frontiers | Frontiers in Conservation Science

TYPE Original Research
PUBLISHED 08 May 2024
DOI 10.3389/fcosc.2024.1386204

OPEN ACCESS

EDITED BY
Shreekar Pant,
Baba Ghulam Shah Badshah University, India

REVIEWED BY
Zishan Ahmad Wani,
Baba Ghulam Shah Badshah University, India
Mohd Tariq,
Parul University, India

*CORRESPONDENCE
Junaid A. Magray
junaidmagray786@gmail.com
Hanan Javid
bhathanan123@gmail.com

RECEIVED 14 February 2024
ACCEPTED 22 April 2024
PUBLISHED 08 May 2024

CITATION
Magray JA, Wani BA, Javid H, Islam T, Ganie AH, Qadir RU and Nawchoo IA (2024) Vegetative propagation of *Phytolacca acinosa* Roxb. by rhizome cuttings: a step towards conservation and cultivation approach. *Front. Conserv. Sci.* 5:1386204. doi: 10.3389/fcosc.2024.1386204

Vegetative propagation of *Phytolacca acinosa* Roxb. by rhizome cuttings: a step towards conservation and cultivation approach

Junaid A. Magray[1]*, Bilal A. Wani[1], Hanan Javid[1]*, Tajamul Islam[1], Aijaz H. Ganie[2], Roof Ul Qadir[1] and Irshad A. Nawchoo[1]

[1]Plant Reproductive Biology, Genetic Diversity and Phytochemistry Research Laboratory, Department of Botany, University of Kashmir, Srinagar, India, [2]Department of Botany, North Campus, University of Kashmir, Baramulla, India

Introduction: *Phytolacca acinosa* Roxb. is a highly valuable multipurpose herb native to the Himalayan region. Unsustainable harvesting of this species due to its diverse uses has resulted in a rapid decline in its population across natural habitats, thereby necessitating its propagation and conservation. To overcome this challenge, the potential of *P. acinosa* rhizomes for *ex situ* regeneration was evaluated.

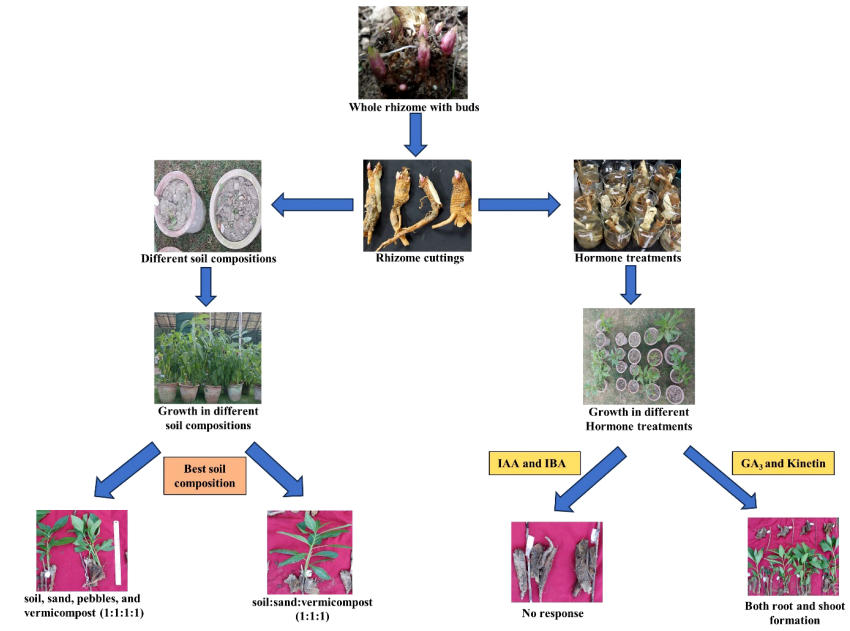
Methods: The current study aims to develop a standard propagation protocol for *P. acinosa*. Rhizome cuttings derived by splitting whole rhizomes were used to study the effect of various hormones and soil compositions on their sprouting and growth performance.

Results: Soil compositions SC10 and SC5 consisting of soil, sand, pebbles, and vermicompost (1:1:1:1) and soil, sand, and vermicompost (1:1:1), respectively, were the most suitable compositions for the optimum growth of this species. The rhizome segments treated with GA_3 (150ppm) induced the highest sprouting percentage (91.67%), with a minimum sprouting time of 23.25 days. The maximum root length (9.25 cm), shoot length (16.5 cm), and leaf number (11.25) were recorded for GA_3 (150ppm) treated rhizome cuttings.

Conclutions: Overall, the results of the present study helped in establishing a cost-effective, rapid, efficient, and simple mass propagation method for the target species. The results of this study will serve as a guide for the large-scale cultivation, effective conservation, and sustainable utilization of this economically valuable medicinal herb.

