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"COMPARATIVE STUDY OF DIFFERENT CONCENTRATION OF KINETIN ON SHOOT INITIATION AND MASS MULTIPLICATION OF *STEVIA REBAUDIANA L.*"

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INTRODUCTION

Stevia is a sweetener and sugar substitute extracted from the leaves of the plant species *Stevia rebaudiana*. The active compounds of *Stevia* are steviol glycosides (mainly Stevioside and Rebaudioside), which have up to 150 times the sweetness of sugar, are heat-stable, pH-stable, and not fermentable.[1] These Steviosides have a negligible effect on blood glucose, which makes *Stevia* attractive to people on carbohydrate-controlled diets. *Stevia's* taste has a slower onset and longer duration than that of Sugar and some of its extracts may have a bitter or Licorice- like after taste at high concentrations. [2-3]

Figures showing the mature seedling of *Stevia rebaudiana*

The legal status of *Stevia* extracts as food additives and supplements varies from country to country. In the United States, *Stevia* was banned in 1991 after early studies found that it

might be carcinogenic; after additional studies, the FDA approved some specific glycoside extracts for use as food additives in 2008.[3-4]

The plant *Stevia rebaudiana* has been used for more than 1,500 years by the Guaraní peoples of South America, who called it "Sweet Herb".[5] The leaves have been used traditionally for hundreds of years in both Brazil and Paraguay to sweeten local teas and medicines, and as a "Sweet Treat".[6] The exact structure of the Aglycone(Steviol) and of the glycoside were published in 1955.

Stevia extracts and derivatives are produced industrially by many companies, and marketed under many trade names like Rebiana,[7] Truvia.[8], PureVia, Enliten, Erylite Stevia.[9-10] Extract like Rebaudioside-A has the least bitterness of all the steviol glycosides in the *Stevia rebaudiana* plant. To produce rebaudioside A commercially, *Stevia* plants are dried and subjected to a water extraction process.

This crude extract contains about 50% rebaudioside A. The various glycosides are separated and purified via crystallization techniques, typically using ethanol or methanol as solvent.[1,7,13]

In 2006, research data compiled in the safety evaluation released by the World Health Organization found no adverse effects.[11] Among these concerns are control of blood sugar and effects on the reproductive, cardiovascular, and renal systems.[12]

Figures showing the Gardening of *Stevia* Plants & Its Drying Process

Domain:	**Eukaryota**
Kingdom:	Plantae
Order:	Asterales
Family:	Asteraceae
Tribe:	Eupatorieae
Genus:	*Stevia*
Species:	*Stevia rebaudiana*

MATERIALS AND METHODS

Selection & Sterilization of Plant

The Plant materials are collected from Grow Tips Biotech, Bhopal & Pallishree Research Farm, Arambagh(West Bengal). We are selecting the plant materials as well as explants on the basis of young and healthy nodal portion and leaf explants.

Explants Sterilization

Explants were primarily washed under running tap water for 20 minutes and then washed with liquid soap to remove the superficial dust particles as well as fungal and bacterial spores. Fresh & washed explants were taken to LAF under sterile condition and again washed twice with sterile double distilled water. The explants were sterilized with 70% ethanol for 2 minutes. The explants were sterilized with 0.1% Mercuric chloride aqueous solution for 2-3 minutes and then rinsed five times with sterile distilled water. Finally the explants were cut into small pieces ranging in size from 4.0 to 5.0 cm long in the LAF Chamber.

S.No.	**Treatment**	**Time**
1.	70% Ethanol + 0.1%$HgCl_2$	2-5 min.
2.	70% Ethanol + 0.1%$HgCl_2$	2-3 min.
3.	70% Ethanol + 0.1%$HgCl_2$	2 min.

Culture Establishment for shoot initiation

After completing the all sterilization and slicing process, the explants were inoculated in MS Medium supplemented with Cytokinins(Kinetin). These test tubes were finally placed in the growth room where Temperature condition maintained 25 ±2ºC and the Photoperiod of 16 hours daylight.

Establishment of Cultures for Multiplication

The initiated plants were taken out of the test tube and the medium adhered to the plants was removed. Plants were transferred to the culture bottles containing autoclaved semi- solid medium and growth regulator.

