Biosciences, Biotechnology Research Asia Vol. **7**(1), 477-480 (2010)

Effect of seed pre-treatment with growth regulators on germination and biomass yield of *Stevia rebaudiana* Bert. - A zero caloric sweetener medicinal plant

PUNJA RAM ENKESHWER and SANDHYA TYAGI

Laboratory of Eco-physiology and Medicinal Plant, Department of Botany,
Mohan Lal Sukhadia, University, Udaipur - 313 001(India).

(Received: November 06, 2009; Accepted: December 20, 2009)

ABSTRACT

Laboratory and net-house studies were carried out to investigates effects of growth regulators on the seeds of *Stevia rebaudiana*. Growth regulators had effect beneficially for germination percent and biomass production in low concentrations up to 10ppm. The results of study could useful to large scale cultivation of *Stevia* plant.

Key words*:* Gibberellic acid, Kinetin, Indole-3- acetic acid, Seed germination, *Stevia rebaudiana***.**

INTRODUCTION

Stevia rebaudiana Bertoni is a herbaceous perennial plant of family Asteraceae. It is zero-caloric natural alternative to the sucrose. The sweet compounds pass through the digestive tract without chemically breaking down, making safe for those who need to control their blood sugar level (Strauss, 1995). Foliage part of *Stevia rebaudiana* is the sourse of diterpene glucose viz. stevioside, rebaudioside-a and b, glucose (Hanson and de Oliveira, 1993). Stevioside is about 110-270 times sweeter than sucrose (Tanka,1982; Ismima and Katyama,1976).The fresh leaves can be good substitute for a diabetics person with no side effect as also a pleasant last like that of liquorices roots as showed by extensive experimental tested on animals (Megeji *et al.*,2005).

Stevia easily propagated by stem cuttings but it is highly a laborious, costly and time consuming process for large scale cultivation. Poor seed germination is the major limiting factor of *Stevia rebaudiana* for large scale cultivation (Gotlemoller and Ching, 1999;). Seed germination can be controlled by many factors among these are natural germination inhibitors . It has been postulated that, seed coat (testa) of many tree species such as *Qurcel coccifera* L. contain considerable amount of germination inhibitor that prevent their germination (EL-Barghathi and EL-Bakkosh, 2005). Germination regulating compounds such as gibberellic acid (GA_3) and kinetin (Yavin *et al.*).

While studying the effect of seed treatment with GA_3 on growth and yield in plant height, number of branches, and number of leaves/plants and shoot dry weight (Khan and Rashid 1983).

The aim of the present studies were to trace out the germination percentages of *Stevia* seed in both laboratory and net-house conditions. In net-house experiment, growth and biomass of *Stevia* plant were also measured.

MATERIAL AND METHODS

Seeds of *Stevia rebaudiana* were collected from Mr. Jeeven Herb Agro farm, Sagar (M.P.) and for net-house experiment, air dried seeds (Black type) were dipped in various test solutions (Table 1). Ten treated seeds (three replicate/treatment) were sown (2.0cm depth; distance between seeds 3.0cm) in nursery pots of equal sizes (i.e. 25cm diameter) with a small hole at the bottom. The pots were filled with sandy-loam soil (34.95 % water holding capacity; 1.43% bulk density; 0.88% organic carbon and 6.8 pH) in equal volume. The experiments were conducted in completely randomized design under a net-house in agro-climatic condition of Udaipur, Rajashan (24°35' NL and 73°41' EL at a height of 582 meters above mean sea level, temperature: 31.3°C max.; 24.4°C min.; R.H: 89 ± 1.92 % max., 78.81 ± 2.1% min.).They were uniformly watered twice a day (morning and evening). Daily observation were recorded, the first germination after two weeks of sowing and first true-leaf emerged after three weeks. After four weeks seedlings were limited mitigate competition, maintaining 4-6 cm distance. After six weeks, seedling growths were assessed by different growth parameters including length of main stem, number of leaf/plant, fresh weight. The oven dried weight was obtained by drying seedlings at 70º C for two days.

