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Growing stevia plants in household condition and their evaluation on the basis of phenotypic attributes

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Abstract: Experiment was carried out to explore the best-suit growing condition of stevia (*Stevia rebaudiana* Bertoni) in household condition during the period from January to December, 2007 in the laboratory of the Department of Biochemistry, Bangladesh Agricultural University, Mymensingh. Plants were grown in six different conditions as T_1-(Hoagland solution, indoor, 15h day length); T_2-(Hoagland solution, indoor, 12h day length); T_3- (Hoagland solution, on roof, 12h day length); T_4-(Pond water, indoor, 12h day length); T_5-(Soil, on roof, 12h day length); T_6-(Soil, in corridor, 12h day length). Light intensities were measured and found to vary from 25000 to 38000 lux in T_1, T_2, T_4; 47000 to 56000 lux in T_3, T_5 and 12000 to 23000 lux in T_6. Deflowering was performed at 15 days interval and matured leaves were collected prior to commencement of flowering. After final harvesting the phenotypic attributes such as biomass content, leaf-stem ratio and leaf area were ascertained and used to evaluate the growing conditions. All these parameters were significantly influenced by the different cultural conditions. Plants growing in Hoagland solution under 15h light regime showed the best performance in terms of leaf-stem ratio and leaf area. Short day length and higher light intensity enhanced the frequency of deflowering. The plants survived when grown in pond water under 12h light regime in a critically deficient nutrient condition. Considering the phenotypic attributes such as flowering incidence, leaf-stem ratio and leaf area, plants grown in Hoagland solution having 15 hours day length of moderate light intensity seemed to be the best suited for the production of stevia plants in house-hold condition.

Key words: Phenotypic attribute, Stevioside, Hydroponic culture

Introduction

Stevia is a perennial plant of the compositae family native to Paraguay (Brian, 2003). It is a popular source of high-potency natural sweetener and dietary supplement. Stevioside is the principal steviol glycoside (ingredient) of stevia leaf which is mainly responsible for the sweetness of the plant and it is 250-300 times sweeter than sucrose (Genus, 2003). Stevia has gained attention with the rise in demand for low-carbohydrate, low-sugar food additive. Stevia has a negligible effect on blood glucose, even enhancing blood glucose tolerance (Curi *et al.,* 1986). Therefore, it is an attractive source of natural sweetener to diabetics as it is proved to be safer compared to the widely used artificial sweetener such as aspartame, saccharine, cyclamate etc.

The stevioside content in leaves can vary 4% to 16% between individual plants depending on the growth conditions and on cultivars (Bian, 1981 and Nakamura, 1985) and hence proper growing condition is required to adopt suitable cultivation techniques. Sensitivity of the plant to water logging condition for a short period and onset of early flowering are the main constraints in growing the plant in field condition in Bangladesh. So, attempts were made to explore suitable growing condition to grow this plant in house-hold condition with sustained production which may ensure their availability as and when necessary especially to diabetic subjects.

Materials and Methods

The experiment was conducted in the laboratory of the Department of Biochemistry, Bangladesh Agricultural University, Mymensingh, during the period from January to July, 2008. Thirty days old stevia seedlings were collected from the nursery of Bangladesh Rural Advancement Committee (BRAC), Gazipur, Bangladesh.

Plant culture: Total twenty four plants were cultured in the whole study. The plants were grown in hydroponic solution as well as in pot soil. Sixteen plants were cultured hydroponically in pot, among them twelve plants were cultured in Hoagland nutrient solution and four plants were grown in normal pond water. Eight plants were grown in soil medium in pot. The plants were grown for a period of 140 days commencing from 20 February, 2007 to 10 July, 2007 as outlined in table 1.

Table 1. Growing conditions of stevia adopted in the whole study

Treatments	Growing media	Location	Day length (hours)
T_1	Hydroponic culture (HC) in Hoagland solution	Indoor, near window	15 (12 + 3*)
T_2		Indoor, near window	12
T_3		On roof	
T_4	HC in pond water	Indoor, near window	
T_5	Pot culture in soil medium	On roof	
		Corridor	

Selection and preparation of pot: Cylindrical shape plastic pots of 16 cm height and 9.5 cm in diameter were selected for the experiment. The internal volume of each pot was about 1.1 liter. The cap of each pot contained two circular openings of around 1cm each in diameter one at the centre and another near periphery.