KEYWORDS

rhizome, conservation, Phytolacca acinosa, Himalaya, propagation, soil composition



GRAPHICAL ABSTRACT

Introduction

The conservation of plant genetic resources involves two main approaches: *in situ*, which involves protecting populations in their natural habitat, and *ex situ*, which involves protecting these resources outside their natural environment. The rising demand from pharmaceutical industries for medicinal plants has led to their increased harvesting from the wild. Anthropogenic threats including overexploitation, habitat degradation, overgrazing, and illegal trade practices have pushed many medicinal plant species to the verge of extinction (Ganie et al., 2019; Chisale et al., 2021; Wani et al., 2022; Wani et al., 2024a). Given the urgency of conserving such species, *ex situ* conservation methods are recognized as a viable and effective option for the conservation and sustainable utilization of threatened plant species (Dávila-Aranda et al., 2016). This serves as both a backup conservation strategy and, in certain instances, a temporary substitute for *in situ* conservation.

Vegetative propagation is an important and promising method for mass cultivation of economically important species, especially those posing difficulty when raised through seeds. Propagation through rhizomes is a convenient and effective method for the rapid multiplication of elite plant populations and for conserving their essential genetic characteristics (Lata et al., 2017; Reddy et al., 2022). It is an easy, cost-effective, and successful technique for the large-scale cultivation, conservation, and regeneration of threatened plant species. While seed germination offers the advantage of producing numerous individuals from a single mother plant, its success is constrained by the need for complex stratification treatments and the slow growth of seedlings in most forest understory plant species (Baskin and Baskin, 1998; Luna, 2001). Thus, vegetative propagation in plants, especially alpine species, is considered more important than sexual reproduction (Brožová et al., 2019). Furthermore,vegetative propagation, as compared to sexual reproduction, enables the development of mature individuals within a year of cultivation. It stands out as a highly effective method for conserving economically important overexploited species, contributing to advancements in propagation techniques, and fostering large-scale commercial cultivation.

Phytolacca acinosa Roxb., commonly known as pokeberry,belongs to the family Phytolaccaceae. It is an economically valuable perennial medicinal plant native to the East Asian and Himalayan regions (POWO, 2023). In the traditional medicine system, it is widely employed to treat eye disorders, body aches, swelling, edema, sores, and as a diuretic drug (Basnet and Kalauni, 2020). Rhizomes of *P. acinosa* contain numerous bioactive compounds, including chochliophilin (A and B), hypaphorine, esculentosides, β-sitosterol, monoglyceride, daucosterol, phytolacacinoside (A, B, and E), phytolaccoside (A, B, and E), esculentoside G, and palmitic acid (Gao et al., 2009; Krishan et al., 2022; Li et al., 2023). Consequently, it serves as an important medicinal herb with diverse pharmacological properties like antibacterial, anti-fungal, anti-inflammatory, antiviral, anti-oxidative, anticancer, immunity-enhancing, anti-parasitic, and insecticidal properties (Gao et al., 2009; Cheng et al., 2017; Bailly, 2021). It has been reported to exhibit cytotoxicity in human cancer cell lines and antimicrobial activity in bacterial culture (Ma et al., 2017, 2019; Krishan et al., 2022). Besides being used as medicine, *P. acinosa* is also used as a source of food, red dye for coloring wool fabric, and for phytoremediation of heavy metals like cadmium (Cd), lead (Pb), manganese (Mn), and zinc (Zn) (Zhao et al., 2014; Wu et al., 2016; Liu et al., 2022). During the last few decades, climate change, habitat destruction, and unsustainable harvesting of *P. acinosa* due to its diverse uses have resulted in a rapid decline in its population across natural habitats (Cheng et al., 2017; Magray et al., 2023; Wani et al., 2024b).

The present study aimed to develop an agro-technique protocol using rhizome cuttings for the vegetative propagation of *P. acinosa*, a multipurpose medicinal plant native to the Himalaya (Magray et al., 2022). Although the species reproduces through both sexual and asexual means, the time-consuming nature of seed germination and vegetative propagation under natural conditions poses a challenge in generating well-developed seedlings (Magray et al., 2023).Vegetative propagation through rhizomes under *in vitro* conditions offers an alternative method for rapid and mass multiplication of the target plant and enables the cultivation of plants with the desired clone. Furthermore, different plant growth regulators are widely used in vegetative propagation to stimulate rooting and improve the growth of cuttings (Yoon et al., 2021).

To the best of our knowledge, the vegetative propagation of *P. acinosa* has not been carried out to date. In this context, the current study was conducted with the following specific objectives: (i) to investigate the effect of different growth hormones on the vegetative propagation potential of *P. acinosa*; (ii) to determine the effect of different soil compositions on sprouting percentage and related growth parameters; and, based on the scientific insights gained, (iii) to outline a reproducible protocol for vegetative propagation of the target species for its conservation, restoration, and mass production for the pharmaceutical industries. This study may serve as a fundamental guide for the restoration of this valuable plant species in Kashmir Himalaya and also provide scientific insights for the restoration of other valuable medicinal plants growing elsewhere in the world.

Materials and methods

Sample collection and experimental location

The fresh rhizomes of *P. acinosa* were harvested during February 2022 from the wild populations of Drung and Gogaldara, Jammu and Kashmir, India (Figure 1). The collected rhizomes were washed thoroughly with running tap water and kept in a dark and cool room (4°C) to avoid the emergence of buds until the experiments started. The experiment was conducted at Kashmir University Botanical Garden (KUBG), University of Kashmir, Srinagar (1590m asl; 34.13254722°N, 74.83755278°E), with a humid temperate climate and a high mean annual rainfall of 1005.5 mm (Dad et al., 2021).