RESULTS AND DISCUSSIONS

As for as seed germinations were concerned, maximum (83.33 %) and minimum (73.33%) germinations were observed in T_3 (GA_3 10ppm), and T_6 (IAA 10ppm) respectively, and least germination (56.66%) was observed in T_6 (Kinetin 10ppm). Order of mean germination percent is; $T_6>T_2>T_5>T_4>T_3$. The length of main stem was

Table 1: The treatment of growth regulators with different concentrations (for net house experiment)

Treatments	Concentrations (ppm)	Growth regulators	Seed soaked time (hrs.)
T_1	-	-	24
T_2	05	GA_3	24
T_3	10	GA_3	24
T_4	10	IAA	24
T_5	10	IBA	24
T_6	10	Kinetin	24

Table: 2. Seed germination in Stevia rebaudiana using pre-sowing growth regulators treatments under nursery condition (net-house)

Treatments	Number of days taken for first seed germination	Mean germination (%)	Mean germination time (days)
T_1	14	53.33	15.66
T_2	13	63.33	12.66
T_3	12	83.33	11
T_4	13	73.33	13
T_5	13	63.66	14
T_6	13	56.66	14
SEM±	0.1929	1.0597	0.1949
CD(P=0.05)	0.5944	3.2653	0.6007
CV(%)	3.43	3.73	3.36

Table: 3. Effect of pre-sowing growth regulators treatment on seedling growth of *Stevia rebaudiana* under nursery condition (net house) after 6th week of sowing

Treatments	Length of main stem (cm)	Number of leaves/plant	Above ground fresh wt. (g)	Below ground fresh wt. (g)	Total fresh wt. (g)	Above ground dry wt. (g)	Below ground dry wt.(g)	Total dry wt.(g)
T_1	26.60	25.16	2.17	1.41	3.58	1.05	0.87	1.92
T_2	36.85	39.33	3.33	2.25	5.58	1.55	1.23	2.78
T_3	47.17	43.00	4.68	3.90	8.66	3.77	2.77	6.54
T_4	38.16	35.00	3.31	1.96	5.27	1.58	1.07	2.65
T_5	36.3	31.33	3.14	1.80	4.94	1.02	0.69	1.71
T_6	30.91	27.00	2.25	1.40	3.65	0.98	0.72	1.70
SEM±	0.7394	0.5219	0.457	0.0303	0.079	0.0213	0.0165	0.0387
CD(P=0.05)	2.782	1.6082	0.1409	0.0932	0.2303	0.657	0.0507	0.1164
CV (%)	4.74	3.60	3.69	3.30	.32	2.97	3.10	3.03

recorded maximum (47.17cm) and minimum (30.91cm) of the plant, raised from the seed treated with GA_3 (10ppm) and Kinetin (10ppm) respectively. Effectiveness of each treatment for length of stem is in the following order; $T_3>T_4>T_2>T_5>T_6$. Maximum number leaves/ plant (43), and highest total fresh weight (8.66g) and total dry weight (6.54) were also found of the plant, raised from the seed treatment T_3 ($GA_3$10ppm). All treatments were better than the control and result indicates that the pre-sowing treatments of *Stevia* seeds withT_2 (GA_3 5ppm), T_3 ($GA_3$10ppm), T_4 (IAA10ppm), T_5 (IBA 10ppm) and T_6 (Kinetin 10ppm) had remarkable beneficial effect on germination, number of leaves/plant, and fresh and dry weight of the plant. When the germination percentage of three growth regulators (Table: 2) were compared, GA_3 (10ppm) was observed more effective, which support the report of Chakrabarti and Mukherji,(2003).The application of another growth regulators could increased the seed germination and other physiological activity by the tolerance to the toxic particles which was found in consistent with the findings of Hoque and Haque, (2002). As from the table 3, data have shown that GA_3 could overcome the adverse effect in *Stevia rebaudiana* than Kinetin and Indole-3-acetic acid in the physiological activity, finding the support the report of Chakrabarti and Mukharji, (2002). It has been confirmed that exogenous application of gibberellic acid were found to promote germination of many seeds (EL-Barghahi and EL-Bakkosh, 2005). Several research reports endorse the enhancement of length of stem by GA_3 application (Vanangamundi et al.,1998).

