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Differential response of *Stevia* leaf-variants to the cutting-mediated clonal propagation

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ABSTRACT

The present study was carried out with an objective to identify most responsive Stevia rebaudiana variant during cutting mediated clonal propagation. A heterogenous population was raised from seeds in the field condition and six different morphological leaf variants of Stevia were selected from the open field condition. The six different four-nodes cuttings were treated with single treatment i.e mixing of 0.5 mg/100 ml of PSB (Phosphate solubilizing bacteria) plus 0.5 mg/100 ml of T21 (Trichoderma asperellum) in 100 ml of solution in laboratory for 1 h followed by planting them in plastic cups filled with soilrite. Out of six morphological leaf variants, the variant-3 showed the highest number of branches and survival in a controlled environment. The largest leaf length, breadth, and the maximum fresh leaf weight, dry leaf weight were recorded in variant-5 on the 42nd day. The variant-3 was the best in performance of biological mass as it produced more branches and higher survival rate over other variants viz., variant-1, variant-2, variant-4, variant-5, variant-6 and it was suitable for the purpose of further cutting mediated clonal propagation.

***Keywords*:** Cutting mediated clonal propagation, Phosphate solubilizing bacteria, Trichoderma, Soilrite.

Stevia rebaudiana (Bert) Bertoni, frequently known as the sweet leaf of Paraguay is an herbaceous perennial plant. It belongs to the family *Asteraceae.* It is a semi-bushy, small perennial shrub. *Stevia rebaudiana* leaves are sweet in taste due to the presence of steviol glycosides (Lemus-Mondaca *et al.,* 2012). Stevioside as a dietary supplement for the human beings has advantages such as it is stable in high temperature and is calorie-free. So, it has significance to diabetic people and diet conscious (Badran *et al.,* 2015). The powdered form of *Stevia* leaves are added to coffee, tea, fruit juices, and extra food products needing sweet taste that possessed both hypoglycemic and bodyweight-reducing effects without any detrimental effects for diabetic patients (Anbazhagan *et al.,* 2010).

Stevia plant shows self-incompatibility, limited pollinator activity, and poor seed set, stevia is often propagated through seed and cutting. This results in the origination of heterozygous plants with different concentrations of glucosides in leaves and a slow rate of multiplication (Gantait *et al.,* 2018). The amount of selfing ranges from 0-0.5 % and the amount of crossing ranges from 0.7-98.7 %. It is a highly cross-pollinated plant and seeds show sterility, low and inconsistent germination (Das *et al.,* 2011; Yadav *et al.,* 2011; Pande and Gupta, 2013; Rock-Okuyucu *et al.,* 2016). *Stevia* can be reproduced sexually by seeds and asexually through vegetative methods such as cutting, grafting, and micro-propagation (Kassahun and Mekonnen, 2011; Singh *et al.,* 2017; Kazmi *et al.,* 2019). Production of new planting materials in *Stevia* depends on multiple factors like the method of propagation, environment, substrata used, etc. *In vitro* clonal propagation, which produces disease-free and uniform plants is one of the most effective ways of vegetative propagation (Pande and Gupta, 2013; Yucesan *et al.,* 2016; Poothong *et al.,* 2018; Pandey *et al.,* 2021).

There is increasing interest in natural sweeteners for the diabetic populations in India in recent years (Siegel *et al.,* 2008). As a result, non-caloric, natural sweeteners that are not dangerous for diabetics are being given more attention now. The response of plants includes production of a greater number of branches as well as survival percentage is important for plant propagation. The artificial soils provide the good environment and basic strength for vegetative growth of plants. Keeping

Short Communication

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Table 1: Mean of different characters of six variants on the 42nd day

	Plant height (cm)	Number of branches	Number of nodes	Number of leaves	Largest leaf length (cm)	Largest leaf breadth (cm)	Fresh leaf weight (mg)	Dry leaf weight (mg)
Variant-1	4.9	1.2	9.2	15.5	2.8	1.1	57.1	14.5
Variant-2	5.8	1.8	9.5	22.3	2.1	0.8	62.4	17.6
Variant-3	8.9	2.7	14.0	28.0	2.4	0.6	39.0	10.4
Variant-4	8.0	2.5	12.5	22.6	2.9	0.6	87.2	26.9
Variant-5	6.4	1.2	8.3	18.0	2.3	0.7	103.7	32.0
Variant-6	7.0	2.2	12.8	25.4	2.1	0.7	35.2	9.9
LSD	2.3	1.1	3.4	7.0	0.5	0.2	18.8	4.7
SE(m)	0.8	0.4	1.2	2.4	0.2	0.1	6.5	1.6
SE(d)	1.1	0.5	1.6	3.4	0.3	0.1	9.2	2.3

Table 2 : Survival percentage of six variants on the 42nd day

	Survival percentage
Variant-1	33.33 %
Variant-2	50.00 %
Variant-3	100.00 %
Variant-4	83.33 %
Variant-5	66.67 %
Variant-6	83.33 %

in view, the study was conducted to identify the ideal plant material for mass multiplication in artificial soil i.e. soilrite for direct regeneration of planting material production from node containing explant.