Effect of different soil compositions

Freshly collected rhizomes were cut longitudinally into different sections, each 4 ± 0.5 g and with 2 buds (Figures 2A, B). Prepared rhizome cuttings were disinfected by a surface wash with a 2% solution of the systemic fungicide, Bavistin. The rhizome cuttings were sown in different soil compositions prepared by putting weighed amounts of soil, sand, pebbles, vermicompost (VC), farmyard manure (FYM), and peat in pots (Table 1). In each composition, five replicates of three cuttings were used. The pots were transferred to the pot house under 60% shade, and irrigation was usually performed by daily watering for the first week of the experiment and subsequently when the soil on the surface started drying. The total sprouting percentage was calculated at the culmination of the experiment for all the treatments. Plants were harvested destructively and were analyzed for various morphological parameters like rhizome length, rhizome breadth, plant height, leaf number, leaf length, leaf breadth, and biomass allocation towards above and belowground parts (Figure 2F).

Growth hormone experiment

To study the effect of different growth hormones on the clonal propagation of *P. acinosa*, the rhizomes were divided longitudinally

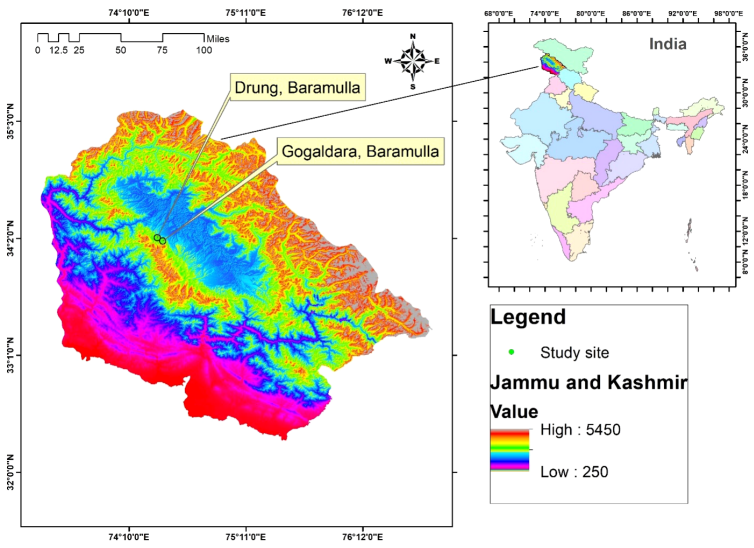


FIGURE 1
Map showing sampling sites in Kashmir Himalaya, India.

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FIGURE 2
(A–F) Effect of different hormone treatments and soil compositions on different growth parameters of *Phytolacca acinosa*. **(A)** Whole rhizome with buds; **(B)** Rhizome cuttings; **(C)** Hormone treatment of rhizome cuttings; **(D)** Harvested material after hormone treatments; **(E)** Plant growth in different soil compositions; **(F)** Record of morphological parameters and biomass accumulation.

into 2-6 sections (each 4 ± 0.5 g and 2 buds) with a sterilized sharp knife, depending on the size and number of apical buds on the parental rhizome. The rhizome cuttings were disinfected by a surface wash with a 2% Bavistin solution followed by treatment with different growth hormones including Kinetin, Indole Butyric Acid (IBA), Indole Acetic Acid (IAA), and Gibberellic Acid (GA_3) (50ppm, 100ppm, 150ppm, and 200ppm), and distilled water (control) for 48 hours (Figure 2C; Supplementary Material S1). The treated rhizome cuttings were planted in pots containing a mixture of soil and sand in a 1:1 ratio. The pots were placed under partial shade (60%) in pot-house at Kashmir University Botanical Garden and irrigated daily for the first few days (10 days) and after that every 3rd day without fertigation. The experiment consisted of 19 treatments (Supplementary Material S1), each repeated four times in a randomized block design with three cuttings per treatment. The experiment was monitored regularly, and growth parameters including root length, shoot length, leaf number,time taken for sprouting (days), and sprouting percentage were recorded (Figure 2F). The average number of leaves per plant was counted manually, while root length (cm) and shoot length (cm) were measured using a scale.

Statistical analysis

Data from the experiment were subjected to analysis of variance (ANOVA). The analyses were carried out using IBM SPSS Statistics software (version 23). ANOVA was performed to evaluate the effect

10.3389/fcosc.2024.1386204

TABLE 1 Different soil compositions used for vegetative propagation of *Phytolacca acinosa*.

