



## ORIGINAL ARTICLE

# The Effect of Various Concentrations of Growth Regulators (Auxins and Cytokinins) On Rooting of Stevia (*Stevia Rebaudiana Bertoni*) In *in Vitro* Conditions

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### ABSTRACT

The plant Stevia with; *Stevia rebaudiana Bertoni*, Scientific name, belongs to the Asteraceae family. Notable features of stevia includes: excess of sweetness in its leaves and the watery extract of it through the presence of glucosides that are several times sweeter than sugar without heating. The micropropagation methods of this plant through tissue culture technique is the only acceptable and standard way to maintain the amount of sugar and to keep native characters of Stevia. Other proliferation methods such as removing welds or stem may change the native quantitative and qualitative characteristics. In this research, the effect of different amounts of auxin hormones includes: IAA(0,1,2mg.l<sup>-1</sup>), IBA(0,1,2 mg.l<sup>-1</sup>) and cytokinin includes: Kin(0,0.15,0.25 mg.l<sup>-1</sup>), BAP(0,1.5,2 mg.l<sup>-1</sup>) was examined in the form of combination and discrete in MS medium zinc on manner and rooting extent of stevia plant. Optimal root production was achieved by the presence of IBA 1 mg.l<sup>-1</sup>. BAP hormone of 2 mg.l<sup>-1</sup> established maximum root length and BAP 1.5 mg.l<sup>-1</sup> and IBA 1 mg.l<sup>-1</sup> established maximum branching out of main roots.

Key Words: Stevia, Rooting, Tissue culture, Auxin, Cytokinin.

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### INTRODUCTION

Stevia plant with the Scientific name of; *Stevia rebaudiana Bertoni*, belongs to the Asteraceae family. It is a Herbaceous plant, shrub, perennial and have medicinal consumptions. Its origin is in the South America, particularly in Paraguay and Brazil (12,13). Notable features of stevia includes excessive sweetness of its leaves and watery extract of it through the presence of glucoside that are several times sweeter than sugar without heating (13). The presence of glucose or Candied compounds contains of it is detrimental for diabetic patients and in the absence of following a proper diet, it will cause them irreparable injuries. But not only the containing glucosides in this plant has no effect on blood sugar level, but also it will repressed a pinch of plasma glucose and it can be consumed in the diabetic person's diet, Hence the proliferation of this plant and the extraction of sugar from that is extremely important as a replace of glucose and other artificial types of sweeteners in food industry (2, 3,11). Accomplished Studies have shown that the best method for proliferation of Stevia is the micropropagation through tissue culture that makes Plants uniformly and with matched stable in glucoside production during the growth years. But the plants with proliferous methods will lose their qualitative characteristics and the production of glocoside will be decreased. also proliferation of Stevia by seed is difficult and has a low germination percentage and obtained plants have not uniformity efficiency of compounds types and sugar amount due to diffraction of traits (6,7,8,9,14). Considering the importance of achieving to maximum amount of growth which obtained from micropropagation plants, it is investigated in this research the effect of auxin and cytokinin hormones individually and hybrid zinc rooting traits in The MS medium.

### MATERIALS AND METHODS

#### *Selection and desinfective explants*

potted bushes of Stevia plant were selected at the time of Preparation explants with complying the specifications include being young , lack of entrance to the Fertility phase and dry foliage (*Stevia rebaudiana* Bertoni). The tail end of stems of these plants with the diameter around two millimeters and the length around 1.5-2 Centimeter were sectioned with sterile scissors and transferred to the laboratory. samples were primary placed in the water containing one percent Tween 20 and were washed immediately under the flowing water for 5 minutes and Placed on the sterile filter paper with aseptic tools under lamynarayrflv device at room temperature. each sample were Initially immersed in hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) %30 and one drop Tween 20 for the term 10 min, then washed with the sterile distilled water three times and placed on sterile filter paper (5,13,15).