In the present experiment six morphological leaf variants of *Stevia rebaudiana* cuttings were collected from the field plants at Uttar Banga Krishi Viswavidyalaya, Pundibari. The *Stevia* population developed from seeds in the field condition, six different leaf morphologies based on morphological observation was selected. The study was conducted in the laboratory under controlled environment, located at the foothill region of the Himalayas, known as the Terai zone in West Bengal, Eastern India. The geographical details were 28°19′N latitude and 89°23′E longitude with an altitude of 43 m above the mean sea level (MSL).

Six morphological leaf variants of *Stevia* were denoted as variant-1, variant-2, variant-3, variant-4, variant- 5 and variant-6. Total four-node containing cuttings (replication= 6) of each variant were collected from the field. Each cutting was dipped for 1 h in a single solution prepared by mixing 0.5 mg/100 ml of PSB (Phosphate solubilizing bacteria) + 0.5 mg/100 ml of T21 (*Trichoderma asperellum*) in 100 ml of solution. During planting the cuttings were touched with power enriched with IBA, NAA, PHB, H_3BO_3, Surfactant, Talc, etc., commercially known as 'Toto-Root' and planted in freshly prepared artificial soil. After planting the cuttings in plastic cups containing artificial soil, the cups were kept in transparent boxes to maintain the humidity for 14 days (Fig.1).

Artificial soil used in the experiment was soilrite. Soilrite contained perlite, peat moss and vermiculite. Perlite was reported for maintaining aeration to ensure an excellent air/water balance which impacts on better root growth including better uptake of nutrients in a more effective manner. Peat moss retains moisture for better plant growth which also saves irrigation frequency. Moreover, it releases water and nutrients to the right proportions for optimum plant growth and reduces the application of manuring. Vermiculite was reported to improve soil porosity as well as to act as a medium for water and nutrient exchange.

The controlled environment provided in the entire experiment was 9000 LUX light intensity for 14 hours per day, 22°C temperature, and 70% relative humidity.

At least six replications were developed for each treatment in a completely randomized design (CRD). The data has been recorded after 6 weeks i.e., 42nd day. The data recorded on various characters were subjected to analysis as per standard statistical methods.

The mean performance of different traits for all the variants included in the experiment was presented in Table 1, and the variants response on the 42nd day was presented in Fig. 2 and 3. The maximum plant height (8.9 cm), number of branches (2.7), number of nodes

