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Evaluating Seed and Stem Cutting Methods for Efficient Propagation of Passiflora foetida

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The escalating medicinal demand for *Passiflora foetida* has led to extensive harvesting from natural habitats, resulting in genetic erosion and posing a significant threat to its survival. This study aimed to develop and evaluate effective propagation methods through seed and stem cutting techniques to facilitate sustainable cultivation and support long-term conservation strategies.

Study Design: A factorial experimental study comprising two separate trials on seed germination and vegetative propagation.

Place and Duration of Study: The study was conducted at the Institute for Agro-technology and Rural Sciences (UCIARS), University of Colombo, Sri Lanka, from August to December 2023.

Methodology: Two factor factorial experiments were carried out in a controlled net house environment. The first experiment assessed the impact of germination-inducing treatments, gibberellic acid (GA_3), hot water soaking, and no treatment on seeds grown in three different media:

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sand, topsoil, and a sand-topsoil (1:1) mixture. Parameters such as germination rate, seedling height, and vigor index were evaluated. The second experiment focused on the rooting performance of softwood, semi-hardwood, and hardwood stem cuttings planted in sand, coir dust, and a sand-coir (1:1) mixture. Root initiation, sprouting rate, and survival percentage were recorded.

Results: GA_3 treatment significantly improved seed germination rate and seedling vigor across all media types (P < 0.05), while hot water treatment in topsoil yielded the highest survival rate. Among cutting types, softwood cuttings exhibited the best rooting success and sprouting performance, particularly in the sand-coir mixture, suggesting optimal aeration and moisture retention. Hardwood cuttings had the lowest performance across all media.

Conclusion: The study establishes baseline propagation protocols for *P. foetida*, demonstrating the effectiveness of GA_3 for seed germination and softwood cuttings in sand-coir media for vegetative propagation. These methods offer flexible solutions for cultivation and conservation of this overexploited medicinal species.

Keywords: Growing media; GA₃; Passiflora foetida; propagation; rooting efficiency.

1. INTRODUCTION

The genus *Passiflora*, commonly known as passion flowers, comprises approximately 550 known species, with only 60 being classified as edible (Ulmer and MacDougal, 2004). This diverse genus is widely recognized for its unique floral morphology, economic significance in horticulture, and its traditional and medicinal applications across various cultures (Vijay et al., 2021). Among these species, *Passiflora foetida L.*, commonly referred to as the stinking passionflower, is one of the lesser-known varieties. Despite its medicinal potential, this species remains largely underutilized and underresearched, particularly in Sri Lanka (Ratnayake et al., 2020).

Phytochemical investigations of P. foetida have revealed the presence of bioactive compounds such as phenols, alkaloids, glycoside flavonoids, and cyanogenic compounds, which exhibit antimicrobial, anti-inflammatory, antioxidant, and anxiolytic properties (Patil et al., 2013: Chinnasamy et al., 2018). Traditionally, the plant been utilized for treating has wounds. inflammation, insomnia, and digestive disorders (Rasool et al., 2011; Anand et al., 2012; Chiavaroli et al., 2020). While local communities continue to use P. foetida in folk medicine, its agronomic potential remains largely unexplored.

The increasing demand for natural medicinal plants has led to extensive harvesting of *P*. *foetida* from wild populations, contributing to its genetic erosion and threatening its conservation (Ghosh et al., 2019). Overexploitation, coupled with habitat destruction, has resulted in a decline in its natural populations, highlighting the urgent

need for sustainable propagation techniques (Ocampo et al., 2010). Although *P. foetida* demonstrates resilience to suboptimal growing conditions and is well-suited for cultivation in dry and intermediate agro-ecological zones, its classification as a weed and the challenges associated with its propagation have contributed to its neglect in commercial agriculture (Takim et al., 2012).

One of the major constraints to cultivating P. foetida is its poor and inconsistent seed germination, which is primarily attributed to dormancy mechanisms, particularly the presence of a hard seed coat (Gutiérrez et al., 2011; Torres, 2018). Seed dormancy, while serving as an adaptive mechanism for survival under unfavorable conditions, poses a significant challenge for large-scale propagation (Baskin and Baskin, 2014). To overcome dormancy and enhance germination rates, various seed pretreatment techniques have been employed, including physical (scarification and hot water), chemical (growth regulators like gibberellic acid), and physiological treatments (cold stratification) (Hartmann et al., 2011; Gilani et al., 2019; Seng and Cheong, 2020; Angelini et al., 2021). Among these, gibberellic acid (GA₃) has been widely recognized for its role in breaking seed dormancy by promoting enzymatic activity, weakening the seed coat, and stimulating embryo growth (Bewley et al., 2013; Domingues Neto et al., 2024a).