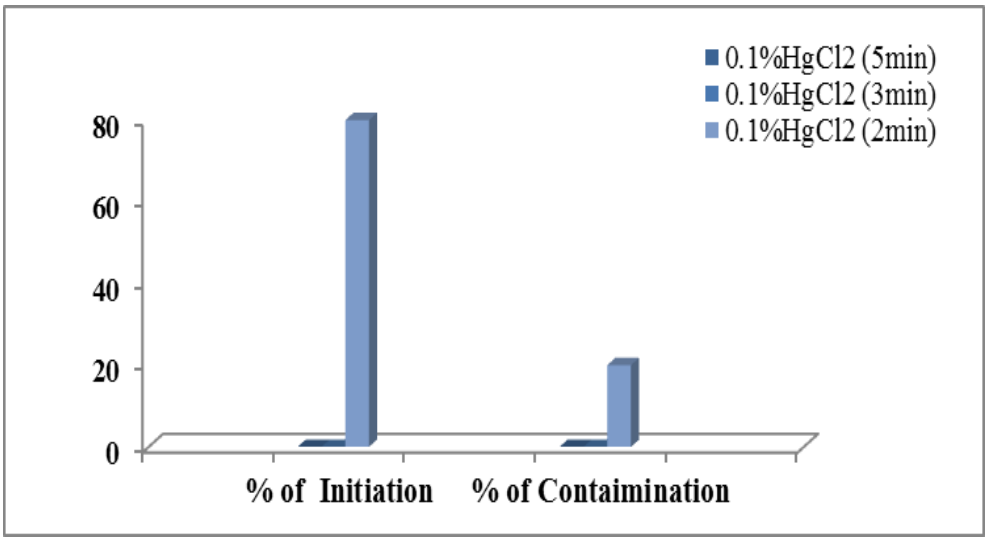
RESULTS AND DISCUSSION

Data Analysis

Various growth data were recorded such as the number of multiple shoot formation, shoot length, and contamination percentage during the time of my research & experiments. All the studies with the mean and standard errors were calculating by using IBM SPSS Statistics software (IBM 2011).[14]

Table showing the Result of Standardization of Surface Sterilizing Agents

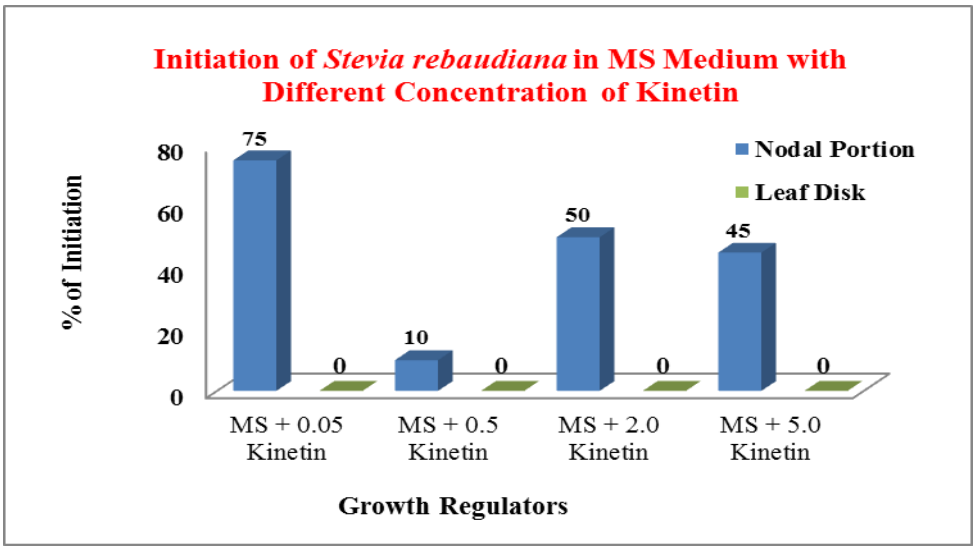
S. No.	Treatment	% of Contamination	% of Shoot Initiation
1	70% Ethanol(2min) + 0.1%$HgCl_2$ (5min)	0	0
2	70% Ethanol(2min) + 0.1%$HgCl_2$ (3min)	0	0
3	70% Ethanol(2min) + 0.1%$HgCl_2$ (3min)	20	80



Graph showing the percentage of Initiation & Contamination of Surface Sterilizing Agents

Table showing the Effects of Different Concentration of Kinetin in MS Medium on Shoot Initiation