ACKNOWLEDGEMENTS

The authors are thankful to Prof. Y.D. Tiagi and Prof. N.C. Aery, for their valuable suggestions and encouragement. We are greatly acknowledged to Mr. Anand Jain (Dr. jeeven Agro Farm, Sagar.) for providing seeds and U.G.C. for the financial help as Meritorious Fellowship.

REFERENCES

1. Chakrabarti, N. and Mukherji, S., Effect of phytohormone pretreatment on metabolic changes in *Vigna radiate* under salt stress. *J. Environ. Biol*., **23**: 295-300 (2002).
2. Chakrabarti, N. and Mukherji, S., Effect of Phytohormone pretreatment on nitrogen metabolism in *Vigna radiate* under salt stress. *Biol. Plant.,* **46**: 63-66 (2003).
3. EL-Bakkosh, A., The effect of mechanicl and chemical treatments on seed germination and seedling growth of some forest trees. *M.Sc Thesis*, Benghazi: Gayounis university (2001).
4. EL-Barghatgi, M. F. and EL-Bakkosh, A., Effect of some mechanical and chemical pre-treatments on seed germination and seedling growth of *Quercus coccifera* (Kemes Oaks). *Jornel of Jerash Private University* (in press) (2005).
5. Gottemoeller, J. and Ching, A., Seed germination in *Stevia rebaudiana* Perspective on New Uses. J. Janik (ed), ASHS Press, Alexandria, VA.pp 510-515 (1999) .
6. Hanson,J. R., and De Oliveira, B. H., Stevioside and related Sweet Diterpenoid Glycoside. *Net. Pod. Rep.* **10:**301-309 (1993).
7. Hoque, M. and Haque, S., Effects of GA_3 and its mode of application on morphology and yield parameters of mungbean (*Vigna radiate* L.). *Pak. J.Biol. Sci*., **5**:281-283 (2002).
8. Ishima, N and Katayama ,O., Sensary Evaluation of Steveioside as a Sweetener. *Rep. Natl. Fwd. Resp. Inst*. **31**: 80-85 (1976).
9. Khan, M.A., and Ungar, I.A., Seed germination and dormancy of *Polygonum aviculare* L. as influenced by salinity, temperature, and gibberellic acid. *Seed Sci. Technol*. **26**: 107-117 (1998).
10. Khan, M.A., Gul, B., and Weber, D. J., Seed germination characteristics of *Halogeton glomeratus*. *Can. J. Bot*. **79**: 1189-1194 (2001c).
11. Megeji, N. W., Ahuja, P. S., Mishra, Nidhi., Introducing *Stevia rebaudiana* anatural zero calorie sweetener. Institute of Himalaya Bioresource Technology, Palmpur. India. *Curreent Science.* **88**(5) (2005).
12. Strauss, S. . The perfect sweetener. *Technol. Rev*. **98**: 18-20 (1995).
13. Tanka , O., Steviol-glycoside: *New Natural Sweeteners Trend. Anal. Chem*. **1**: 246-248 (1982).
14. Uddin, S. Mohd., *In vitro* propagation of *Stevia rebaudiana* Bert. *In Bangladesh. African J. Biotech.* **5(13**): 1238-1240 (2006).
15. Vanangamundi, K., Venktesh, A., Jaiprakash, Mallika Vanangamundi , Umarani, R. and Vinayari, R. S., Effect of pre-sowing treatments on germination and seedling growth in *Syzygium cumini* (L). Skeeis. *Journal of Tropical Forest Science,* **8**: 77-87 (1998).
16. Yaniv, Z., Lisker, N., and Corbineau, F., Germination potential of *Sinapsis alba* seeds collected in Israel. *J. Arid Environ.* **29**: 293-303 (1995).