Code	soil composition	Ratio
SC1	Soil	100%
SC2	soil:sand	(1:1)
SC3	soil:sand	(3:1)
SC4	soil:sand	(1:3)
SC5	soil:sand:vermicompost	(1:1:1)
SC6	soil:pebbles	(1:1)
SC7	soil:pebbles	(3:1)
SC8	soil:pebbles	(1:3)
SC9	soil:pebbles:vermicompost	(1:1:1)
SC10	soil:sand: pebbles:vermicompost	(1:1:1:1)
SC11	soil:farmyard	(3:1)
SC12	sand:soil:farmyard	(1:1:1)
SC13	soil rite	100%
SC14	soil rite:soil	(3:1)
SC15	soil:soil rite	(1:1)
SC16	soil:peat	(3:1)
SC17	soil:peat	(1:1)
SC18	soil:sand: peat:vermicompost	(1:1:1:1)

of different soil compositions and growth hormones on different growth parameters and morphological traits like sprouting percentage, time taken for sprouting, rhizome length, rhizome breadth, plant height, leaf number, leaf length, leaf breadth, and biomass allocation towards above and belowground parts. Tukey's test (at $p \leq 0.05$) was conducted for statistical mean comparisons.

Results

Effect of different soil compositions on growth and survival of rhizome cuttings

The sprouting percentage varied significantly among different soil compositions (Table 2). The highest sprouting percentage of rhizome cuttings was recorded in SC10 treatment (100%) consisting of soil, sand, pebbles, vermicompost (1:1:1:1) followed by SC5 (91.67%) having soil, sand, vermicompost in the ratio of 1:1:1, and SC9 (83.33%) treatment having soil, pebbles, vermicompost in 1:1:1 ratio (Table 2; Figure 2E,). The sprouting time of rhizome cuttings also varied significantly with different soil compositions. The shortest sprouting time was recorded in the SC10 treatment (26 days), followed by the SC5 treatment (28.75 days) (Table 2). All morphological parameters also showed significant variation among the various treatments (Table 2; Figures 3A, B). Maximum rhizome length and rhizome breadth of 15.38±0.59cm and 3.13±0.31cm, respectively, were observed for soil composition SC10, followed by composition SC5 (Table 2; Figure 3A). Similarly, maximum plant height (34.2±0.86 cm) and leaf number (49.65±1.03) were recorded in SC10 composition(Table 2; Figure 3A). The leaf length was highest in the SC10 treatment (8.83±0.44 cm), followed by SC5 (7.53±0.51 cm). Likewise, the leaf breadth was highest in the SC5 treatment (2.73±0.4 cm), followed by SC10 (2.63±0.13 cm) (Table 2; Figure 3A). The analysis of variance also exhibited a significant variation in the above and belowground biomass among all the treatments (Table 2). Both the highest aboveground biomass of 11.65±0.66 g and belowground biomass of 31.79±0.88 g were estimated in SC10 composition followed by SC9 with above and belowground biomass of 9.95±0.8 g and 30.05±0.86 g respectively (Table 2; Figure 3B).

The rhizome cuttings treated with different phytohormones showed significant variation in all the growth parameters (Table 3; Figures 2D, F). The highest sprouting percentage (91.67%) was observed in the rhizome divisions treated with GA_3 (150ppm), followed by (83.33%) treated with GA_3 (100ppm) as compared to the control with 33.3% of sprouting (Table 3, Figure 2D). The auxins used (IAA and IBA) failed to stimulate the sprouting of rhizome cuttings. The sprouting time varied among different treatments, ranging from 5.25 to 9.25 (Table 3). GA_3 at a concentration of 150ppm was most effective in reducing the sprouting time of rhizome cuttings. GA_3 (150ppm) was most effective in reducing the sprouting time of rhizome cuttings. The highest sprouting percentage was recorded in 150ppm GA_3 (91.67%) and 100ppm GA_3 (83.33%); sprouting time was also attained rapidly with 23.25 and 25 days, respectively; however, the sprouting percentage of 33.33% in control was completed with a sprouting time of 35.75 days (Table 3). Further, treatment of rhizome cuttings with gibberellic acid also significantly increased the shoot length, root length, and leaf number on plants. The maximum number of leaves (11.25±0.63) was observed in GA_3 (150ppm) followed by 50ppm GA_3 (9.75±0.63). The highest root length (9.25±0.48 cm) and shoot length (16.5±0.65 cm) were also recorded in rhizome cuttings treated with GA_3 (150ppm) (Table 3).

Discussion

Vegetative reproduction offers an efficient means for mass multiplication of germplasm and cultivation for species exhibiting poor regeneration through sexual means (Deepak et al., 2016; Sreekissoon et al., 2021). The frequent weather changes at high elevations result in reproductive failures at various developmental stages of plants (Carbognani et al., 2018). *Phytolacca acinosa*, a typical sub-alpine plant, exhibits poor seed germination within its wild habitats (Magray et al., 2023). The present study demonstrated multi-approaches for the propagation of *P. acinosa* through rhizome cuttings to compensate for this reproductive bottleneck. In vegetative propagation, the application of plant growth regulators and various chemicals has been extensively employed to enhance the rooting and subsequent growth of rhizome cuttings (Nadeem et al., 2000; Butola and Badola, 2007; Bisht and Bhatt, 2014). During the present study, the vegetative growth and

10.3389/fcosc.2024.1386204

TABLE 2 Effect of different soil combinations on growth and morphological parameters of rhizome cuttings.

