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## STEVIA NODAL EXPLANT INJECTED WITH 6-BENZYLAMINOPURINE SHOWED NEW EMERGENCE IN THE ARTIFICIAL SOIL MEDIA

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### Abstract

A number of reports are available to show successful micropropagation in *Stevia rebaudiana* Bertoni. Mainly, MS (Murashige and Skoog, 1962) media supplemented with different types of hormone was followed under aseptic condition for new emergence and subsequent hardening the plantlets. In the present study, the *Stevia* explant having single and double nodes were considered directly for injection with different concentration, mg/mL and mg/L dose and fixed volume (1.2 µl) of 6-Benzylaminopurine (BAP) with the help of microinjection. The node injected by 0.50 mg/mL of BAP only showed response and emergence is recorded. The mg/L dose of BAP does not work. This is an initial observation. Further experiment will be required for repetitions as well as optimization of factors for achieving efficient new plant emergence bypassing MS based tissue culture mediated new planting material production.

**Key Words :** Stevia, 6-Benzylaminopurine (BAP).

### Introduction

Stevia, botanically known as *Stevia rebaudiana* Bertoni belongs to family- *Asteraceae* is a sweet herb. The leaves are mild green and intensely sweet. It is a perennial, photoperiod sensitive, insect-pollinated, self-incompatible bushy shrub (Yücesan et al., 2016). The sugar present in stevia is a non-caloric and used as an alternative to artificially produced sugar substitute (Ahmed et al., 2007). Stevia leaf is 300 times sweeter than sugar, obtained from sugar beet & sugar cane with zero caloric value (Richman et al., 1999). It has attracted economic and scientific interests due to its sweetness and therapeutic properties present in leaves. Despite the need of good production with high active component content, it has poor germination rate (Goettemoeller and Ching 1999).

Over the many years researchers mainly focused on the direct and indirect organogenesis in invitro condition (Sreedhar et al. 2008; Ahmad et al. 2011; Mathur and Shekhawat 2013; Khalil et al. 2014; Gantait et al. 2015; Ramírez-Mosqueda and Iglesias-Andreu 2015; Yadav et al., 2016; Thilakarathne et al., 2019). But, in the present study mainly focus on bypassing of MS artificial media dependent aseptic culture for clonal proliferation. This will also bypass the down line hardening of the tissue culture derived plant. Moreover, the present process is a very low-cost process for proliferating suitable superior line.

## 2. Materials and Methods

### 2.1 Selection of source material

The explants of *Stevia rebaudiana* were collected from the field plants at Uttar Banga Krishi Viswavidyalaya, Pundibari. The investigation was carried out at a laboratory situated in 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 (141.076 ft) above the Mean Sea Level (MSL).

### 2.2 Preparation of explants

All the treatments used in the present experiment was presented in Table.1. Various amounts of 6-Benzylaminopurine (BAP) are dissolved in the required amount of solvent i.e., 1N NaOH. Then the volume makes up to 1 ml and 1 liter by adding the required amount of Millipore water. After that 1.2 µl of each treatment of BAP is injected into the one node and two-node cuttings of stevia and planted in the square pot contains artificial soil. A total of six nodes were injected for each treatment. Later they were kept in transparent boxes to maintain humidity in the boxes for 14 days.

**Table-1 : List of treatments used in the present experiment.**

Sl. No.	Treatments	Sl. No.	Treatments
1	0.25 mg/ml BAP	9	0.25 mg/L BAP
2	0.50 mg/ml BAP	10	0.50 mg/LBAP
3	0.75 mg/ml BAP	11	0.75 mg/L BAP
4	1.00 mg/ml BAP	12	1.00 mg/L BAP
5	1.25 mg/ml BAP	13	1.25 mg/L BAP
6	1.50 mg/ml BAP	14	1.50 mg/L BAP
7	1.75 mg/ml BAP	15	1.75 mg/L BAP
8	2.00 mg/ml BAP	16	2.00 mg/L BAP

### 2.3 Place of Experiment carried out

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

### 2.4 Statistical analysis of data

At least six cultures were developed for each treatment in a Completely Randomized Design. The data recorded on various characters subjected to analysis as per standard statistical methods.

## 3. Results and Discussion

**Stevia single node injection :** The data recorded for various traits is presented in Table.2.

**14<sup>th</sup>-day data:** Only one treatment responded i.e., 0.50 mg/ml BAP. The plant height (1.3 cm) and the number of leaves (4.0) were recorded by the stevia 1 node injected with 0.50 mg/ml BAP.