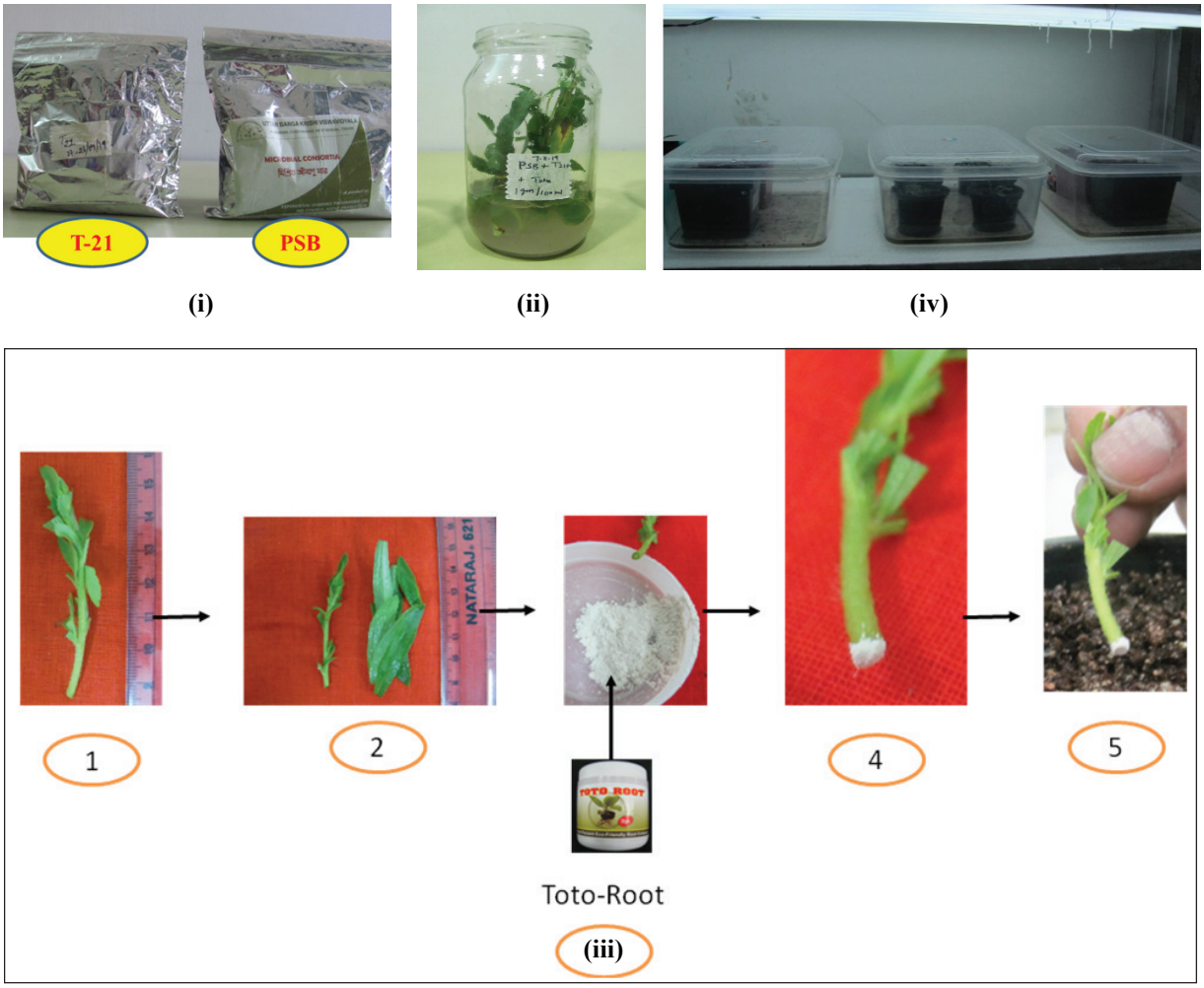

Fig.1. **Preparation of Explants.** (i) T21 (*Trichoderma asperellum*), PSB (phosphate solubilizing bacteria); (ii) PSB (0.5 mg) + T21 (0.5 mg) in 100 ml water; (iii) The process of incubation of hormone enriched powder to the cut end of the harvested cutting and (iv) After planting in artificial soil the pots covered with cups for 14 days.

(14.0), and number of leaves (28.0) was observed in variant-3 on the 42nd day. The maximum leaf length (2.9 cm) was reported in variant-4 on the 42nd day. The maximum leaf breadth (1.1 cm) was reported by variant-1. Highest fresh leaf weight (103.7 mg) and dry leaf weight (32 mg) was reported in variant-4. The highest survival percentage was reported in variant-3 (100.00%) on the 42nd day (Table 2).

The leaf variant-3 was the most perfect combination of more branch production and survival percentage among six leaf variants. Thus, more research will be required for fine-tuning the process for quality planting material production constantly throughout the year and without need of any sophisticated techniques. The present finding and subsequent fine tuning of the process might be explored for quality planting material production.

The major aim of the present experiment is to identify the best available variant for delivering the branches for the next round of multiplication with better survival percentage in artificial soil i.e. soilrite. The current study used a nodal cutting which incubates in the humidified environment with controlled light intensity. In the present experiment, for the mass production of clonal explants, the variant-3 was the best as it produced more branches as well higher survival percentage.

