



RESEARCH ARTICLE

Comparative Germination of Stem-cuttings vs Seed of *Stevia rebaudiana*

Syed Shabab Hussain¹, Abdur Rauf^{1,*}, Wajid Khan², Tanweer Kumar², Muhammad Arif², Kashmala Jabbar¹, Guleena Khan¹, Wisal Khan¹, Ruby Wali Khan¹, Majeed Ullah¹, Asghar Ali¹, Ameer Sultan¹, Farhan Ullah¹ and Ikramullah Khan¹

¹Garden Campus, Abdul Wali Khan University, Mardan, Pakistan

²Sugar Crops Research Institute (SCRI), Mardan, Khyber Pakhtunkhwa, Pakistan

*Corresponding author: rauf77@awkum.edu.pk

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ABSTRACT

Stevia rebaudiana, a perennial shrub known for its sweet leaves, is gaining global recognition as a high-potency natural sweetener due to its steviol glycosides (SvGs), which are 300 times sweeter than sucrose. This research focuses on enhancing the cultivation of *Stevia rebaudiana* for sustainable production of steviol glycosides, with a particular emphasis on optimizing growth parameters through a novel clonal propagation method. Two groups of *Stevia rebaudiana*, one from stem cuttings and the other from seeds, were subjected to identical experimental setups in beds enriched with a mixture of pure soil and agricultural waste. The growth parameters, including plant height, leaf area, root length, stem diameter, and number of roots, were meticulously measured at regular intervals from 40 and 120 DAP (days after plantation). Our findings highlight the effectiveness of the stem tip clonal propagation over the seed-grown method in establishing a genetically uniform population of *Stevia rebaudiana*. Throughout this comprehensive study, the technique of stem cutting consistently demonstrated its dominance, highlighting superior growth characteristics in various parameters in fostering enhanced plant height, root length, leaf area, number of roots, and stem diameter. The sustained dominance of stem cutting across various parameters underscores its efficacy in promoting robust *Stevia* plant growth over an extended 120-day period. The study contributes valuable insights into optimizing *Stevia rebaudiana* cultivation for sustainable and high-yielding production of steviol glycosides.

Key words: Stem-cuttings, Seed and *Stevia rebaudiana*

INTRODUCTION

Stevia rebaudiana is an emerging economic species throughout the world (Sreedhar et al., 2008) that belongs to the family Asteraceae. *Stevia* is a sweet herb commonly known as “honey leaf” in the Indian market (Rayaguru and Khan, 2008). *Stevia*, a small, semi-bushy, perennial shrub native to the valley of the Rio Monday of Paraguay, is rapidly gaining popularity worldwide as a high-potency natural sweetener (Katayma et al., 1976). The Guaraní people of Paraguay have used *stevia* leaves for centuries to sweeten their drinks and foods. *Stevia* is one of 154 species in the genus *Stevia*, but only *Stevia rebaudiana* Bertoni produces significant amounts of stevioside, a diterpenoid glycoside that is 200-300 times sweeter than sucrose (Hwang, 2006). Stevioside, rebaudioside A, rebaudioside C, and

dulcoside A, are the four major steviol glycosides, which are natural sweeteners found in the leaves of the *Stevia rebaudiana*. Stevioside is the sweetest of the four, at 210 times sweeter than sucrose, followed by rebaudioside A at 242 times sweeter, and rebaudioside C and dulcoside A at 30 times sweeter. The yield of steviol glycosides in leaf tissues can vary depending on the method of propagation (Tamura et al., 1984; Rauf et al., 2022).

S. rebaudiana Bertoni produces sweet diterpene glucosides, such as stevioside and rebaudioside A, which have been used as natural sweeteners in Japan. Because the plant is highly self-incompatible and genetically heterozygous, sexually propagated plants exhibit significant variation in growth and glucoside contents. To create a genetically uniform population, a stem tip clonal propagation method for *Stevia* was

developed (Tamura et al., 1984). Stevia species are widely used to treat diabetic patients because their natural steviol glycosides cannot enter the bloodstream due to a lack of absorption receptors. The sweet substances present in Stevia are not harmful to diabetics, making it a valuable resource for this large population. Stevia can also contribute to the national economy by replacing sugar largely. Stevia is a promising alternative to sugar for people with diabetes, obesity, and other health conditions. Steviol glycoside does not metabolize in the body, so it has no calories and does not raise blood sugar levels. Stevia is also heat-stable, making it suitable for use in a variety of food and beverage products. Stevia is cultivated in over 50 countries around the world, and its production is increasing rapidly. The global stevia market is expected to reach \$3.5 billion by 2028. Stevia is a sustainable crop that requires less water and fertilizer than traditional crops, and it is relatively resistant to pests and diseases (Soejarto et al., 1982).

Seed germination of Stevia is poor, sometimes less than 10%. This is due to several factors, including the hard seed coat, the need for light for germination, and the sensitivity of Stevia seedlings to drought and salinity (Miyazaki and Wantenabe, 1974). Therefore, the current research project was designed to use stem cuttings as an alternate source for future breeding programs.