**28<sup>th</sup>-day data:** Only one treatment responded i.e., 0.50 mg/ml BAP. The plant height (2.1 cm), number of nodes (4.0), number of leaves (6.0), largest leaf length (0.3 cm), and largest leaf breadth (0.2 cm) were recorded by the stevia 1 node injected with 0.50 mg/ml BAP.

**42<sup>nd</sup>-day data:** Only one treatment responded i.e., 0.50 mg/ml BAP (Picture.1). The plant height (3.4 cm), number of nodes (6.0), number of leaves (10.0), largest leaf length (0.8 cm), and largest leaf breadth (0.6 cm) were recorded by the stevia 1 node injected with 0.50 mg/ml BAP.

**Table-2 : Stevia single node injection**

	Treatments (mg/ml)	Plant Height (cm)	Number of Nodes	Number of Leaves	Number of Branches	Largest leaf Length (cm)	Largest leaf Breadth (cm)
		Mean	Mean	Mean	Mean	Mean	Mean
14 <sup>th</sup> Day	0.50 mg/ml BAP	1.3	4.0	4.0	-		
28 <sup>th</sup> Day	0.50 mg/ml BAP	2.1	4.0	6.0	-	0.3	0.2
42 <sup>nd</sup> Day	0.50 mg/ml BAP	3.4	6.0	10.0	-	0.8	0.6



**Pic-1 : Stevia at 42th day from single node injection experiment.**

**Stevia double node injection:** The data recorded for various traits are presented in the Table.3.

**14<sup>th</sup>-day data:** Only one treatment responded i.e., 0.50 mg/ml BAP. The plant height (3.4 cm), number of nodes (4.0), number of leaves (7.0), largest leaf length (1.6 cm), and largest leaf breadth (0.8 cm) were recorded by the stevia 2 node injected with 0.50 mg/ml BAP.

**28<sup>th</sup>-day data:** Only one treatment responded i.e., 0.50 mg/ml BAP. The plant height (5.0 cm), number of nodes (6.0), number of leaves (12.0), number of branches (1.0), largest leaf length (2.1 cm), and largest leaf breadth (1.1 cm) was recorded by the stevia 2 node injected with 0.50 mg/ml BAP.

**42<sup>nd</sup>-day data:** Only one treatment responded i.e., 0.50 mg/ml BAP (Picture.2). The plant height (6.2 cm), number of nodes (9.0), number of leaves (18.0), number of branches (1.0), largest leaf length (2.7 cm), and largest leaf breadth (1.3 cm) was recorded by the stevia 2 node injected with 0.50 mg/ml BAP.

**Table-3 : Stevia double node injection**

	Treatments (mg/ml)	Plant Height (cm)	Number of Nodes	Number of Leaves	Number of Branches	Largest leaf Length (cm)	Largest leaf Breadth (cm)
		Mean	Mean	Mean	Mean	Mean	Mean
14 <sup>th</sup> Day	0.50 mg/ml BAP	3.4	4.0	7.0	0.0	1.6	0.8
28 <sup>th</sup> Day	0.50 mg/ml BAP	5.0	6.0	12.0	1.0	2.1	1.1
42 <sup>nd</sup> Day	0.50 mg/ml BAP	6.2	9.0	18.0	1.0	2.7	1.3



Pic-2 : Stevia at 42th day from double node containing explant injected with hormone.

#### 4. Conclusion

The main objective of the present study is to bypass the aseptic environment mediated tissue culture which requires huge initial cost for creating the facility as well as maintaining the facility. The present study considers the nodal explant for direct injection with hormone and incubate the injected nodal explant initially in humid condition under controlled environment and light intensity. Surprisingly, it was evidenced that only one dose was working on nodal explant mediated new emergence. This pilot scale experiment indicates that hormone could be directly injected into nodal explant and produce planting material without following the aseptic mediated new plant emergence which requires long term hardening in addition to costly tissue culture chemicals and process. This is very pilot scale experiment which requires repetitions and optimization of the process for achieving efficient plant material production. The present is an observation which have huge scope for fine tuning.

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#### 6. Compliance with ethical standards (e.g., Conflict of interest)

The communicating author (HAM) declared that there is no conflict of interest.

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