Gibberellic acid (GA₃) has been widely recognized for its role in promoting seed germination across various *Passiflora* species by breaking dormancy and enhancing seedling vigor (Ferrari et al., 2008). In parallel, hot water treatment has proven effective as a pre-sowing technique, facilitating water uptake by softening the hard seed coat, thereby improving germination rates (Jasper et al., 2019). Despite these established methods, research on their specific applicability to Passiflora foetida remains limited, especially under the unique agro-climatic conditions of Sri Lanka. This lack of localized data underscores the need for targeted investigations into the efficacy of germinationinducing treatments and optimal propagation strategies tailored to P. foetida within this regional context.

In addition to seed pre-treatment methods, the choice of growing medium plays a critical role in seedling establishment and early growth (Paula et al., 2020). An ideal potting medium should provide a balance of aeration, moisture retention, and nutrient availability to support seedling development. Commonly used growing media include topsoil, sand, and organic matter-based mixtures. While topsoil is nutrient-rich and supports plant nutrition, it may retain excess moisture, leading to fungal infections. In contrast, sand facilitates good drainage but lacks essential nutrients (Paixão et al., 2021). A combination of topsoil and sand has been shown to provide a balanced substrate. improving seedling establishment in various Passiflora species (Ghosh et al., 2019). Optimizing these growing media conditions is crucial for enhancing the survival and growth of *P. foetida* seedlings.

In addition to seed propagation, vegetative propagation through stem cuttings offers an alternative approach, especially for maintaining genetic uniformity and faster plant establishment. However, the type of cutting, softwood, semihardwood, or hardwood, along with the rooting medium plays a critical role in determining rooting success and survival.

In Sri Lanka, *P. foetida* naturally thrives in dry and intermediate zones, demonstrating resistance to pests and tolerance to poor soil conditions (Rathnayake et al., 2020). However, due to the lack of targeted research and propagation efforts, this species remains neglected in commercial agriculture. Farmers and researchers have yet to explore its full agronomic potential, limiting its large-scale cultivation despite its adaptability.

This study aims to develop a standardized macro-propagation protocol for *P. foetida* through seed propagation. Specifically, it seeks to identify

the most effective seed pre-treatment method and the optimal growing medium to enhance germination rates and seedling growth. By providing crucial insights into the propagation of *P. foetida*, this research will support conservation efforts by reducing reliance on wild populations and promoting sustainable cultivation practices. Furthermore, the study aligns with broader biodiversity conservation strategies, ensuring the sustainable utilization of medicinal plant resources.

2. MATERIALS AND METHODS

2.1 Experimental Location

The experiment was conducted from August to December 2023 in a farm net house at the Institute for Agro-technology and rural Sciences University of Colombo (UCIARS), Weligatta, Hambantota, Sri Lanka, belongs to low country dry zone and has a tropical wet and dry climate (As) according to the Köppen climate classification

2.2 Planting Materials

Selection of plant material carried out for two experiments. For the first experiment, *P. foetida* seeds obtained from well-ripened fruits from the UCIARS premises were used and for the second experiment softwood, semi-hardwood, and hardwood cuttings obtained from plants within UCIARS premises were used (Thimba & Itulya, 1982).

2.3 Experiment 01

Experiment 1 was conducted to identify the best germination inducing agent with different potting media for seed propagation of *P. foetida*. The experimental was arranged in a two-factor factorial Complete Randomized Design (CRD) with nine treatment combinations with two germination inducing agents and three potting media type. Each treatment was replicated four times and each replicate consisted of 10 seeds. The treatments were as follows;

- T1 Gibberellic Acid + Sand
- T2 Gibberellic Acid + Top soil
- T3 Gibberellic Acid + Sand and Top soil (1:1)
- T4 Hot water + Sand
- T5 Hot water + Top soil
- T6 Hot water + Sand and Top soil (1:1)
- T7 No treatment + Sand

T8 – No treatment + Top soil T9 – No treatment + Sand and Top soil (1:1)

2.3.1 Seed treatment

All seeds were thoroughly washed with tap water at room temperature and soaked in water for 24 hours and then air dried (Angelini et al., 2021). Then all the seeds were divided into three parts, and seed treatment was done.