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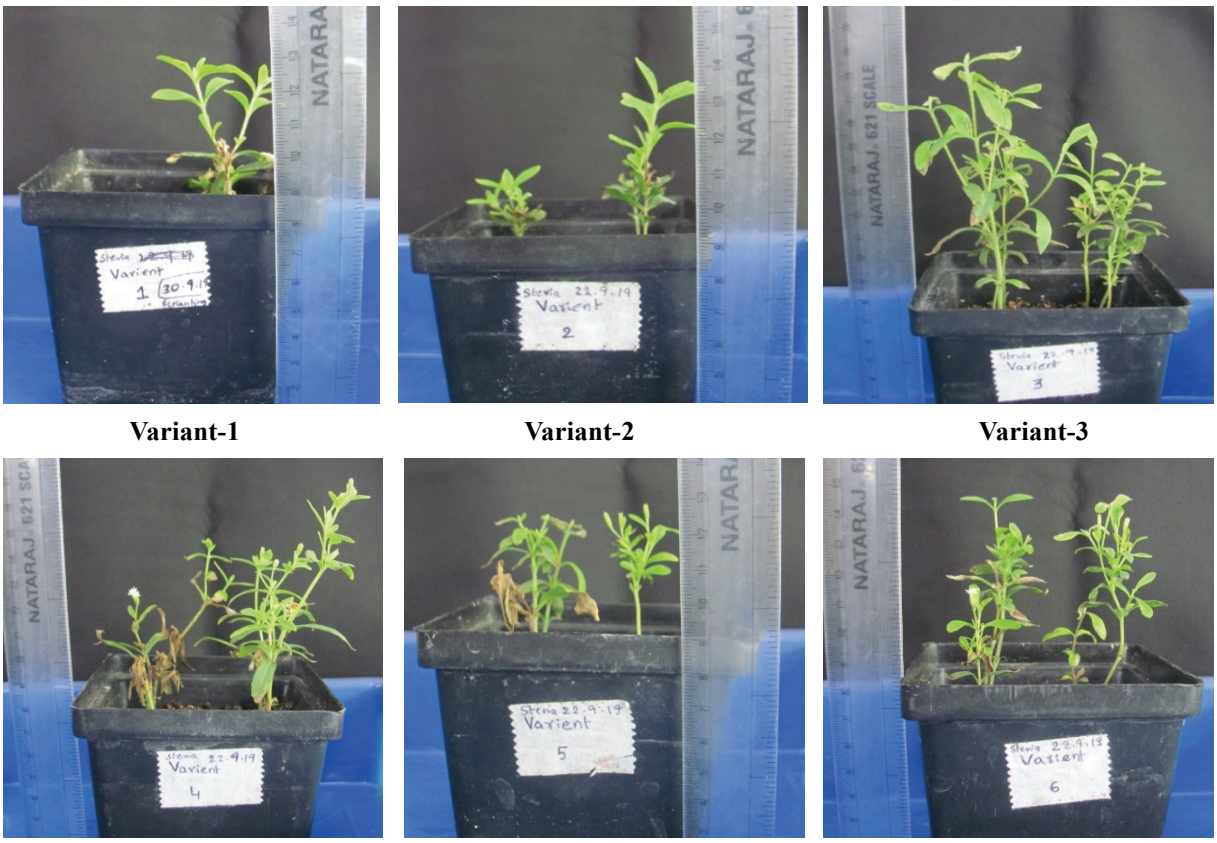

Fig. 2 : *Stevia* four-node cutting variants response at 42nd day in the controlled environment