S. No.	Medium	Conc. Of Kinetin	% of Initiation in Nodal Portion	% of Initiation in Leaf disk
1	**MS Media**	0.05 Mg/lit	75%	Nil
2	**MS Media**	0.5 Mg/L	10%	Nil
3	**MS Media**	2.0 Mg/L	50%	Nil
4	**MS Media**	5.0 Mg/L	45%	Nil



Graph showing the Initiation of *Stevia rebaudiana* in MS Medium with different concentration of Kinetin

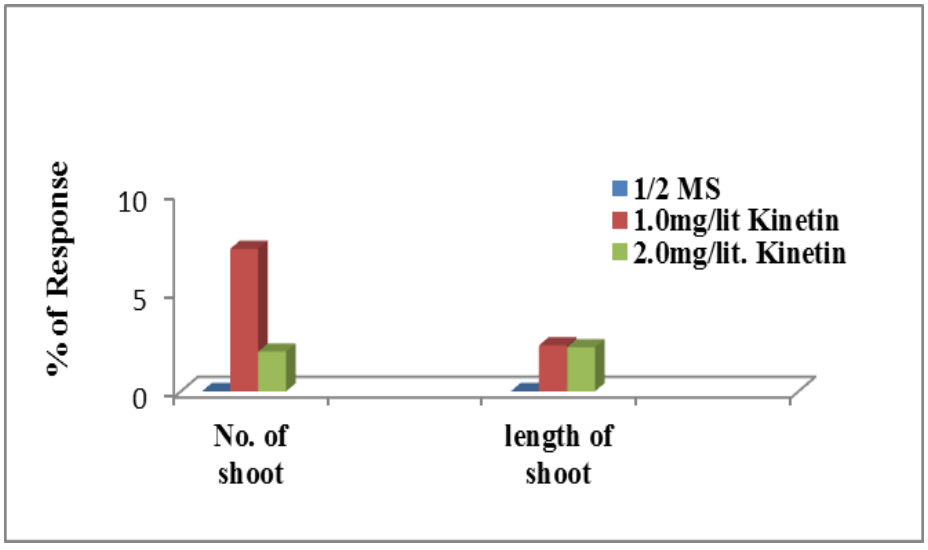
Initiation in 0.5 Mg/I Kinetin

nitiation in 5.0 Mg/I Kinetin

Initiation in 2.0 Mg/I Kinetin **Initiation in 0.05 Mg/I Kinetin**

Tables showing the Effects of Different Concentration of Kinetin in MS Medium on Shoot Multiplication

S.No.	Medium	Conc. of Kinetin	No of Shoots (Mean± SD)	Length of Shoot (Mean ± SD)
1.	½ MS Media	Nil	No response	No response
2.	**MS** Media	**1.0 mg/L**	**7.2 ± 1.0**	**2.32 ± 1.61**
3.	MS Media	2.0 mg/L	2.0 ± 0.0	2.23 ± 1.31



Graph showing the percentage of responses on different Concentration of Kinetin

Figure showing the Shoot Multiplication of *Stevia rebaudiana* in 2.0Mg/L Kinetin

Figure showing the Shoot Multiplication of *Stevia rebaudiana* in 1.0Mg/L Kinetin

CONCLUSION

In the Present investigation protocols for shoot initiation and mass multiplication of *Stevia rebaudiana* L. have successfully been standardized and changes according to the Lab conditions. As the sweet herb, is an important medicinal plant, it is becoming an endangered species due to its infertile and small sized seed. The methods of vegetative propagation are not efficient to save this rare plant. Protocols for regeneration through somatic embryogenesis and anther culture need to be developed as it can help in producing true to the type and homozygous plants with improved quality. Improved genotypes with a high content of Rebaudioside-A need to be developed and propagated as it has most desirable flavor characteristics. Further investigations are required to develop techniques for the commercial production of desirable glycosides of *Stevia* in a suitable bioreactor system. The fundamental research was carried out on Grow Tips Biotech, Bhopal and School of Biological & Chemical Sciences, Raipur. From my study, it was finally screened that in *Stevia* tissue culture, Shoot

initiation was observed on MS medium with the different concentrations of hormone (Kinetin). The best results of initiation were obtained in MS medium with 0.05 mg/L Kinetin which gave 75%. Also screened In multiplication stage the development that we obtained during the initiation and establishment phase continued and it was observed that MS media with 1.0 mg /L Kinetin give best results for number of shoots i.e. 7.2 and for explants length i.e.2.32 cm.

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