In gibberellic acid seed treatment, the seeds were treated for 24 hours with gibberellic acid (400 mg L⁻¹ ppm) (Gil et al., 2015). In hot water treatment, seeds were immersed in water in 70 °C for 10 seconds and then were immediately sowed in the seed trays (Niwarinda & Nyamweha, 2019). No treatment was performed to the control treatment.

2.3.2 Media preparation and sowing

For the experiment, three potting media (top soil, sand, top soil: sand=1:1) (Jone, 2017) were selected. All potting media and nursery trays were sterilized using captan fungicide (1L water for 2g captan). Each nursery tray hole was filled with a planting medium according to the experimental layout. The treated seeds were then sown in nursery trays and water was supplied in the morning and evening.

2.3.3 Data collection

Data collection started as soon as seedlings started to emerge. Accordingly, germination percentage, days taken for 50% germination, survival percentage, and number of leaves were measured. A ruler was used to measure the height of the seedlings. Germination was confirmed by the emergence of at least 2 mm of the radicle (Hadas, 1976).

Germination percentage determined by $G = (N/A) \times 100$, where G is the germination percentage, N is the number of germinated seeds, and A is the total number of seeds tested. Survival percentage (S) calculated by S = (Number of surviving plants at end of study / Number of planted seeds) * 100.

2.4 Experiment 02

Experiment 2 was conducted to identify the best cuttings type with media for rooting of cuttings propagation of *P. foetida*. The experiment was carried out in a CRD factorial (two-factor) design and 09 treatments with four replicates. Each

replication consisted of 05 plants and treatments were as follows;

- T1 Softwood cuttings + Sand media
- T2 Softwood cuttings + Coir dust media
- T3 Softwood cuttings + Sand and Coir dust (1:1) media
- T4 Semi hardwood cuttings + Sand media
- T5 Semi hardwood cuttings + Coir dust media
- T6 Semi hardwood cuttings + Sand and Coir dust (1:1) media
- T7 Hardwood cuttings + Sand media
- T8 Hardwood cuttings + Coir dust media
- T9 Hardwood cuttings + Sand and Coir dust (1:1) media

2.4.1 Cuttings preparation

Softwood cuttings, semi-hardwood cuttings, and hardwood cuttings were obtained based on their color, and the root of the cutting was prepared using a 45-degree cut with three pulling nodes (Thimba & Itulya, 1982).

2.4.2 Media preparation and cutting establishment

For the second experiment, three types of potting media (sand, coir dust, and a 1:1 mixture of sand and coir dust) (Bemkaireima et al., 2019; Bhardwaj and Kumar, 2020; Paula et al., 2020) were selected. All media and nursery trays were sterilized using Captan fungicide (2g per 1L water) and prepared three days prior to planting. Polybags (4cm \times 4.5cm) were filled with the prepared media and arranged in a net house.

P. foetida stem cuttings were collected early in the morning and immediately placed in clean water-filled baskets. Only pest-free, healthy cuttings with three root nodes were selected and cut using sterilized scissors and planted. Cuttings were categorized as softwood, semi-hardwood, or hardwood and labeled accordingly (Thimba & Itulya, 1982). Once planted, water and fungicide solutions (2g per 1L water) were applied and then the cuttings were placed in a multipropagator of 2 feet in height, 2 feet in width, and 3 feet in length covered with 500-gauge transparent polythene (Moura et al., 2020).

2.4.3 Data collection

Data for this experiment was collected two weeks after planting the cuttings, with additional observations recorded at 2, 4, and 6 weeks for the three types of propagators. The key parameters measured included rooting percentage, survival percentage, number of sprouts, root length, and root dry weight.

2.5 Data Analysis

Analysis of variance (ANOVA) was performed for all the data at the 5% probability level using the statistical analysis system (SAS 9.1.3) software. Duncan's Multiple Range Test (DMRT) was used for mean separation.