MATERIALS AND METHODS

Study Area and Experimental Materials

This research project was executed at the Sugar Crops Research Institute (SCRI), Mardan, Pakistan. The region experiences an average annual rainfall of 696 mm, with 488 mm occurring during summer and 208 mm in winter. Summer temperatures average 39.8 °C, while winter temperatures average 13.4°C. The average relative humidity is 60.8% (SCRI). A total of 30 mature Stevia plants were selected for stem cuttings (6-8 inches) and seed germination. The seeds were collected, washed with a mixture of ethanol and detergent, and left to dry for 24 hours.

Experimental Setup and Soil Composition

Two beds of identical size and length were prepared for the experiment, with each bed having dimensions of 101 inches in length and 36 inches in width. Plants were arranged in rows and columns: Row-wise: 3 plants per row and Column-wise: 10 plants per column. The Row-wise distance between plants is 12 inches, and the interplant distance is 10.1 inches. Both groups were planted in a mixture of 70% pure soil and 30% agricultural waste (animal waste). The same soil composition was used for both stem cuttings and seed-grown plants.

Germination Process

Seeds were germinated on a petri dish with tissue paper. Germinated seeds were transferred to the greenhouse for 40 days. After 40 days in the

greenhouse, both stem-cutting-grown and seed-grown plants were shifted to the prepared beds for further growth and observation.

Agronomic Parameters Data

1-Plant Height: Plant height was measured from the surface of the soil to the tip of the last leaf, and 6 plants were randomly selected. The data were recorded from 40 days after plantation (DAP) to 120 DAP, while data records were done three times, each with a one-month gap.

2-Leaf Area: Leaf area was measured by using image analysis software to quantify the leaf surface area from digital images.

3-Root Length: Root length was measured in standard research by employing digital image analysis software to quantify the length from captured root images.

4-Stem Diameter: Stem diameter was measured using digital calipers to obtain precise measurements of the stem's cross-sectional width.

5-Number of Roots: The number of roots was measured per plant, and the average was calculated.

RESULTS AND DISCUSSION

A comparative evaluation of Stevia plants over a 120-day period revealed clear differences between propagation methods, with stem cutting generally outperforming seed germination across most growth traits.

Plant Height

Stem cutting plants were consistently taller than seed-derived plants at all stages. Heights averaged 119.06 cm vs. 77.20 cm at 40 DAP, 137.28 cm vs. 96.20 cm at 80 DAP, and 140.28 cm vs. 118.29 cm at 120 DAP (Fig. 1). This progressive advantage aligns with earlier findings that vegetative propagation enhances shoot vigor and reduces variability compared to seed propagation (Yadav et al., 2011; Patel et al., 2015).

Root Length

Cuttings also exhibited longer roots throughout, increasing from 14.41 cm at 40 DAP to 17.00 cm at 120 DAP, while seed plants ranged from 9.05–11.45 cm (Fig. 2). Longer roots provide stronger anchorage and greater access to soil nutrients and water, supporting superior growth (Das et al., 2007; Kalpana & Ramesh, 2019).

Leaf Area: Foliar development favored cuttings, with leaf areas rising from 12.58 cm² to 17.38 cm², compared with 9.01–13.01 cm² for seeds (Fig. 3). Larger leaves enhance photosynthetic efficiency, thereby contributing to higher biomass and steviol glycoside accumulation (Debnath, 2008; Kumar et al., 2014).

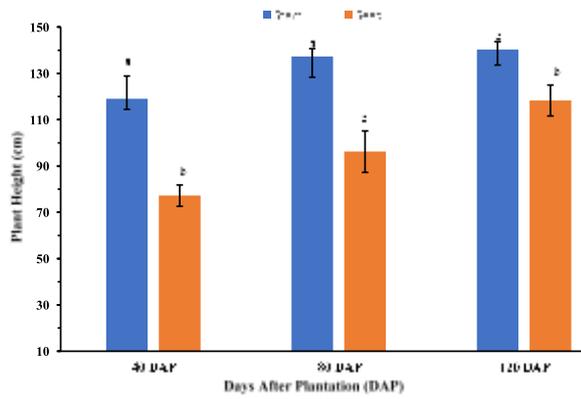


Fig. 1: Data on plant height is shown after seed and stem cutting germination for Stevia. Plant height was recorded at 40, 80, and 120 days after plantation (DAP). The maximum plant height obtained in stem cutting (Blue Bar) vs seed germination (Orange Bar) at different time intervals.

