Different responses can be observed in plants grown in different soil types; therefore, these factors are not exclusive to hydroponic conditions (Sharma *et al*., 1980; Resh, 2013). Studies on soilless systems have increased, suggesting upward progress in the use of water culture (Gibeaut *et al*., 1997; Arteca and Arteca, 2000; Siedlecka and Krupa, 2002; Schlesier *et al*., 2003; Huttner and Bar-Zvi, 2003; Norén *et al*., 2004; Smeets *et al*., 2008). Although the water-dependent method is a quick cycle, the quick screening and year-round manufacturing of this approach make it useful, and the development of low-cost, simple-to-use, and maintainable approaches is crucial for the effective deployment of commercial hydroponic technology (Sharma *et al*., 2018).Water-borne illnesses can be readily transferred from one plant to another in a hydroponic system because all plants share the same nutrients (Ikeda *et al*., 2002). Studies on the chemical composition difference between the plant grown in hydroponics to normal plants have been reported Telgote *et al*. (2022).

This can be improved based on the report of Sambo *et al*. (2019); Stegelmeier *et al*. (2022) by using beneficial microorganisms, such as plant growth-promoting rhizobacteria (PGPR), a new developing technology known as soilless agriculture, which may help manage soilless cropping systems more effectively. Macropropagation techniques have been investigated for different commercially valuable plant species, notably uncommon, endangered, and threatened plant species (Jamir *et al*., 2016), and can aid in the development of plant improvement programs. Therefore, this hybrid method is beneficial for plants that are difficult to root in the soil. Singh and Chettri (2013) highlighted that compared to the traditional method of multiplication by terminal stem cuttings, this innovative *in vitro* method of propagation by leaf cuttings boosted the rate of propagation of the plant by 10-15 times. Therefore, this method will help to overcome the minimal availability of herbal and medicinal plant samples through further improvements.





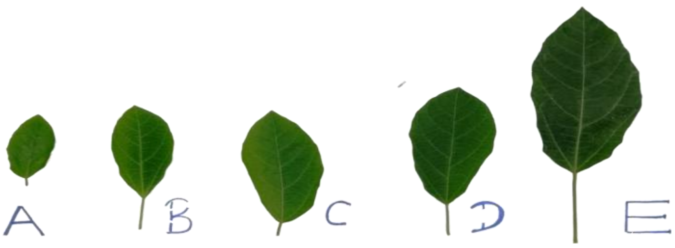
Graph 1a & b: Effect of explant age and plant growth regulators on Survival and rooting rate of *Baliospermum montanum* after 15 days of growth.

Table 1: Effect of different growth regulators on rooting of *Baliospermum montanum* leaves after 15 days & 30 days of culturing water (15 minutes of explant immersion in PGRs).

PGR	Observation after 15 days				Observation after 30 days			
	Rooting %	**Root Length (cm)**	**No. of Roots**	**Leaf Health Score**	**Rooting %**	**Root Length (cm)**	**No. of Roots**	**Leaf Health Score**
Control	0±0	0±0	0±0	-	0±0	0±0	0±0	-
IAA (200 ppm)	**66.66**	3.7±0.15^{b}	4.6±0.21^{ba}	+++	**50**	5.6±0.17^{c}	8.8±0.12^{c}	++
IAA (500 ppm)	**83.33**	4.0±0.13^{a}	**5.7±0.18^{a}**	+++	**66.66**	7.3±0.19^{a}	**12.5±0.19^{a}**	++
IAA (1,000 ppm)	**75**	3.6±0.22^{ba}	**4.9±0.12^{b}**	+++	**58.33**	6.2±0.14^{b}	**11.1±0.22^{b}**	++
IBA (200 ppm)	41.66	**6.65±0.19^{c}**	2.5±0.17^{ba}	+++	33.33	**9.8±0.16^{c}**	2.6±0.19^{ba}	+
IBA (500 ppm)	**75**	**7.5±0.16^{b}**	2.6±0.23^{b}	+++	41.66	**12.4±0.21^{b}**	2.9±0.13^{b}	++
IBA (1,000 ppm)	**75**	**8.3±0.22^{a}**	3.3±0.14^{a}	+++	**58.33**	**13.6±0.19^{a}**	3.2±0.16^{a}	++
NAA (200 ppm)	0	0±0	0±0	+	8.33	0.5±0.23^{ba}	1.2±0.22^{ba}	+
NAA (500 ppm)	16.66	0.7±0.15^{b}	1.3±0.19^{a}	++	8.33	0.9±0.17^{b}	2.3±0.18^{a}	+
NAA (1,000 ppm)	16.66	1.3±0.13^{a}	0.8±0.24^{b}	++	8.33	1.3±0.20^{a}	1.5±0.14^{b}	+