#### **Transfer explants to the Culture medium containing hormone**

Under a sterile lamynarayrflu dvice , each aseptic sample divided to the explants with 0.5-0.8 cm length to the node single mode with a surgical blade and were transferred to the inbound glass with sterile forceps contained Thirty milliliters MS basal medium with hormone and became maintained under the light of fluorescent bulb in the growth chamber with 2800 lux intensity, 16 hours light and 8 hours of darkness, with 26 ±2 centigrade heat degrees and 75-80 percent moisture. different amounts of the group of auxin hormones includes: Indol-3- acetic acid (IAA) 0,1,2mg.l<sup>-1</sup>, Indol- 3- butyric acid (IBA) 0,1,2 mg.l<sup>-1</sup> and the group of cytokinin hormones includes: Kinetin( Kin) 0,0.15,0.25 mg.l<sup>-1</sup> , 6-Banzyl amino purin (BAP) 0,1.5,2 mg.l<sup>-1</sup> was selected in the form of total 31 treatments (Table 1) discrete and composition mode a long a hormone-free witness on MS medium. There were six repeats for each treatment and for each repeats, it was considered one glass containing three explants( 5, 13 ).

**Table 1:** Different concentrations growth regulators mode to discrete and composition on MS medium

| Treatment | growth regulators (According mg.l <sup>-1</sup> ) |      |       |     |
|-----------|---|------|-------|-----|
|           | Cytokinin   |      | Auxin |     |
|           | BAP   | Kin  | IAA   | IBA |
| 1         | 2   | 0.25 | 2     | 2   |
| 2         | 2   | 0.25 | 2     | 0   |
| 3         | 2   | 0.25 | 0     | 2   |
| 4         | 2   | 0.25 | 0     | 0   |
| 5         | 2   | 0    | 0     | 0   |
| 6         | 2   | 0    | 2     | 2   |
| 7         | 2   | 0    | 2     | 0   |
| 8         | 2   | 0    | 0     | 2   |
| 9         | 1.5   | 0.15 | 1     | 1   |
| 10        | 1.5   | 0.15 | 1     | 0   |
| 11        | 1.5   | 0.15 | 0     | 1   |
| 12        | 1.5   | 0.15 | 0     | 0   |
| 13        | 1.5   | 0    | 0     | 0   |
| 14        | 1.5   | 0    | 1     | 1   |
| 15        | 1.5   | 0    | 1     | 0   |
| 16        | 1.5   | 0    | 0     | 1   |
| 17        | 0   | 0.15 | 1     | 1   |
| 18        | 0   | 0.15 | 1     | 0   |
| 19        | 0   | 0.15 | 0     | 1   |
| 20        | 0   | 0.15 | 0     | 0   |
| 21        | 0   | 0    | 1     | 1   |
| 22        | 0   | 0    | 1     | 0   |
| 23        | 0   | 0    | 0     | 1   |
| 24        | 0   | 0.25 | 2     | 2   |
| 25        | 0   | 0.25 | 2     | 0   |
| 26        | 0   | 0.25 | 0     | 2   |
| 27        | 0   | 0.25 | 0     | 0   |
| 28        | 0   | 0    | 2     | 2   |
| 29        | 0   | 0    | 2     | 0   |
| 30        | 0   | 0    | 0     | 2   |
| 31        | 0   | 0    | 0     | 0   |

#### **Survey the growth traits explants in the different treatments**

Samples was taken out from the growth chamber after 40 days cultured, and the traits including root numbers, branching of the main root length was measured and after normalization and data conversion through the taking square, analysis of data variance was performed by using the SAS statistical software and comparison the mean by method of Duncan with 95 percent probability.

## **RESULT AND DISCUSSION**

### **The effect of different amounts growth regulators of auxin group( iaa,iba) and cytokinin ( kin, bap) on the zinc rooting stevia explants**

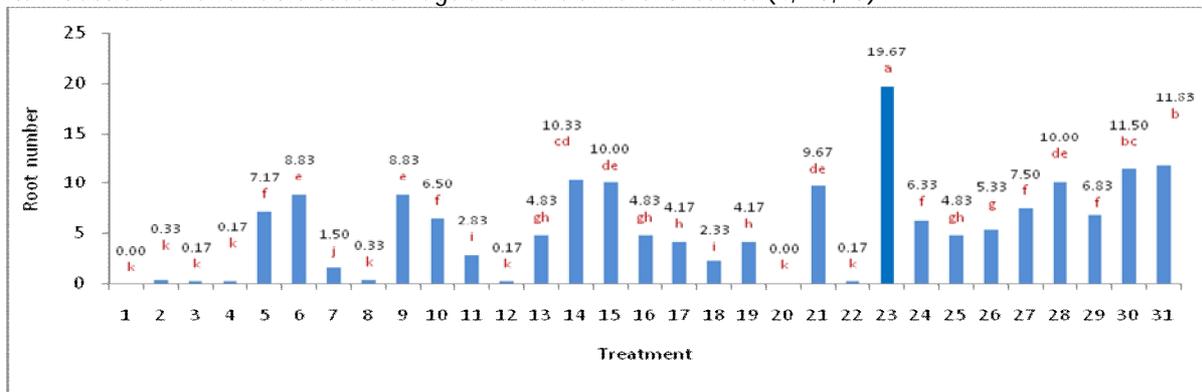
Results of variance analysis showed that there is significant difference between the treatments at level %1 and coefficient variation (CV) indicate desired accuracy experiments (Table 2).