Soil composition	SP	Time taken for sprouting (days)	RL	RB	PH	LN	LL	LB	AGB	BGB
SC1	58.33 ±8.33^{*abc}	37±0.71^{i}	4.7 ±0.25^{a}	1.75 ±0.15^{abc}	23.18 ±0.72^{ab}	27.38 ±0.9^{a}	6.2 ±0.34^{abc}	1.6 ±0.17^{ab}	8.83 ±0.52^{def}	24.75 ±0.63^{g}
SC2	75 ±8.33^{abc}	31.5±0.29^{bcd}	7.28 ±0.58^{bcd}	2.13 ±0.18^{abcd}	29.2±0.65^{f}	36.25 ±0.75^{cdef}	6.75 ±0.28^{bcd}	1.73 ±0.17^{ab}	9.5 ±0.32^{efg}	25.78 ±0.7^{g}
SC3	58.33 ±8.33^{abc}	33.5±0.65^{defgh}	6.83 ±0.66^{abc}	2.15 ±0.06^{abcd}	27.5 ±0.77^{def}	34.5 ±0.65^{cde}	5.6 ±0.23^{abc}	1.48 ±0.18^{a}	9.43 ±0.3^{efg}	24.9 ±0.87^{g}
SC4	50±9.62^{ab}	33.75±0.48^{defgh}	6.28 ±0.25^{ab}	1.9 ±0.07^{abc}	24.33 ±0.71^{abcd}	32 ±0.71^{bc}	5.9 ±0.16^{abc}	1.55 ±0.17^{ab}	9.13 ±0.27^{defg}	23.78 ±0.6^{g}
SC5	91.67 ±8.33^{bc}	28.75±0.63^{ab}	14.18 ±0.57^{g}	2.73 ±0.4^{cd}	29.33 ±0.96^{f}	43.25 ±0.85^{g}	7.53 ±0.51^{cd}	2.73 ±0.4^{b}	8.33 ±0.48^{cdef}	19.45 ±0.69^{ef}
SC6	75 ±8.33^{abc}	31.75±0.25^{bcde}	7.78 ±0.46^{bcde}	2.03 ±0.27^{abc}	27.63 ±0.62^{def}	36.4 ±0.77^{def}	7.23 ±0.5^{bcd}	2.4 ±0.36^{ab}	6.88 ±0.58^{abcde}	26.58 ±0.6^{g}
SC7	66.67 ±0^{abc}	32.25±0.63^{cdef}	5.43 ±0.29^{ab}	2.13 ±0.13^{abcd}	28.4 ±0.83^{ef}	35.5 ±0.87^{cde}	7.23 ±0.48^{bcd}	2.03 ±0.25^{ab}	8.73 ±0.58^{def}	26.38 ±0.61^{g}
SC8	50±9.62^{ab}	32.5±0.5^{cdefg}	5.63 ±0.43^{ab}	2 ±0.16^{abc}	24.93 ±0.94^{abcde}	42.75 ±0.85^{g}	7.1 ±0.52^{bcd}	1.95 ±0.21^{ab}	7.68 ±0.85^{bcdef}	26.53 ±0.76^{g}
SC9	83.33 ±9.62^{abc}	30.25±0.48^{bc}	11.13 ±0.55^{f}	2.65 ±0.12^{bcd}	28.93 ±0.91^{f}	40.33 ±0.62^{fg}	7.5 ±0.51^{cd}	2.13 ±0.18^{ab}	9.95 ±0.8^{fg}	30.05 ±0.86^{h}
SC10	100±0^{c}	26±0.41^{a}	15.38 ±0.59^{g}	3.13 ±0.31^{d}	34.2 ±0.86^{g}	49.65 ±1.03^{h}	8.83 ±0.44^{d}	2.63 ±0.13^{ab}	11.65 ±0.66^{g}	31.79 ±0.88^{h}
SC11	58.33 ±8.33^{abc}	36±0.41^{hi}	8.88 ±0.35^{cdef}	2.2 ±0.28^{abcd}	24 ±0.61^{abcd}	34 ±0.71^{cde}	7.43 ±0.41^{cd}	2.35 ±0.17^{ab}	7.9 ±0.46^{bcdef}	17.3 ±0.55^{def}
SC12	66.67 ±0^{abc}	34.75±0.63^{efghi}	9.3 ±0.49^{cdef}	1.9 ±0.22^{abc}	27.4 ±0.55^{cdef}	35.25 ±0.75^{cde}	6.75 ±0.27^{bcd}	2.1 ±0.25^{ab}	9.65 ±0.42^{fg}	20.33 ±0.76^{f}
SC13	41.67 ±8.33^{a}	36.25±0.48^{hi}	10.58 ±0.51^{f}	2.2 ±0.31^{abcd}	23.58 ±0.82^{abc}	35.5 ±1.04^{cde}	6.08 ±0.21^{abc}	1.85 ±0.25^{ab}	4.88 ±0.42^{a}	17.79 ±0.65^{def}
SC14	41.67 ±8.33^{a}	35.5±0.87^{ghi}	9.28 ±0.55^{cdef}	1.35 ±0.05^{a}	29.83 ±0.72^{f}	29.25 ±0.85^{ab}	5.13 ±0.41ab	1.65 ±0.19^{ab}	7.85 ±0.41^{bcdef}	7.87 ±0.26^{a}
SC15	50±9.62^{ab}	36.5±0.65^{hi}	7.83 ±0.47^{bcde}	1.45 ±0.12^{a}	26.65 ±1.01^{bcdef}	35.25 ±1.03^{cde}	4.53 ±0.67^{a}	1.57 ±0.37^{ab}	5.85 ±0.44^{abc}	13.03 ±0.54^{b}
SC16	50±9.62^{ab}	35.5±0.87^{ghi}	9.15 ±0.51^{cdef}	1.53 ±0.14^{a}	21.33 ±0.52^{a}	33 ±0.82^{bcd}	5.2 ±0.33^{ab}	1.73 ±0.18^{ab}	5.53 ±0.35^{ab}	13.55 ±0.15^{bc}
SC17	66.67 ±8.33^{abc}	35±0.71^{fghi}	10.23 ±0.44^{ef}	1.65 ±0.21^{ab}	24.18 ±0.51^{abcd}	37.5 ±0.87^{ef}	5.78 ±0.44^{abc}	1.65 ±0.12^{ab}	9.25 ±0.52^{efg}	16.8 ±0.51^{cde}
SC18	66.67 ±0^{abc}	34.75±0.48^{efghi}	9.58 ±0.51^{def}	1.35 ±0.1^{a}	23.33 ±0.43^{ab}	35.75 ±0.85^{cde}	6.5 ±0.41^{abc}	2.03 ±0.18^{ab}	6.6 ±0.41^{abcd}	15.18 ±0.46^{bcd}