3. RESULTS AND DISCUSSION

3.1 Experiment 01 - Evaluation of the Effect of Seeds Germination Inducing Agent and Media Type for *Passiflora foetida* Seeds

3.1.1 Germination percentage of seeds

The statistical analysis revealed a less significant interaction (P > 0.05) between the tested factors, specifically the germination inducing agent and types of media, concerning the germination of P. foetida seeds. Notably, the germination inducing agent exhibited a highly significant effect (P < 0.023, P < 0.000, P < 0.002) during the 7th, 8th, and 9th days, significantly influencing the germination percentage. Among the three germination-inducing agents evaluated. GA3 germination demonstrated the highest percentage (72% and 79%), whereas hot water (59% and 62%) and the control group (50% and 62%) exhibited the lowest germination percentages during the 7th and 8th days respectively (Fig. 1.a).

Gibberellic acid plays a crucial role in germination by stimulating the production of enzymes that break dormancy and activate metabolic processes essential for germination. This includes promoting the synthesis of proteins, carbohydrates, and other compounds vital for the plant's initial development, as well as regulating the hydrolysis of energy reserves through the induction of new α -amylase, the enzyme responsible for starch hydrolysis (Taiz et al., 2017; Sousa et al., 2020). The efficacy of gibberellic acid in increasing the germination rate is corroborated by previous studies, (Domingues Neto at al., 2024a; Domingues Neto at al., 2024b), although the responses may vary depending on the species and doses used.

Furthermore, a significant difference (P < 0.006, P < 0.009) was observed among various media types during the 7th and 8th days of the experiment (Fig. 1.b). Specifically, topsoil and sand + topsoil media displayed the highest germination percentages, while sand germination vielded lowest media the percentage of 54%. These findings contribute valuable results into the factors influencing the germination process of P. foetida seeds, emphasizing the significant role of germinationinducing agents and the type of media used.

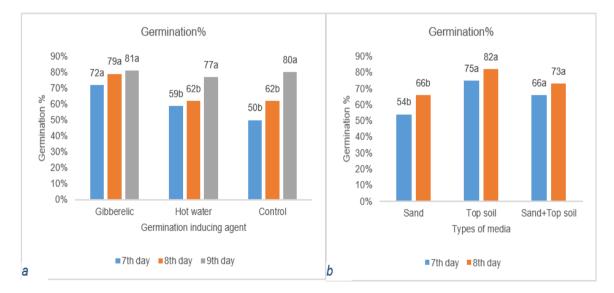


Fig. 1. Effect of seed germinating agent (a) and media type (b) on germination percentage of *P. foetida* seeds

3.1.2 Days taken for 50% of germination

There is no significant interaction (P > 0.05) was found between germination-inducing agents and media types (Fig. 2). However, significant differences were observed among treatment methods. GA₃ treatment reduced the time for 50% germination to eight davs. in contrast to nine days for hot water treatment and control ten davs for the group. This finding underlines the efficiency of GA₃ in expediting the germination process, aligning with broader studies on dormancy-breaking treatments across Passiflora species (Löffler et al., 2022).

3.1.3 Number of leaves

While no significant interaction was detected (P > 0.05) between treatments with respect to the number of leaves of seedlings after two weeks, GA₃-treated seedlings developed a significantly higher number of leaves (average of four), while hot water-treated seedlings produced fewer leaves (Fig. 3). Similarly, seedlings grown in topsoil or the sand-topsoil mix displayed superior leaf development compared to those in sand-only media. These observations compatible with the work of Ożarowski (2011), who noted the positive influence of GA₃ on vegetative growth parameters in medicinal plant species.

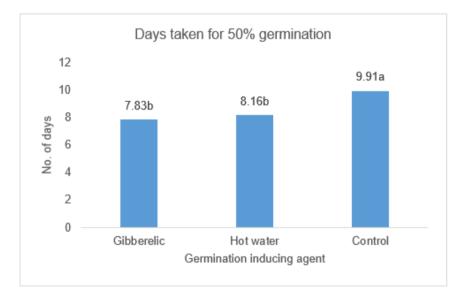


Fig. 2. Effect of seed germinating agent and media types on days taken for 50% germination of *P. foetida* seeds

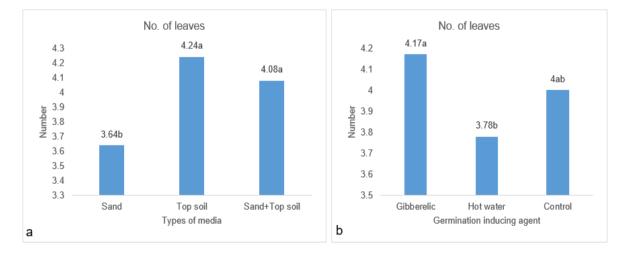


Fig. 3. Effect of seed germination inducing agent (a) and media type (b) on number of leaves of *P. foetida* seedlings after two weeks