**Table 2:** variance analysis different amounts growth regulators on rooting *Stevia rebaudiana* Bertoni) In vitro conditions

| Sources changes               | Freedom degree | Mean-square (MS) |                   |                  |
|-------------------------------|----------------|------------------|-------------------|------------------|
|                               |                | Root number      | Branching of root | Main root length |
| Treatment                     | 30             | 6.52**           | 0.129**           | 0.367**          |
| Error                         | 155            | 0.03             | 0.004             | 0.013            |
| Coefficient of Variation(C.V) |                | 7.85             | 8.24              | 10.21            |

**Root number**

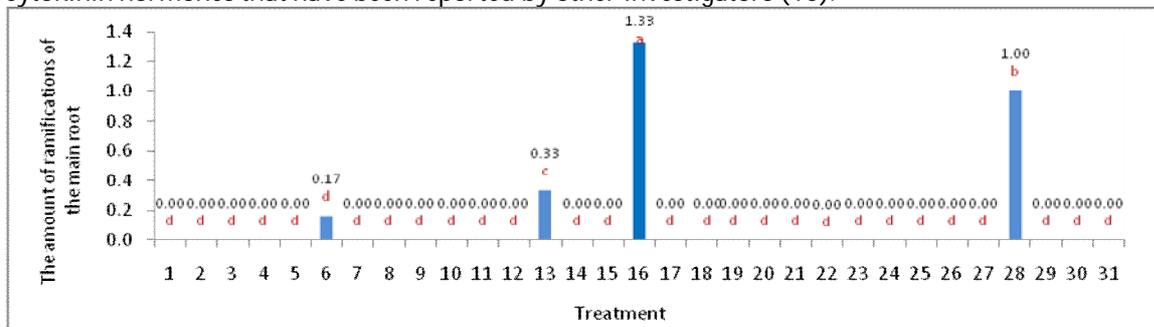
Comparison between data averages Showed that the MS medium containing IBA 1 mg .l<sup>-1</sup> amounts with 19.67 root mean in each explant was statistically significant and the best treatment than the others (Figure A). After the above treatment, the witness treatment (MS medium without hormone) with 11.83 average of roots in each explants was superior than other treatments and produced more roots, But the other treatments produced fewer roots than the witness treatment. the treatments with BAP 2 mg, Kin 0.25mg, IAA 2 mg ,IBA2 mg.l<sup>-1</sup>and Kin 0.15mg.l<sup>-1</sup> an average of zero were not able to produce root in each explant (diagram 1). Sometimes one certain auxin is the factor of growth and cell division. But if the same material be used in high concentration, it will be the cause of death and the loss of the plant. The other effective factors of auxin performance is the physiological age of the plant. So concentrations of auxin and its induction time variable based on age and have different results (4, 10,15) .



**Diagram 1:** Average number of root in hormonal treatments with MS medium

**The amount of ramifications of the main root**

The highest amount of branching out of the main root with 1.33 average at BAP 1.5 mg.l<sup>-1</sup> IBA 1 mg.l<sup>-1</sup> concentration was seen with meaningful difference in %1 probability level than other treatments (Figure B). After the above treatment, treatments include: IAA 2 mg IBA2 mg, BAP 1.5 mg.l<sup>-1</sup> Singly and the combination of three hormone concentrations contains BAP 2 mg, IAA 2 mg and IBA2 mg .l<sup>-1</sup> are respectively with the averages 1, 0.33 and 0.17 produce more root ramifications.in other treatments there was no obtained ramification from root (Diagram 2). The obtained results indicate the main role of auxins in lateral root formation. Auxins in high concentrations will cause to form lateral roots and cytokinins are mostly used to stimulate development of growth (1, 15). Lack of lateral roots production in other treatments and excessiveness of ramifications number in this composition of hormonal amounts indicates different sensitivity of this type of plants relative to the concentration between two groups of auxin and cytokinin hormones that have been reported by other investigators (10).