SP, Sprouting percentage; RL, rhizome length; RB, rhizome breadth; PH, plant height; LN, leaf number; LL, leaf length; LB, leaf breadth; AGB, aboveground biomass; BGB,belowground biomass. *Mean ± SE, Means with the same superscript letters in the same column are not significantly different while those with different superscript letters are significantly different at $p \leq 0.05$Effect of different growth hormones on the vegetative propagation of Phytolacca acinosa.

sprouting percentage of the plant was found to be highest at intermediate hormone concentrations (Table 3). The best results were recorded for GA_3 150ppm and kinetin 150ppm.Gibberellic acid is an important phytohormone, as it is at the core of many plant growth and developmental processes. Gibberellic acid (GA_3)-treated rhizome cuttings showed a significant increase in root and shoot development (Ma and Huang, 2016). It is reported to enhance the sprouting percentage and improve the growth and survival of vegetatively propagated plants under *in vitro* conditions (Shabir et al., 2010; Hassan et al., 2020). In blueberries, gibberellic acid induces plant growth and enhances leaf development as compared to the control (Zang et al., 2016). Consistent with earlier studies (Tuna et al., 2008; Wen et al., 2010; Bose et al., 2013), GA_3 application enhanced various plant growth characteristics like leaf number, root length, and shoot length in *P. acinosa*. Our results conform to those of Shabir et al. (2010), who also reported a significant increase in growth-related parameters in *Inula racemosa* when treating rhizomes with GA_3.Gibberellins can influence plant growth by accelerating the movement of cytokinins to developing buds and also by altering carbohydrate metabolism (Salimi et al., 2010). Further, similar to our findings, cytokinins have been reported to increase the shoot length and leaf

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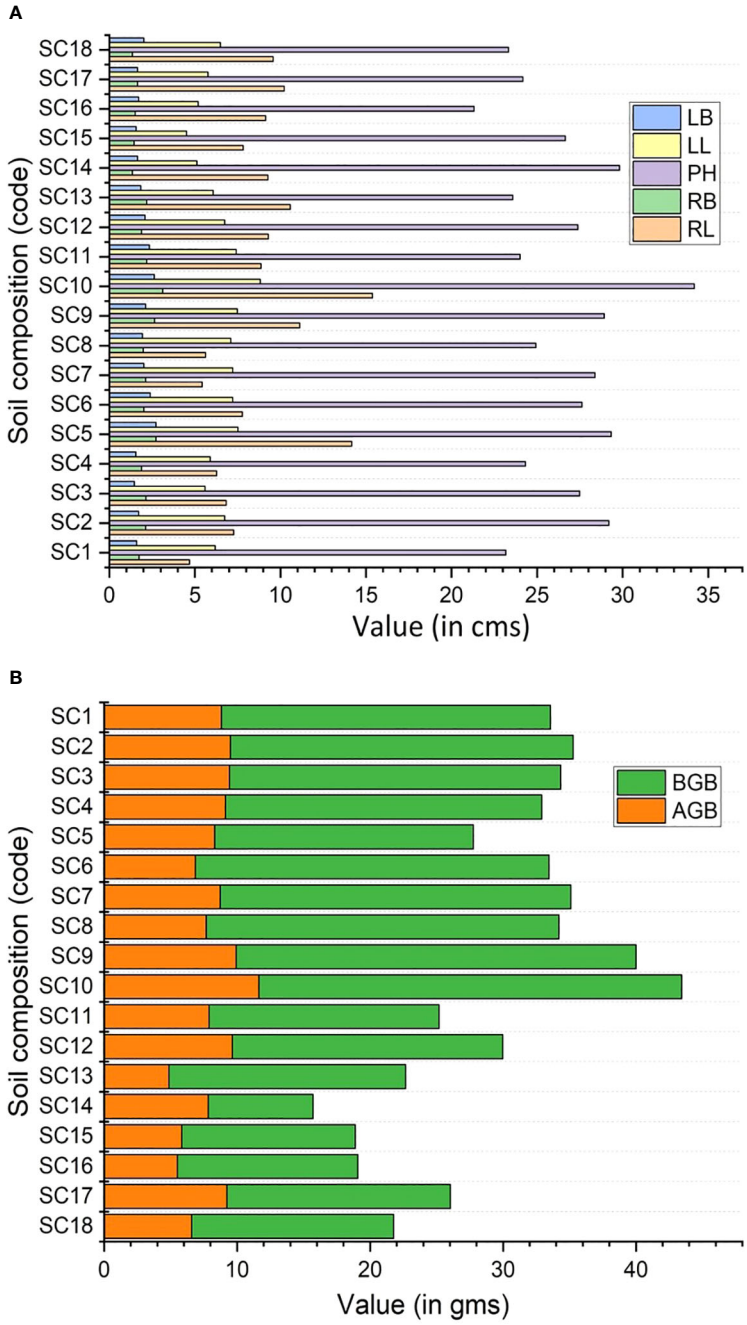