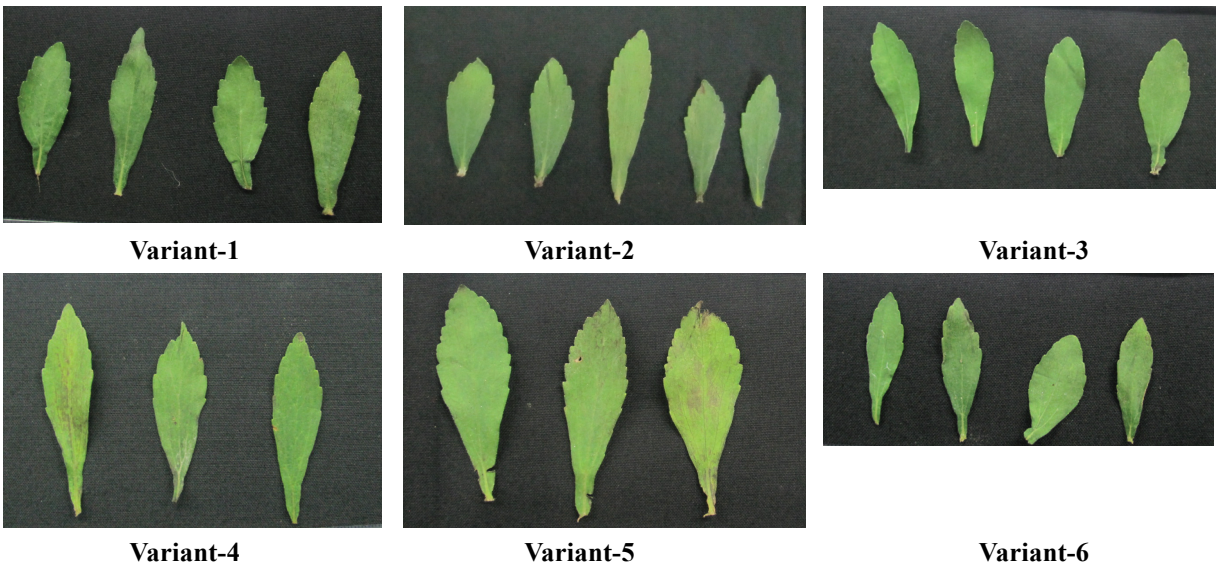

Fig. 3 : *Stevia* single largest leaf at 42nd day from all variants

REFERENCES

Anbazhagan, M., Kalpana, M., Rajendran, R., Natarajan, V. and Dhanavel, D. 2010. In vitro production of *Stevia rebaudiana* Bertoni. *Emirates J. Food Agric.*, **22**: 216-222.

Badran, A. E., Abd Alhady, M. R. and Hassan, W. A. 2015. In vitro evaluation of some traits in *Stevia rebaudiana* (Bertoni) under drought stress and their relationship on stevioside content. *Am. J. Plant Sci.*, **6**:746-752.

Das, A., Gantait, S. and Mandal, N. 2011. Micropropagation of an elite medicinal plant: *Stevia rebaudiana* Bert. *Int. J. Agric. Res*., **6**:40-48.

Gantait, S., Das, A. and Banerjee, J. 2018. Geographical distribution, botanical description and self-incompatibility mechanism of genus Stevia. *Sugar Tech.*, **20**:1-10. https://doi.org/10.1007/s12355-017-0563-1

Kassahun, B. M. and Mekonnen, S. A. 2011. Effect of cutting position and rooting hormone on propagation ability of Stevia (*Stevia rebaudiana* Bertoni). *The African J. Plant Sci. Biotechnol.*, **6**: 5-8.

Kazmi, A., Khan, M. A., Mohammad, S., Ali, A. and Ali, H. 2019. Biotechnological production of natural calorie free Steviol glycosides in *Stevia rebaudiana*: an update on current scenario. *Curr. Biotechnol.*, **8**: 70-84.

Lemus-Mondaca, R., Vega-Gálvez, A., Zura-Bravo, L. and Ah-Hen, K. 2012. *Stevia rebaudiana* Bertoni, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional and functional aspects. *Food Chem.*, **132**:1121-1132. https://doi.org/10.1016/j.foodchem.2011.11.140

Pande, SS. and Gupta, P. 2013. Plant tissue culture of *Stevia rebaudiana* (Bertoni): A review. *J. Pharmacogn. Phytotherapy*, **5**:26-33.

Pandey, D. K., Konjengbam, M., Dwivedi, P., Kaur, P., Kumar, V., Ray, D., Ray, P., Nazir, R., Kaur, H., Parida, S. and Dey, A. 2021. Biotechnological interventions of in vitro propagation and production of valuable secondary metabolites in *Stevia rebaudiana*. *Appl. Microbiol. Biotechnol.*, **105**: 8593-8614. https://doi.org/10.1007/s00253-021-11580-9

Poothong, S., Khen, T. and Chumphukam, O. 2018. In vitro mineral nutrition for improving growth and multiplication of Stevia. *Agric. Natural Resour.*, **52**: 477-483.

Röck-Okuyucu, B., Bayraktar, M., Akgun, I. H. and Gurel, A. 2016. Plant growth regulator effects on in vitro propagation and stevioside production in *Stevia rebaudiana* Bertoni. *Hort. Sci.*, **51**:1573-1580.

Siegel, K., Narayan, K. V. and Kinra, S. 2008. Finding a policy solution to India's diabetes epidemic. *Health Affairs*, **27**: 1077-1090.

Singh, M., Saharan, V., Dayma, J., Rajpurohit, D., Sen, Y. and Sharma, A. 2017. In vitro propagation of *Stevia rebaudiana* (Bertoni): An overview. *Int. J. Curr. Microbiol. Appl. Sci.*, **6**: 1010-1022.

Yadav, A. K., Singh, S., Dhyani, D. and Ahuja, P. S. 2011. A review on the improvement of Stevia [*Stevia rebaudiana* (Bertoni)]. *Can. J. Plant Sci*., **91**: 1-27.

Yücesan, B., Büyükgöçmen, R., Mohammed, A., Sameeullah, M., Altuð, C., Gürel, S. and Gürel, E. 2016. An efficient regeneration system and steviol glycoside analysis of *Stevia rebaudiana* Bertoni, a source of natural high-intensity sweetener. *In Vitro Cellular Dev. Biol. Plant*, **52**: 330-337.