Placement of seedlings

i) **In Hoagland solution:** Rooting media of twelve healthy and uniform stevia seedlings were washed out slowly by tap water. One liter Hoagland solution (Hoagland, D.R. and Arnon, D.I. 1950) was taken in each pot. One seedling was planted in each pot at the central hole and the second hole was left for adding nutrient solution or distilled water. Seedling was fixed properly in the hole with the help of a small piece of foam. The second hole was also covered with foam to protect evaporation. Constant volume of the nutrient solution was maintained by adding required amount of distilled water in every week to keep the nutrient concentration almost unchanged. By keeping 100mL of old (taken first time) nutrient solution, the remaining whole (900mL) nutrient solution of each pot was changed in every one month.

ii) **In pond water:** Following the same procedure as that of hydroponic culture in Hoagland solution, four plants were subjected to grow in pond water.

iii) **In soil:** Soil collected from Bangladesh Agricultural University campus of agro-ecological zone-9 under the Old Brahmaputra Floodplain (UNDP and FAO, 1988) was sun dried and ground properly. The nutrient status of the soil was enriched by mixing cowdung at the rate of 7.5 kg per 100 kg of soil. Medium size pots (0.049 m^2) were used for planting. Each pot was filled with 7kg of soil. Rooting media of the seedlings were removed by gentle jerking and planted one plant in each pot. Required amount of water was added in each pot as and when necessary.

Artificial lightening: In case of treatment T_1, three hours artificial lightening was added with the normal day time to make total fifteen hours day length. For artificial lightening, three fluorescence tube lights of total light intensity 6000 lux were hanged on four inches above from the top of the plants. Illumination of tube lights was controlled by an automatic timer (theben timer 026).

Measurement of light intensity: Light intensity was measured at noon (1pm) once in every five days by a Lux-meter (testo-0500, Germany) and was recorded properly commencing from 01 March to 30 June, 2008.

Deflowering: Flowering of the plants was monitored carefully, deflowered manually at fifteen days interval and recorded properly.

Harvesting and drying: Matured lower leaves of plant were harvested prior to flowering when stevioside content in leaves was maximum (Sumida, 1980 and Xiang, 1983). The weights of the leaves were recoded properly. Final harvesting was done on 10 July, 2008. Leaves were dried in an oven at 45-50^o Celsius and preserved in air-tight condition.

Determination of biomass: Initial weight of the whole plant was taken individually before planting in growing medium. All the leaves and stems of individual plant collected from plantation to final harvesting were weighed freshly and recorded properly. The addition of all the values along with the root gave the accumulated weight. Accumulated weight minus initial weight gave the value of biomass increment of the individual plant during the experimental period.

Measurement of leaf-stem ratio: Fresh weights of all the leaves and stems of an individual plant produced during the whole life span were taken separately. From these two values leaf-stem ratio was calculated.

Determination of leaf area: Leaf area was determined from the length and breath measurements of leaf according to the method followed by Michael *et. al*. (2000) and Rawson (1984).

Results and Discussion

Phenotypic characters: The phenotypic attributes considered to evaluate the effects of cultural conditions were biomass content, leaf-stem ratio and leaf area. All these parameters were significantly influenced by the different cultural conditions (Table-2). The maximum amount of biomass was observed in the treatment T_5 (24.20 g) followed by the treatment T_2 (22.66 g) and treatment T_1 (20.02g). The lowest biomass was noticed in the treatment T_4 (15.15 g). The highest leaf-stem ratio occurred in treatment T_1 (2.644) and was significantly different from the rest. Treatment T_6 resulted the lowest leaf-stem ratio (1.232). The highest leaf area was noticed in treatment T_1 (4.34 cm^2) and was significantly different from those of the rest. Plants growing in treatment T_6 produced the lowest leaf area (2.04 cm^2).

