Research Article

Effect of gibberellic acid foliar spray on growth and stevioside content of *ex vitro* grown plants of *Stevia rebaudiana* Bertoni

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ABSTRACT

Stevia rebaudiana Bertoni is a shrub which is known for non-caloric natural sweetener called stevioside, which is the main component for its sweetness. In present investigation *ex vitro* studies have been conducted to analyze effect of different concentrations of plant growth hormone, gibberellic acid (GA_3). Foliar spray was used to mediate the experiment. Biochemical parameters (moisture, crude protein, total soluble sugars, chlorophyll content, calcium, total phenols, and antioxidant activity) and stevioside content have been estimated from *ex vitro* grown plants. Different concentrations (15, 30 and 60 µM) of gibberellic acid were applied as a foliar spray on *in vivo* vegetatively propagated plants of Stevia. Stevioside content in the leaf of *ex vitro* (control) grown plant was recorded 7.58 % on dry weight basis which was found to be increased significantly up to 18.08 % on dry weight basis. **[Medicinal Plants 2011; 3(2) : 00-00]**

Keywords : Gibberellic acid, *Stevia rebaudiana*, Stevioside.

INTRODUCTION

Stevia rebaudiana Bertoni, native to Paraguay, is an herbaceous perennial (2n = 22) shrub of the *Asteraceae* family. The shrub contains stevioside as a sweetener principle and thus, is having tremendous application to sweeten soft drinks, soy sauce, yoghurt and other foods in Japan, Korea and Brazil (Tadhani *et al.,* 2007). *Stevia* and its products are widely consumed by the Japanese all over the world (Taylor, 2005). It is recommended for diabetes and has been used by humans with no side effects (Megeji *et al.,* 2005). Due to its sweetener components the plant will get the place in natural food market in the near future (Starratt and Gijzen, 2004). Khan *et al.* (2006) reported that foliar spray of gibberellic acid on two cultivar of tomato (Hyb-SC-3 and Hyb–Himalata) showed increase in plant height, leaf area, leaf P content, fruit number, fruit yield and fruit lycopene content. They also observed the enhancing capacity of treated plants for biomass production which was reflected as the increase in the fresh and dry weight of plants and the observed leaf P content. Afroz *et al.* (2005) studied the exogenous application of gibberellic acid on mustard previously treated with NaCl. Treatments of NaCl resulted in a decrease in dry mass, leaf chlorophyll content, carbonic anhydrase activity (E.C. 4.2.1.1), nitrate reductase activity (E.C. 1.6.6.1) and net photosynthetic rate at the 60-day stage, and pod number and seed yield at harvest. However, spray application of GA_3 neutralized the ill effect of soaking treatment in NaCl (1 or 10 mM). Stevioside is a diterpene glycoside having two sites for the attachment of glycone moiety. Structure of stevioside is very similar to that of gibberellins, a plant growth regulator. Stridner *et al.* (2004) fed GA_3 to cell suspension cultures of *Stevia rebaudiana* which showed fast conversion to stevioside.

During the present investigations we have studied the effect of gibberellic acid foliar spray with the aim of observing changes in the biochemical parameters including stevioside content in *Stevia rebaudiana*.

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MATERIAL AND METHODS

Stevia rebaudiana Bertoni were grown in the Botanical garden of Department of Botany, Anand Agricultural University. Plants were grown in March, 2008 through vegetative propagation and used for experiment after one month. In pot grown plants of *Stevia rebaudiana,* three different concentrations (15, 30 and 60 mM) of gibberellic acid were sprayed on 30 – day old plants. Sampling was carried out after two days of treatment.

Biochemical Parameters and Stevioside Content

To study growth of *Stevia rebaudiana* several biochemical parameters were analyzed. These biochemical parameters include moisture, crude protein, total soluble sugar, chlorophyll content, calcium content and total phenol and antioxidant activity. Stevioside content was also determined with biochemical parameters.

Moisture content and crude protein content from leaf of *Stevia rebaudiana* was determined by the method described by Sadasivam and Manickam (1992) with minor modifications. Total soluble sugars from leaf of *Stevia rebaudiana* was determined by phenol-sulphuric acid method described by Dubois *et al.* (1956). The method developed by Hiscox and Israelstam (1979) was used for determination of chlorophyll from plant leaf. Total calcium was determined using acid extract as per the method described by Sadasivam and Manickam (1992). Total phenols and antioxidant activity of *Stevia rebaudiana* leaves were measured using method described by Tadhani *et al.* (2007). Total stevioside content was measured using method developed by Ludmila *et al.* (1998) with minor modifications.

Statistical Analysis

Observation for all the biochemical data was taken in three replications in the experiment, which were analyzed using completely randomized design (CRD) at 5 % level of C.D.

