*International Journal of Farming and Allied Sciences*
Available online at www.ijfas.com
©2014 IJFAS Journal-2014-3-7/782-785/ 31 July, 2014
ISSN 2322-4134 ©2014 IJFAS

# Effect of Different Culture Media and Plant Growth Regulators on Callus Induction of Stevia Rebaudiana

**K.Keighobadi*, M.Golabadi and F.Mortazeenezhad**

Agriculture faculty, Khorasgan (Isfahan) Branch , Islamic Azad University , Isfahan , Iran

***Corresponding author:*** K.Keighobadi

**ABSTRACT:** The main objectives of this present study were to develop the optimal concentratioand combination of auxin and cytokinin for optimized callus induction through seeds implanting in MS medium. stevia is cultivated by seeds or stem cutting, but Seed germination of stevia is very poor. In this investigation, seeds of stevia were evaluated for in vitro callusing and demonstrated that callus induction in medicinal plant Stevia rebuadiana was affected by seed explants and different level of plant growth regulators. Callus induction was initiated trough seeds on MS media having auxins & cytokinins and responses were varied according to the plant growth regulators treatment. Whereas the application of IBA+BAP (3 or 3.5+2.5 mg/l) and NAA+IBA+BAP+KIN (0.5+3+1.5+1 mg/l) indicated most satisfactory performance on callus initiation and induction but the highest callusing (85%) was achieved on MS medium enriched with NAA+IAA+BAP (1.5+2.5+1.5 mg/l). Data showed positive correlation between presence of BAP and results proved that media with IBA+BAP (3.5+2.5mg/l) and NAA+IAA+BAP (1.5+2.5+1.5 mg/l) were better for callus induction of stevia respectively as compared to others.

***Keywords*:** cytokinins, explants, germination, in vitro, plant growth regulators

## INTRODUCTION

Stevia rebaudiana Bertoni, belonging to the family Asteraceae. The leaves of stevia are the source of the diterpene glycosides, vis. Stevioside and rebaudioside (Lemus-mondaca, 2012, Ramesh, 2006). They are estimated to be 40 to 250 times sweeter than sucrose. It is recommended for diabetes and has been extensively tested on animals and has been used by humans with no side effects (Ramesh, 2006). The seeds of stevia show a very low and poor germination percentage (Puri, 2011, Ramesh, 2006). Therefore, there are basically two options for multiplication: tissue culture and stem cutting (Puri, 2011, Ramesh, 2006, shukla, 2009). Shoot apex, nodal, leaf and also seed explants of stevia rebaudiana (Bertoni) can regenerate shoots when cultured on murashige and skoog (MS) medium supplemented with different combination growth regulators (Ramesh, 2006).

## MATERIALS AND METHODS

The present experiment for callus induction in stevia rebuadiana was performed at the tissue culture laboratory of Saba Sekeh institute in Esfahan Research & Knowledge Town, Iran, during January 2013 to April 2013. The procedures of this paper are described below.

### *Plant materials & Sterilization*

Seeds of stevia rebuadiana were collected as the source of explants. These collected explants were used for the callus induction. The seeds brought to the laboratory and then they were first cleaned thoroughly under a

running tap water for 20 min. The explants were immersed in detergent solution and then rinsed three times with autoclaved water. For further surface sterilization of seeds, first ethanol solution (70%) at 1.5 min and Sodium hypochlorite (20%) at 7.5 min were applied and then for 3-5 times, explants were immersed in double- distilled water. Eventually the seed explants were dried with autoclaved filter paper. Due to importance of explants inoculation step in tissue culture technique, this procedure was conducted in the laminar air flow. The material preparation was conducted following by the method of Verma, (2001) with some alteration.

***Media preparation***

Various MS (Murashige & Skoog) media treatments used for callus induction. These media fortified with various concentrations of auxins & cytokines, like Indole Buteric Acid (IBA), Indole-3-Acetic Acid (IAA), 1-Naphthaleneacetic Acid (NAA), 2,4-Dichlorophenoxyacetic Acid (2,4-D) and 6-Benzylaminopurine (BAP) and Kinetin (KIN). pH of the media was adjusted at 5.77 – 5.83 prior to autoclaving. Dried seeds were then inoculated into test glass under aseptic condition in laminar flow cabinet. Each treatment was represented by 10 cultures and the experiment was repeated three times.