FIGURE 3
(A, B) Effect of soil composition on morphological parameters and biomass allocation of *Phytolacca acinosa*. **(A)** RL, rhizome length; RB, rhizome breadth; PH, plant height; LN, leaf number; LL, leaf length; LB, leaf breadth; **(B)** AGB, aboveground biomass; BGB, belowground biomass.

number in *Phytolacca dodecandra* (Chishimba et al., 1999). Kinetin also improved the shooting and leaf numbers in *Phytolacca americana* (Trunjaruen et al., 2022). The efficacy of applying cytokinins (kinetin) and GA_3 in promoting sprouting responses, root and shoot development among rhizome explants has been reported in several plant species, such as *Pongamia pinnata* (Sugla et al., 2007); *Jurinea dolomiaea* (Banday et al., 2014); *Talinum triangulare* (Swarna and Ravindhran, 2013); *Picrorhiza kurroa* (Patial et al., 2012) and *Euphorbia wallichi* (Hassan et al., 2020). In *Nardostachys jatamansi*, vegetative propagation by rhizome splitting was found to be successful and proved to be a better and more rapid means of multiplication, as well as higher production than cultivation through seedlings (Nautiyal and Nautiyal, 2004).

The current study revealed that different soil combinations significantly affected the sprouting and growth performance of *P. acinosa*. Rhizome cuttings exhibited maximum sprouting percentage with minimum sprouting time in soil:sand:pebbles: vermicompost (1:1:1:1) composition (Table 2). In natural habitats, the species is mainly found growing along roadsides and forest margins with a soil texture consisting mainly of soil, sand, and

10.3389/fcosc.2024.1386204

TABLE 3 Effect of different growth hormones on vegetative propagation of *Phytolacca acinosa*.

Treatment	Concentration	Sprouting percentage	Root length (cm)	Shoot length(cm)	Leaf number	Time taken for sprouting (days)
Kinetin	50ppm	41.67±8.33*[a]	5.5±0.29[ab]	5.38±0.38[a]	7.13±0.31[ab]	31.5±0.65[d]
Kinetin	100ppm	50±9.62[ab]	5.75±0.48[abc]	5.63±0.55[a]	7.5±0.29[abc]	31.5±0.87[d]
Kinetin	150ppm	58.33±8.33[abc]	6.25±0.48[abc]	6.25±0.25[ab]	8.25±0.48[bc]	32.25±0.63[d]
Kinetin	200ppm	41.67±8.33[a]	5.5±0.29[ab]	5.75±0.48[a]	7.25±0.63[ab]	33.5±0.65[de]
IBA	50ppm	0±0	0±0	0±0	0±0	0±0
IBA	100ppm	0±0	0±0	0±0	0±0	0±0
IBA	150ppm	0±0	0±0	0±0	0±0	0±0
IBA	200ppm	0±0	0±0	0±0	0±0	0±0
IAA	50ppm	0±0	0±0	0±0	0±0	0±0
IAA	100ppm	0±0	0±0	0±0	0±0	0±0
IAA	150ppm	0±0	0±0	0±0	0±0	0±0
IAA	200ppm	0±0	0±0	0±0	0±0	0±0
GA3	50ppm	75±8.33[bcd]	7.5±1.04[bcd]	12±0.91[d]	9.75±0.63[cd]	26.5±0.87[bc]
GA3	100ppm	83.33±9.62[cd]	7.75±0.85[cd]	10±0.58[cd]	8±1.35[abc]	25±0.71[ab]
GA3	150ppm	91.67±8.33[d]	9.25±0.48[d]	16.5±0.65[e]	11.25±0.63[d]	23.25±0.75[a]
GA3	200ppm	50±9.62[ab]	7.5±0.29[bcd]	9.25±0.48[c]	7.5±0.65[abc]	28.5±0.87[c]
Control	–	33.33±0[a]	5.25±0.48[a]	8±0.71[bc]	5.75±0.25[a]	35.75±0.85[e]