**Diagram 2:** Average number of ramifications from the main root in hormonal treatments with MS medium

### The length of main root

Based on data averages, maximum length of explant's main root was formed at the amount of two mg.l<sup>-1</sup> (Figure C) with 1.82cm average in the MS medium containing BAP. Roots were not formed in a culture medium which contained 0.15 mg.l<sup>-1</sup> of Kin and in a situation that simultaneously was used from highest amount of one of the four growth regulators. (BAP 2 mg, Kin 0.25 mg, IAA 2 mg, IBA 2 mg.l<sup>-1</sup>) roots were not formed (Diagram 3). Although increase of the root size normally attributed to existence of auxin group hormones But it should be mentined to this point that the increase of length or extent of root number in the explants of *Stevia* is related to increase of the group of auxin hormones in the presence of the group of cytokinin hormones (15). Without cytokinin, the existence of the group of auxin hormones alone has not lead to increase root elongation of *Stevia* (1). When these two types of hormone simultaneously attended, their ratio will determine the growth. If the amount of cytokinin was higher than auxin concentrations, the auxin hormone would moved the terminal bud in to down and it would have inhibited the activity of the lateral buds. So, cytokinin will affect on the terminal bud and activate that and finally it will cause to the growth of shoot longitudinally. In the absence of auxin, cytokinin can not alone causing the increase of shoot growth (10). The Increase root length in the presence of higher concentrations of BAP than other treatments can be related to the role of cytokinin in the increase of cell division and root organs formation (4).

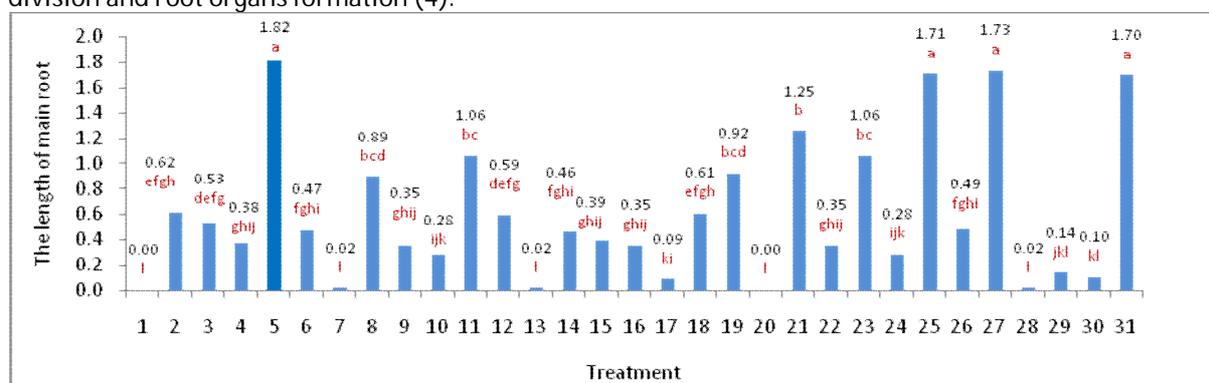


Diagram 3: Average main root length hormonal treatments in the MS medium

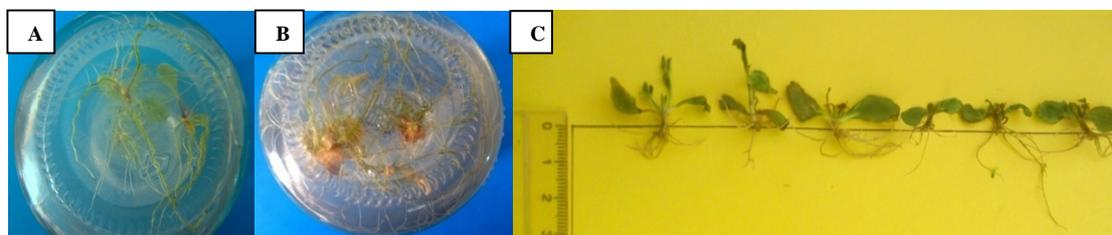


Figure A: Number root in hormonal treatments IBA 1 mg.l<sup>-1</sup>, Figure B: The ramifications of the main root in hormonal treatments BAP 1.5 mg, IBA 1 mg.l<sup>-1</sup>, Figure C: main root length in hormonal treatments BAP 2 mg.l<sup>-1</sup>.

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