***Inoculation and incubation of callus***

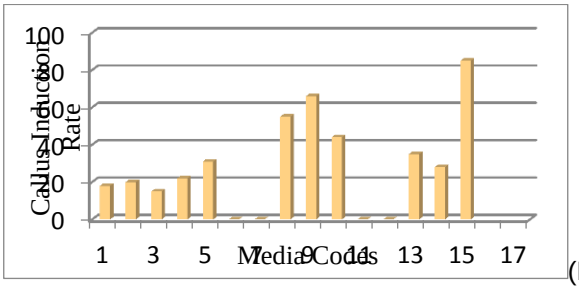
The explants were inoculated into Murashige and Skoog`s (1962) culture medium (MS). MS culture medium was supplemented with different combinations of growth regulators for callus induction. Various MS media along with auxins like: IAA (1.5, 2, 2.5, 3 and 4 mg/l), IBA (3 and 3.5 mg/l), NAA (0.5, 1.5 mg/l) and 2, 4-D (0.5 and 1 mg/l) and also cytokinin hormones like: kinetin at (o.5, 1, 1.5 and 2 mg/l) and BAP (1.5, 2, 2.5, 3 and 5 mg/l) were used in combination. Inoculated cultures were incubated at 25±3 C under the influence of fluorescent lamp for 16 hours photoperiod. Callus induction and initiation of stevia seeds were observed after 8 days. The data of callus induction frequency were recorded for seed explants on 15 different media with different levels of plant growth regulators. Callused micropropagules were thoroughly washed to remove the adhering gel. All the produced cali were examined periodically up to five weeks of culture and the morphological changes were recorded on the basis of visual observations. The mean percentage of cultures producing callus were recorded after 5 weeks. The amounts of callus were estimated by a 0-4 Scale scoring system (table 1).

Table1. A 0-4 Scale scoring system

| Score | Description |
|---|---|
| 0 | No visible callus |
| 1 | Small proliferation |
| 2 | Up to 10mm callus |
| 3 | 10-25mm callus |
| 4 | >25 mm callus |

## RESULTS AND DISCUSSION

The effect of different growth regulator on callusing and on callus growth was the objective of this study. Risk of contamination was much low when the explants were treated with (20 %) Sodium hypochlorite and (70%) Ethanol. The ANOVA analysis showed that Due to concentration fluctuation of plant growth regulators in all media, the callus induction frequency was ranged from 15% to 85% (Fig.1).



(B)

(A)

Figure 1. Callus induction in seed of stevia through different media formulations

Callus induced from seed explants were different at various level of BAP, IBA, IAA and NAA. Maximum callus induction (85%) was obtained for explants when seeds were cultured on MS medium enriched with NAA+IAA+BAP

(table 1&2). Lowest callus of (15%) was observed for seeds, when MS media are supplemented with IAA+BAP (2.5+1.5). It is noteworthy that potential of BAP in combination with other auxins was higher than 2, 4-D. This result showed positive correlation between presence of BAP and induction and negative for 2, 4-D. results proved that media with IBA+BAP (3.5+2.5mg/l) and NAA+IAA+BAP (1.5+2.5+1.5 mg/l) were better for callus induction of stevia respectively as compared to others. And also media with combination of BAP and 2, 4-D at 1mg/l had no response.

Table 2.effect of different media culture on morphological traits of callus in stevia rebaudiana