Table 2. Mean values of different phenotypic characters of stevia plants

Treatments	Biomass (g)	Leaf-stem ratio	Leaf area (cm^2)
T_1	20.02 c	2.644 a	4.34 a
T_2	22.66 b	1.517 bc	2.57 c
T_3	18.10 d	1.813 b	3.24 b
T_4	15.15 f	1.346 c	2.43 cd
T_5	24.20 a	1.708 b	3.07 b
T_6	16.19 e	1.232 c	2.04 d
LSD	0.609	0.410	0.396

T_1 = Hoagland solution, indoor, 15h; T_2 = Hoagland solution, indoor, 12h; T_3 = Hoagland solution, roof, 12h; T_4 = Pond water, indoor, 12h; T_5 = Soil, roof, 12h; T_6 = Soil, corridor, 12h

Plants grown in soil under 12 hours light regime (T_5) synthesized maximum amount of biomass (24.20 g) but those grown in hydrophonic culture under 15 hours light regime (T_1) showed the best performance in terms of leaf-stem ratio (2.644) and leaf area (4.34cm^2). Therefore, it was noticed that extended day length has contributed to attain highest leaf area and leaf-stem ratio in the treatment T_1.

Light intensity: Table-3 presents that the range of variation of light intensity during experimental period was 25000-38000lux for the treatments T_1, T_2 and T_4; 47000-56000lux for treatments T_3 and T_5 and 12000-23000lux for the treatment T_6. Treatment T_1 had comparatively lower light intensity (25000-38000 lux) than direct sunshine (47000 to 56000 lux) of treatment T_5. Increased light intensity can expedite vegetative growth. T_5 treatment had 47000 to 56000 lux intensity which might

have resulted the production of maximum biomass. However, the higher leaf-stem ratio of T_1 may compensate the biomass effect of T_5 in terms of stevioside content as leaf contain maximum amount of stevioside.

Table 3. Applied light intensity in different growing conditions

Treatments	Light intensity (x×1000 lux) at noon (1 pm)				Range of variation during experimental period
	March	April	May	June	
T_1	25-29	28-32	31-35	34-38	25-38
T_2	25-29	28-32	31-35	34-38	25-38
T_3	47-49	48-50	50-53	54-56	47-56
T_4	25-29	28-32	31-35	34-38	25-38
T_5	47-49	48-50	50-53	54-56	47-56
T_6	12-14	15-17	17-20	20-23	12-23

Unless it is noticeably poor, fertility of the growing medium is less important for offering desirable phenotypic attributes and shading has been shown to reduce growth (Slamet and Tahardi, 1988). For this reason, in treatment T_6 where the plants were grown in soil receiving minimum light intensities (12000-23000 lux) showed the lowest values for biomass increment (16.19g), leaf-stem ratio (1.232) and leaf area (2.04 cm^2). In treatment T_4 light intensities were reasonable (25000-38000 lux) but the growing medium (pond water) was deficient of all the nutrients. It was astonishing that the plants survived in such a deficient nutrient condition.

Deflowering: Deflowering was practiced to speed up the vegetative growth of the plant and also to restrict the reduction of stevioside accumulation in leaves (Sumida, 1980 and Xiang, 1983). The highest number of deflowering was done in case of treatment T_5 (seven times) followed by T_3 (five times) and four times deflowering was performed in case of other treatments (Table-4). It indicates that plants those were grown under direct sunlight needed more deflowering. Higher light intensity (47000-56000 lux) along with short day length (12h) might have induced these sorts of flowering. Similar finding was also reported by Brandle (1998) who mentioned that flowering was photoperiod dependent and was enhanced by reducing day length.