3.1.4 Survival percentage of seedlings

Seedling survival rates showed a significant interaction between seed treatment and media type (P < 0.05). Interestingly, the highest survival rate (92%) was recorded in seeds treated with hot water and planted in topsoil. Conversely, the lowest survival rates were observed in seeds treated with hot water and planted in sand (60%) and untreated seeds grown in topsoil (65%) or the sand-topsoil mix (67%) (Fig. 4). These results suggest that although GA₃ improved germination and leaf production, hot water treatment in nutrient-rich media like topsoil may better support seedling survival, possibly due to less stress accelerated physiological from changes (Ożarowski, 2011).

3.2 Experiment 02 - Evaluation of the Effect of Different Cutting Types and Different Media Type for *Passiflora foetida* Cuttings Propagation

3.2.1 Rooting percentage

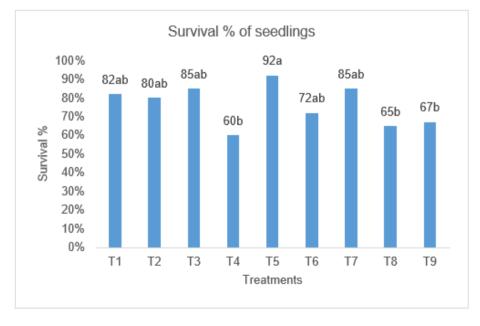
Rooting percentage showed no significant interaction between cutting type and media; however, cutting type alone had a significant effect (P < 0.05). Softwood cuttings displayed the highest rooting percentage compared to semihardwood and hardwood cuttings (Fig. 5). These findings are supported by earlier studies (Bemkaireima et al., 2019; Bhardwaj and Kumar, 2020; Paula et al., 2020), which reported that softwood cuttings, being physiologically active and less lignified, roots grow more effectively due to higher auxin sensitivity.

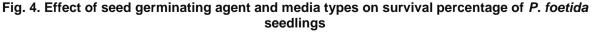
3.2.2 Survival percentage

A significant interaction (P < 0.05) was found between cutting type and media for survival percentage at week four. Softwood and semihardwood cuttings planted in sand-coir dust mixtures or sand alone exhibited higher survival rates, while cuttings planted in coir dust alone had the lowest survival (Fig. 6). The results indicate that the coir dust media may retain excessive moisture, which could lead to decay in tender softwood cuttings. These findings are consistent with the recommendations by Jone (2017), highlighting that medium aeration and drainage are critical for vegetative-propagation.

3.2.3 Number of sprouts

Sprout development also varied with cutting type. During the second week, hardwood cuttings produced the most sprouts, while by the sixth week, softwood cuttings had the highest sprout count (Fig. 7). Hardwood cuttings later showed reduced sprouting, possibly due to depletion of internal reserves. These dynamics reflect the varying physiological stages of cuttings and their capacity for sustained growth (Moura et al., 2020).





Bamunusinghe et al.; Asian J. Res. Crop Sci., vol. 10, no. 2, pp. 98-109, 2025; Article no.AJRCS.134462

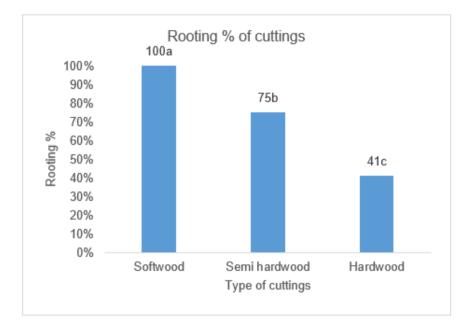
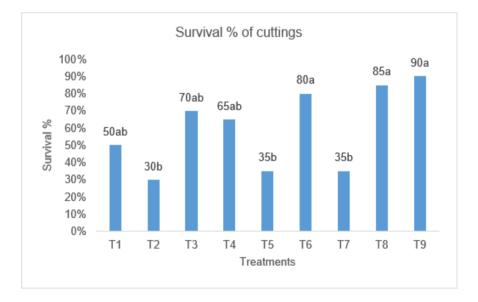
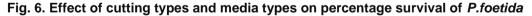