RESULTS AND DISCUSSION

Biochemical Parameters

Data for all the parameters (and stevioside content) studied are presented in detail (Table 1). Due to foliar application of gibberellic acid the water content in the leaf was decreased significantly. Maximum water content was recorded in control plants (84.51 %) and the amount decreased with increasing concentrations of gibberellic acid and found lowest in 60 µM (75.40 %). Decrease in water content of the cells suggested that foliar spray of gibberellic acid may increase the cellular biomass inside the leaf tissue. Subedi and Bhattarai (2007) studied the effect of gibberellic acid on reserve food mobilization of maize endosperm during germination and found that the dry matter of the growth axis significantly increased after foliar spray of gibberellic acid of different concentration. In case of transplanted plants, it might be possible due to increased uptake of essential nutrients from soil and thus dry matter of the cell increased. In the gibberellic acid treated plants the highest protein content was found in control plants (20.57 %) and the amount was reduced

Table 1. Growth parameters and stevioside content in fresh leaf of gibberellic acid treated plants.

Treatment	Moisture (%)	Crude Protein* (%)	Total Soluble Sugars (%)	Total Chlorophyll mg/g of fresh weight	Calcium (%)	Total Phenols (%)	Antioxidant Activity mg equivalent of ascorbic acid/gm of dry weight	Stevioside Content (%)
Control	84.51	20.57	0.22	14.1	0.18	1.33	10.32	7.58
15 µM GA_3	80.50	16.78	0.14	12.2	0.34	1.16	11.75	9.48
30 µM GA_3	78.87	17.95	0.15	13.8	0.36	1.40	12.49	11.48
60 µM GA_3	75.40	18.82	0.25	17.5	0.38	3.50	13.19	18.08
S. Em.	0.328	0.038	0.013	0.153	0.055	0.015	0.002	0.111
$C.D._{0.05}$	1.167	0.136	0.047	0.543	0.197	0.053	0.009	0.395
CV %	6.416	3.328	4.654	6.784	9.284	5.819	1.594	5.267

significantly in treated plants, however the protein content increased significantly within the treatments. The biochemical function of gibberellic acid on protein content is still not clear from the experiment. Sharaf *et al.* (2003) studied the effect of GA_3 on growth characteristics, earliness, yield, carbohydrates and protein in *Cynara cardunculus* and found that the application showed no direct influence on carbohydrates as well as protein content. However, they also found that the protein content in treated plants were lower than the untreated plants. Total soluble sugar content was found lowest in 15 µM application and highest in 60 µM application. However, increase in the content was not significant between 15 and 30 µM application of GA_3. Highest amount of chlorophyll content was observed in 60 µM applications (17.5 mg/g of fresh weight) whereas lowest was observed in 15 µM applications of gibberellic acid (12.2 mg/g of fresh weight). Almost 28 % enhancement in chlorophyll content was observed in GA_3 treated plants as compared to control. It is possible that this effect may be through an interaction with phytochrome, since chlorophyll is influenced by phytochrome (Mathis *et al.,* 1989). They also studied the effect of gibberellic acid on greening of pea seedlings and found that the chlorophyll content was significantly increased due to foliar spray of gibberellic acid. Exogenous application of gibberellic acid as foliar spray on leaf of *Stevia rebaudiana* with different concentrations showed that the highest calcium content was recorded 0.38 % in 60 µM treatments of gibberellic acid sprayed plants and the lowest were recorded 0.18 % in control plants. The calcium content was increased significantly due to treatments and the enhancement was also significant within the treatments. Increase in calcium content due to treatments suggested that foliar spray of gibberellic acid enhances the uptake of calcium from the soil and transport to the leaf but the exact mechanism is still unclear. Both total phenols and antioxidant activity were increased significantly in the exogenous application of gibberellic acid. Similar result was observed by Sharaf *et al.* (2007). They studied the effect of gibberellic acid on Globe artichoke at different concentrations at different time intervals and found that the application of 60 ppm spray gave significant increase in phenolic compounds after 2 weeks of transplanting in leaves. Increased antioxidant activity can be correlated with amount of phenolic compounds. As the total phenols increased the total antioxidant activity also increased.

Stevioside Content

The chromatogram of HPLC showed that stevioside was detected at retention time of 5.9 minutes (Figure 1). The results of the foliar spray showed maximum amount of stevioside in 60 µM application (18.08 %) and minimum in control (7.58 %) on dry weight basis. The stevioside content was significantly increased in treated plants. Gibberellic acid and stevioside shares a common metabolic pathway (Brandle and Telmer, 2007). Due to uptake of gibberellic acid through foliar spray the amount of gibberellic acid increases within the cell which may lead to shut down the process of gibberellins biosynthesis by feedback inhibition and the pathway is diverted to steviol glycoside accumulation.

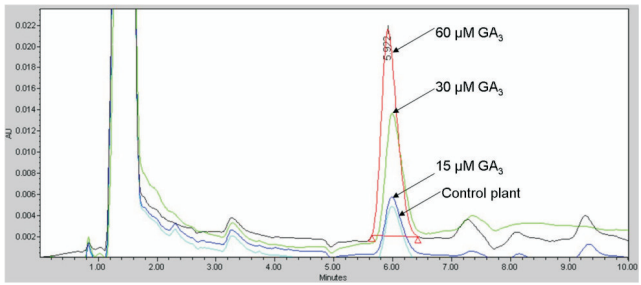

Fig. 1. Chromatogram of stevioside content measured in control and treated plants detected at retention time of 5.9 minutes.

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