Leaf Health Score; '+' for Pale Green/Yellow; '++' for Pale Green; '+++' for Green; '-' for No response and leaves dried.

Fig. 1. Collected *Baliospermum montanum* L. plant



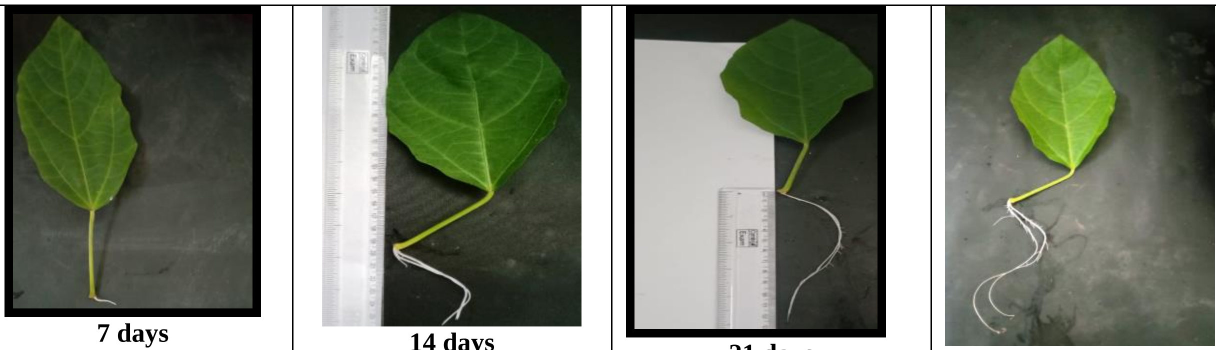
Different Size of Leaves (A - Small/Young; B, C & D – Mature; E - Old)
Fig. 2. Different aged leaves of *Baliospermum montanum*

Fig. 3. Leaf culture of *Baliospermum montanum* inoculated in water

Fig. 4. Rooted leaves in water.

 **14 days**	 **30 days**
Fig. 5. Rooted leaf treated with NAA.	

 **7 days**	 **14 days**	 **21 days**	 **30 days**
Fig. 6. Rooted leaf exposed to IAA.			



7 days | 14 days | 21 days | 30 Days

Fig. 7. Rooted leaf treated with IBA.

Fig. 8. *Baliospermum montanum* rooted single leaves cultured in soil (IAA).

CONCLUSIONS

B. montanum is a regionally vulnerable plant. Regeneration of this plant by all possible means is the need of the hour. Therefore, alternative approaches to the conventional system will be a useful technique to raise these plants in large numbers. Conservation of this vulnerable plant using an innovative leaf propagation technique is tried in the present study. An attempt is made in the present study to efficiently regenerate the entire plant from a single leaf which serves as a remarkable technique without sacrificing the plant.

FUTURE SCOPE

We experimented with a new vegetative propagation method in water followed by soil for *Baliospermum montanum* L. The hydroponic-type method is quick, easy, and capable of producing roots in leaf culture. The rooting potential of *B. montanum* in the soil becomes difficult; therefore, we tried this method of initiating in water and later transplanting it to the soil. As this type of study is at an early stage of development, further improvements will have a great impact on the propagation of other herbal and medicinal plants. Additionally, the analysis of chemical profiling can be done and compared to its mother plants.

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Conflict of Interest. None.

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