*Mean±SE.
Different letters in the same column indicate means that are significantly different among different treatments (Tukey test: $p \leq 0.05$).

pebbles. Porous sandy soil is best for successful mass cultivation as it allows the plant to develop a thick, deep root system (Chauhan, 1999). According to Banday et al. (2014), porous sandy soil allows enough aeration and easy penetration of roots and, therefore, is suitable for propagating and developing robust rootstock in *Jurinea dolomiaea*. Many researchers (Devkota and Jha, 2009; Deshmukh, 2010; Hassan et al., 2020) also reported that sandy loam soils result in maximum growth and higher yields in various economically important medicinal plant species. Similarly, in *Paris polyphylla*, maximum sprouting and rooting were found in sandy soils (Kavita et al., 2015). Chauhan and Bhatt (2000) found that sand particles, being larger than soil particles, hold more oxygen and less water due to rapid drainage, resulting in improved aeration compared to compactly packed soil particles.

Our study also reflected a positive growth response of rhizomes to adding vermicompost. Rhizome cuttings planted in the soil, sand, and pebbles mixed with vermicompost lead to a significant increase in morphological parameters such as rhizome length, rhizome breadth, plant height, leaf number, and leaf dimensions. The aboveground and belowground biomass also increased significantly in the soil:sand:pebbles:vermicompost (1:1:1:1) combination (SC10), indicating the importance of vermicompost in generating a higher biomass yield. Our results are in line with those obtained for *Angelica glauca* and *Heracleum candicans* (Butola and Badola, 2007), which reported a significant influence of sandy loam soil mixed with vermicompost on the cultivation and growth parameters of these species. The addition of vermicompost to soil enhanced germination, root biomass, and pod number in pea crops (Bhadauria et al., 2014). In *Polygonatum cirrhifolium*, vermicompost addition to the soil leads to increased biomass production (Lohani et al., 2011). Plant growth is significantly impacted by the availability of soil resources, particularly nutrients (Bazzaz and Bazzaz, 1996). Several researchers (Lohani et al., 2011; Rather et al., 2022) also reported an increase in plant biomass with the application of vermicompost. The composted organic matter supplies key nutrients like nitrogen and phosphorus essential for the proper growth and development of plants (Sánchez et al., 2017). Incorporating organic materials such as compost, manure, and animal dung into the soil not only boosts crop yield but also contributes to maintaining soil fertility for longer periods of time. Additionally, organic fertilizers enhance both soil nutrient retention and water-holding capacity (Ali et al., 2018). These fertilizers also promote microbial growth and activity, which is crucial for breaking down soil nutrients and making them readily available to plants (Chen, 2006).

The development of efficient integrated procedures for the vegetative propagation of plants is of great importance for their cultivation, regeneration, and conservation. Considering the urgent need to restore the diminished natural populations of *P. acinosa*, our research could serve as a foundational reference. This could enable conservationists to implement a multifaceted approach in

devising prompt restoration and regeneration strategies for this multipurpose medicinal herb.

Conclusion

Vegetative propagation is one of the most effective methods to regenerate and sustain plant diversity. The knowledge of vegetative reproduction requirements, growth hormone effects, and soil compositions is important for mass cultivation, restoration, and *ex situ* conservation of medicinally valuable species. Our study is the first report on the successful propagation of *P. acinosa* through rhizomes. The current study revealed that the application of hormones, especially Gibberellic acid and Kinetin, proved effective in reducing sprouting time and promoting sprouting percentage, rhizome, and shoot length in rhizome cuttings. The plants also showed maximum growth performance in highly porous, loosely packed soils consisting of soil, sand, pebbles, and vermicompost (1:1:1:1). The addition of vermicompost to the soil enhanced the vegetative growth of the plant. Thus, propagation through rhizome cuttings proves a convenient and cost-effective method for large-scale cultivation and conservation of the target species under *ex situ* conditions. The present study provides a basic guide for the regeneration and conservation of *P. acinosa*, along with scientific insights for the restoration of threatened endemic biodiversity elsewhere in the world. Its implications are significant for achieving successful germination, and large-scale cultivation and serve as a sustainable approach for utilizing and conserving the target species, thereby reducing its exploitation in wild habitats.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

JM: Conceptualization, Data curation, Formal analysis, Methodology, Software, Visualization, Writing – original draft. BW: Writing – review & editing. HJ: Data curation, Formal analysis, Writing – review & editing. TI: Data curation, Formal analysis, Writing – review & editing. AG: Conceptualization, Investigation, Methodology, Supervision, Validation, Writing – original draft. RQ: Writing – original draft. IN: Conceptualization, Investigation, Supervision, Validation, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

We are grateful to the research scholars of PRBGPR Laboratory, Department of Botany, University of Kashmir, for their kind assistance and support during the present study. Author (JM) highly acknowledges the Maulana Azad National Fellowship (MANF), Govt. of India, for funding under MANF-201920-JKO416200268, during the study period.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor SP declared a past co-authorship with the author TI.

The reviewer ZW declared a past co-authorship with the author TI to the handling editor.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcosc.2024.1386204/full#supplementary-material

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