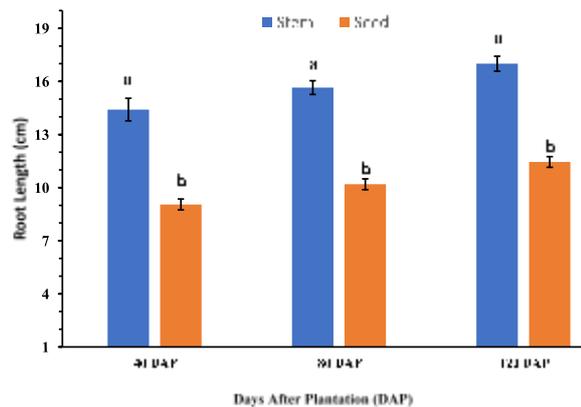


Fig. 2: Data on Root Length is shown after seed and stem cutting germination for Stevia. Root Length was recorded at 40, 80, and 120 days after plantation (DAP). The maximum Root Length obtained in stem cutting (Blue Bar) vs seed germination (Orange Bar) at different time intervals.

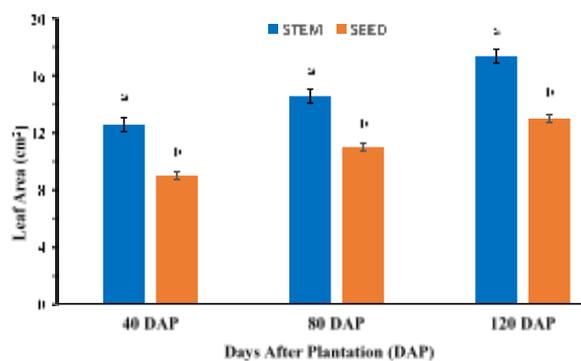


Fig. 3: Data of Leaf Area is shown after seed and stem cutting germination for Stevia. Leaf Area was recorded at 40, 80, and 120 days after plantation (DAP). The maximum Leaf Area obtained in stem cutting (Blue Bar) vs seed germination (Orange Bar) at different time intervals.

Number of Roots: The only trait favoring seeds was root number, with averages of 115.4, 124.9, and 126.5 across the three stages compared with 94.5, 101.5, and 112.5 in cuttings (Fig. 4). However, despite greater root

counts, seed plants developed shorter and thinner roots, a pattern observed in other studies comparing seed- vs. cutting-derived Stevia (Jain et al., 2009). This suggests that higher root numbers alone do not guarantee superior aboveground growth.

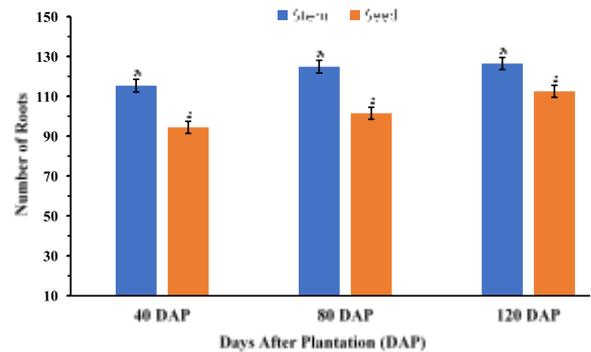


Fig. 4: Data of the Number of Roots shown after seed and stem cutting germination for Stevia. The number of Roots was recorded at 40, 80, and 120 days after plantation (DAP). The maximum Number of roots obtained in stem cutting (Blue Bar) vs seed germination (Orange Bar) at different time intervals.

Stem Diameter: Stem cutting plants exhibited thicker stems (0.751–0.833 cm) than seed-derived plants (0.475–0.565 cm) (Fig. 5). Increased stem girth provides better mechanical strength and resilience, which is critical for field establishment and long-term productivity (Ramesh et al., 2006; Kumar et al., 2014). Overall, stem cutting proved more effective than seed germination for most growth traits. It promoted greater height, root length, leaf area, and stem thickness, while seed propagation only excelled in root number. These findings reinforce the suitability of stem cutting as a reliable propagation method for Stevia, ensuring uniformity, vigor, and enhanced agronomic performance (Yadav et al., 2011; Patel et al., 2015).

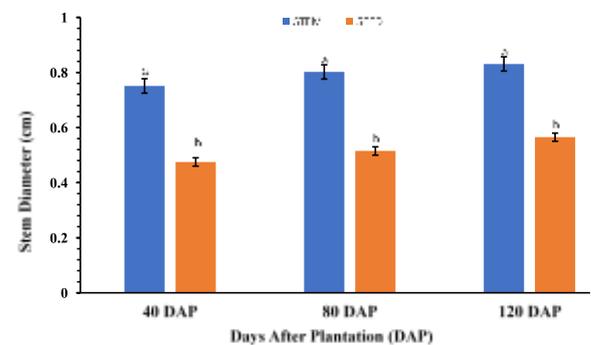


Fig. 5: Data on Stem Diameter is shown after seed and stem cutting germination for Stevia. The Stem Diameter was recorded at 40, 80, and 120 days after plantation (DAP). The maximum Stem Diameter obtained in stem cutting (Blue Bar) vs seed germination (Orange Bar) at different time intervals.

Conclusion

In conclusion, the findings of this study provide compelling evidence that stem cutting emerged as the

superior propagation method for cultivating Stevia plants over 120 days. The consistent dominance of stem cutting over seed germination across multiple parameters, including plant height, root length, leaf area, number of roots, and stem diameter, underscores its holistic efficacy in promoting robust and comprehensive plant growth.

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