| Callus induction experiment | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Media codes | PGRs | Conc. (Mg/l) | Callus induction frequency(%) | Callus diameter(cm) | Texture | colour | Fresh weight (gr) | Dry weight (gr) | decription |
| M1 | IAA+BAP | 2.5+2.5 | 18 | 0.71 | Compact | Fluorscent green | 1.94 | 0.355 | 2 |
| M2 | NAA+IAA+BAP | 0.5+2.5+2.5 | 20 | 0.95 | Soft | Brown | 1.75 | 0.33 | 2 |
| M3 | IAA+BAP | 2.5+1.5 | 15 | 1.14 | Compact | green | 1.52 | 0.286 | 3 |
| M4 | IAA+BAP | 4+2 | 23 | 2.93 | Compact | yellowish | 2.42 | 0.456 | 4 |
| M5 | NAA+IAA+BAP | 0.5+4+2 | 31 | 0.66 | Friable | Pale green | 2.11 | 0.395 | 2 |
| M6 | IAA+BAP+KIN | 2+2+1.5 | 0 | --- | --- | --- | --- | --- | 0 |
| M7 | IAA+KIN | 1.5+2 | 0 | --- | --- | --- | --- | --- | 0 |
| M8 | IBA+BAP | 3+2.5 | 55 | 2.86 | Compact | green | 3.04 | 0.522 | 4 |
| M9 | IBA+BAP | 3.5+2.5 | 66 | 1.83 | Compact | Fluorscent green | 2.88 | 0.518 | 3 |
| M10 | NAA+IBA+BAP+KIN | 0.5+3+1.5+1 | 44 | 1.52 | Soft | Light green | 2.41 | 0.438 | 3 |
| M11 | 2,4-D+IAA+BAP | 1+3+2.5 | 0 | --- | --- | --- | --- | --- | 0 |
| M12 | 2,4-D+IAA+BAP | 1+4+5 | 0 | --- | --- | --- | --- | --- | 0 |
| M13 | 2,4-D+IAA+BAP | 0.5+2.5+3 | 35 | 0.79 | Friable | yellowish | 2.17 | 0.4 | 2 |
| M14 | 2,4-D+IAA+BAP+KIN | 0.5+2+2+0.5 | 28 | 0.31 | Soft | Green | 2.01 | 0.373 | 1 |
| M15 | NAA+IAA+BAP | 1.5+2.5+1.5 | 85 | 3.27 | Excellent Compact | light green | 3.11 | 0.583 | 4 |

## CONCLUSION

Our results are in disagreement with those of Ali Kemal and Cirak (2005) who reported that BA & 2,4-D at different concentrations promoted callus induction. Their findings showed that culturing seeds in media supplemented with BA & 2,4-D resulted green & friable callus induction and they approved that MS media fortified those plant growth regulators had a significant effect on callus induction. Using different concentration of formulation in all media causing to variation between in texture,colour and also weights of cali (table.2).

The overall purposes of the present study were has been to indicate a methodology for the callus initiation and induction of stevia rebuadiana through implant of seed explants on different media. Some authors reported that different explants of stevia such as nodal, leaf and shoot tip can produce callus and some others noted that seed explants in other plant were tested. The results of the investigation and earlier research clearly support the induction of callus of stevia. Shazia et al (2005) indicated that excellent callus was achieved by implanting rice seed explants on MS media fortified 2,4-D and KIN and also showed that concentration of auxins and cytokinins could increase in callus mass. In our findings, results of statistical analysis showed that 73.33% of all media had responses on callus induction. As far as we know, very limited data is available regarding the callus induction from seed explants of stevia and hence the results achieved through this assessment are of high importance. Nonetheless, Islam et al (2004) investigated on the callus induction through inoculating of rice seed on various MS media and their results showed that plant growth regulators have an important role on induction. The result, show that media with IBA+BAP (3.5+2.5mg/l) and NAA+IAA+BAP (1.5+2.5+1.5 mg/l) were better for obtained excellent callus. Variations are the basis for improvement and sometimes this variation is heritable. Callus induction is the best way to create somaclonal variations in plant.

## REFERENCES

Ayan AK and Cirak C. 2005. Callus induction in some Hypericum species growing in Turkey. Turk Journal of Field Crops. 10:23-28.

Islam MM, Khan WS A and SaKU. 2004. Studues On Callus Induction and Regeneration from Dehusked Rice (Oryza sativa L.). Seeds. Plant Tissue Culture. 14: 155-160.

Lemus-Mondaca R, Vega-Galvez 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 Chemistry. 132(3):1121-1132.

Puri M, Sharma D and Tiwari AK. 2011. Downstream Processing of Stevioside and its Potential Applications. Biotechnology Advances. 29(6):781-791.

Ramesh K, Singh V and Megeji NW. 2006. Cultivation of Stevia rebaudiana (BERT.): A Comprehensive Review. Advances in Agronomy. ( 89):137-177.

Shazia B, Fazal MJR and Ihsan I. 2005. Callus Induction and Regeneration in Seed Explants of Rice (*ORYZA SATIVA* CV.SWAT.2). Pakikistan Journal of Botany.( 37): 829-836.

Shukla S, Mehta A, Bajpai VK and Shukla S. 2009. In Vitro Antioxidant Activity and Total Phenolic Content of Ethanolic Leaf Extract of Stevia Rebaudiana Bert. Food and Chemical Toxicology.47(9):2338-2343.

Verma S, Yadav K and Singh N. 2011. Optimization of the Protocols for Surface Sterilization, Regeneration and Acclimatization of Stevia Rebaudiana Bertoni. American-Eurasian Journal Agriculture & Enviroment. 11(2):221-227.