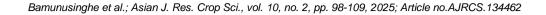
Fig. 5. Effect of cutting types on percentage rooting of P. foetida





3.2.4 Roots length

A significant interaction (P < 0.05) of cutting types and media types was observed on roots length of *P. foetida* during 2nd week (Fig. 8). Softwood cuttings planted all the type of media showed higher root length values. This is because softwood cuttings generally possess higher levels of endogenous auxins and active meristematic tissues, which promote rapid root initiation and elongation (Hartmann et al., 2011). The consistent rooting success of softwood cuttings across various media also indicates their adaptability. Media composition greatly influences rooting by affecting aeration, water retention, and nutrient availability (Angami, et al., 2019). The rooting media used in this study may have created favorable physical and chemical conditions that enhanced root elongation in softwood cuttings. Previous studies on *Passiflora* spp. and other tropical plants have shown that well-drained, aerated media like sand, cocopeat, or their combinations support better root development (Salehi 2014; Angami, et al., 2019).



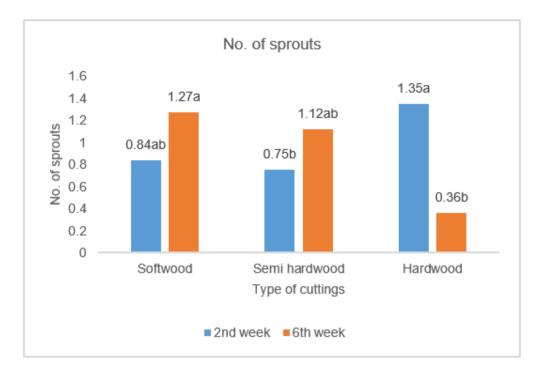
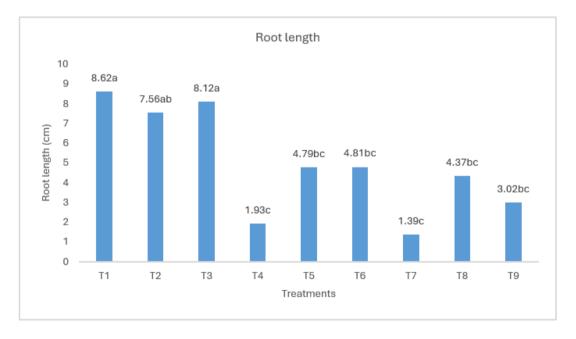


Fig. 7. Effect of cutting types on number of sprouts





3.2.5 Roots dry weight

There is no significant interaction (P>0.05) average roots dry weight produced in P. foetida (Fig. 9). But, there is a significant difference between different cutting types during 2nd and 6th week. Higher weight of roots dry weight by

softwood and semi hardwood cutting for both weeks and lowest weight of roots dry weight by hardwood cuttings during 2nd and 6th week. A study on (Moura et al., 2020) vegetative propagation by cuttings of Passiflora species support these findings.

Bamunusinghe et al.; Asian J. Res. Crop Sci., vol. 10, no. 2, pp. 98-109, 2025; Article no.AJRCS.134462

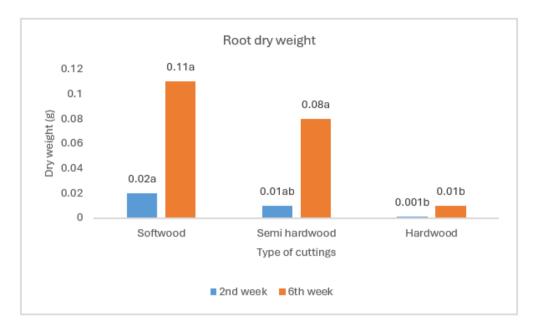


Fig. 9. Effect of cutting types on roots dry weight

4. CONCLUSIONS

In seed-based propagation, gibberellic acid (GA₃) treatment emerged as the most effective method for improving germination percentage, reducing the time required for 50% germination, and promoting vegetative growth. However, for seedling survival, hot water-treated seeds sown in nutrient-rich topsoil demonstrated the best potential results, suggesting trade-off а between rapid germination and seedling vigor. Vegetative propagation through stem cuttings proved to be a viable alternative, particularly using softwood cuttings, which showed the highest rooting and sprouting performance. The combination of softwood cuttings with a sand-coir dust medium provided an ideal balance of moisture retention and aeration, leading improved survival rates and root to development.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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