Table 4. Incidence of deflowering in different cultural conditions

Treatments	Days of deflowering after planting						
	40	55	70	85	100	115	130
T_1	-	+	-	+	+	-	+
T_2	-	+	-	+	-	+	+
T_3	-	+	+	+	+	-	+
T_4	-	+	-	+	-	+	+
T_5	+	+	+	+	+	+	+
T_6	-	+	-	+	-	+	+

"+" stands for deflowering, and "–" stands for no deflowering

Metivier and Viana (1979) reported that the concentration of stevioside in the leaves of stevia increases when the plants are grown under long days and Yermakov *et al. (*1996) also concluded that increasing day length to 16 hours can increase vegetative growth and stevioside levels. The leaves of stevia plants grown in treatment T_1 might have produced more stevioside as the day length was 15 hours. Tateo *et al.,* (1998) reported that the total stevioside was positively correlated with leaf-stem ratio. The plants of treatment T_1 had produced the highest leaf-stem ratio and might be suitable for more stevioside accumulation. Treatment T_1 that means growing the plants in Hoagland solution having 15 hours day length of moderate light intensity (25000-38000 lux) showed the best performance for the production of stevia plants in house-hold condition by considering the phenotypic attributes namely flowering incidence, and relative stevioside content in terms of leaf-stem ratio and leaf area.

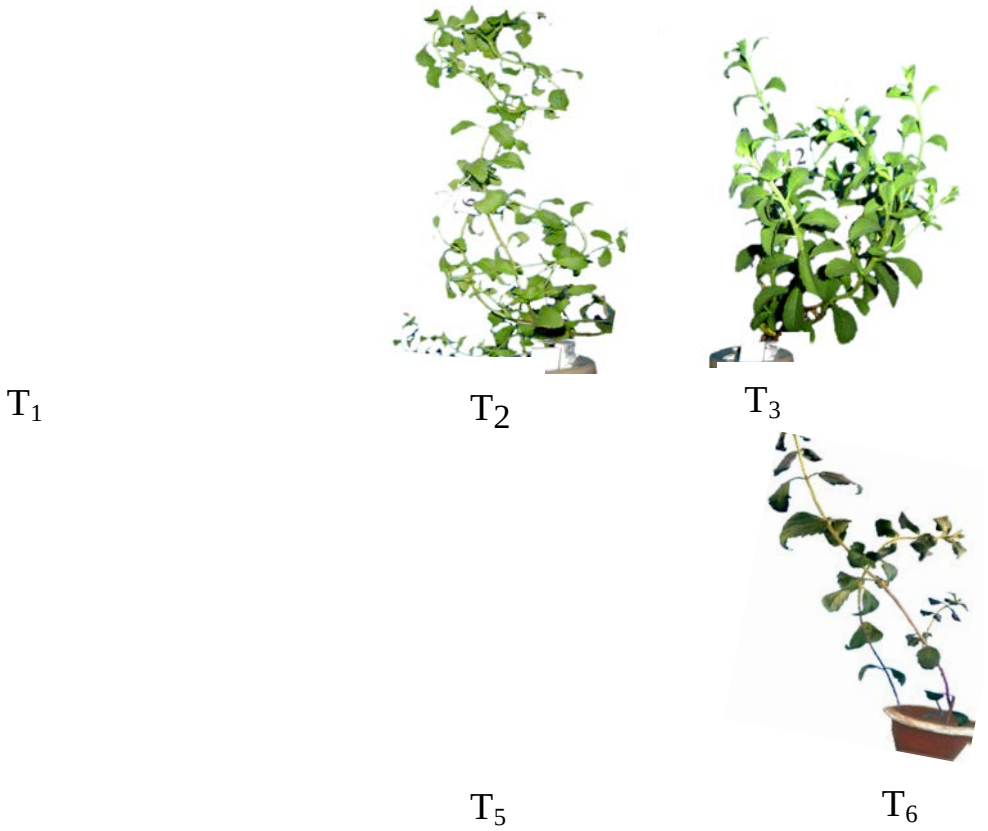

Fig.1. Stevia plants were grown in different cultural conditions(T_1 = Hoagland solution, indoor, 15h; T_2 = Hoagland solution, indoor, 12h; T_3 = Hoagland solution, roof, 12h; T_4 = Pond water, indoor, 12h; T_5 = Soil, roof, 12h; T_6 = Soil, corridor, 12h)

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