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Vegetative propagation of Stevia (*Stevia rebaudiana*)

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Abstract: The experiment was carried out in the BINA (Bangladesh Institute of Nuclear Agricultural), Mymensingh (24^o N and 98^o S latitude, 11 m Elevation) during March'08 to June'08 to find out the protocol of propagation of Stevia (*Stevia rebaudiana* Bert.), which is compatible with the socio-economic condition of Bangladesh. Three consecutive experiments were done to complete this experiment. In the first experiment, from mother plant, 21 to 25 days old disease free branches were selected and 8-10 cm long terminal part containing 8-10 leaves were cut off with the help of a sharp knife in the afternoon. Immediately after cutting, the cuttings were placed in the small pots of plastic tray containing different medium (viz. soil, sand, saw dust, soil + sand, soil + saw dust, and sand + saw dust). A randomized complete block design was used with two factors (Factor A: Medium of planting viz. soil, sand, saw dust and their combinations and Factor B: glass house, polythene shade, straw shade and under the open sky) and three replications. The highest survivability was found from soil as a media under the straw shade. Based on the results of the first experiment another experiment was set up considering the survivability percent. It was set up directly in the field. A randomized complete block design was used with two factors. Factor A: open sky, polythene shade and straw shade and factor B: cuttings (with leaves and without leaves) and three replications. The block size was 2.5 m × 4 m × 1 m. Each plot was 1m^2 (1m x 1m). From this experiment the cuttings with leaves under straw shade showed distinctly superior performance compared to others. The highest percent survivability (90.33%) was obtained from cuttings with leaves under the straw shade which was significantly different from all others combinations. In case of third experiment randomized complete block design was used with two treatments and three replications. In this experiment, only straw shade was used due to it's outstanding performance in case of first one and two experiments by maintaining same distance. Cuttings with leaves produced highest percent (93.67%) final survivability followed by 38.67% from cuttings without leaves.
Key words: Stevia, Vegetative propagation, **S**urvivability

Introduction

Stevia rebaudiana Bert. is one of 154 members of the genus Stevia and one of only two that produce sweet steviol glycosides (Robinson 1930; Soejarto *et al.* 1982, 1983). It is native to the valley of the Rio Monday in highlands of Paraguay, between 25 and 26 degrees south latitude, where it grows in sandy soils near streams (Katayama *et al.* 1976). Stevia is a calorie free natural sweetener, botanically known as *Stevia rebaudiana* Bert. The sweetness in leaves due to presence of sweetening agent called stevioside and about 300 times sweeter than sucrose (Geuns, 2003). The principle importance of stevia is due to the possibility of substituting it for saccharine (Lewis,1992.). Stevia had wide antimicrobial, antibacterial activity against virus and yeast. It is used as a natural sweetener, for diabetes, for high blood pressure, for cavity prevention, as a weight loss aid, increases urination, dilates blood vessels (Frederico, *et al*., 1996). Moreover, diabetic are a serious problem throughout the world. In our country about 80 lakhs of 20 to 69 years old peoples are suffering from this serious disease which is about 5.9% of total population. But the most alarming message is a number of children aged from 8 to 20 years old are also suffering from this serious disease. Under this situation stevia can help these large number of people and in addition to this stevia can contribute to our national economy.

Stevia is a new crop in Bangladesh. It has been introduced by Bangladesh Sugarcane Research Institute (BSRI) in 2001 from Thailand. After that some research works has been done in BSRI and BRAC. But still now suitable method of cultivation at field level has not been developed. Even the most important thing that is propagation involves tissue culture which is not feasible for the farmer of our country. Worldwide reviews on tissue culture and about safety for human consumption are abundant but reviews on propagation of Stevia are scanty. As tissue culture requires highly sophisticated technologies and equipments. this research was aimed to develop a Protocol of propagation of Stevia (*Stevia rebaudiana*) which is compatible with the socio-economic condition of Bangladesh.

Materials and Methods

The experiment was carried out in the BINA (Bangladesh Institute of Nuclear Agricultural), Mymensingh (24o N and 98o S latitude, 11 m Elevation) during March'07 to June'07 to find out the protocol of propagation of Stevia (*Stevia rebaudiana*), which is compatible with the socio-economic condition of Bangladesh. The experimental area was characterized by non-calcareous dark grey floodplain soil belonging to the Sonatola Soil Series under the Old Brahmaputra Floodplain (AEZ-9) (UNDP and FAO 1988). Soil of the experimental site was more or less neutral with a pH value of 6.8, low in organic matter content and fertility level. The land type was medium high with silty loam texture. The experimental site was under the subtropical climate, which was characterized by high temperature, high humidity and heavy precipitation with occasional gusty winds during April to September and Scanty rainfall associated with moderately low temperature from October to March. Stevia is a new crop in Bangladesh and considerable research work has not been conducted yet. This was the first and worthy research work on Stevia in Bangladesh at field level. Based on research works conducted in BSRI and some international works this experiment was designed.

First experiment

From mother plant, 21 to 25 days old disease free branches were selected and 8-10 cm long terminal part containing 8-10 leaves were cut off with the help of a sharp knife in the afternoon. Immediately after cutting, the cuttings were placed in the small pots of plastic tray containing different medium (viz. soil, sand, saw dust, soil + sand, soil + saw dust, and sand + saw dust). A randomized complete block

design was used with two factors and three replications as folows.

Factor A: Medium of planting. The sub-factors are as follows:

i.	Soil	iv. Soil (50%) + sand (50%)
ii.	Sand	v. Soil (50%) + saw dust (50%)
iii.	Saw dust	vi. Sand (50%) + saw dust (50%)

Each tray contains 64 small holes and all holes of a tray were filled up with the same type of medium. Thus 24 trays were prepared. In each tray cuttings were planted in 16 holes with an alternate fashion of planting and empty at each side.

Factor B: Environment.

Four different environments were selected to place the trays containing the cuttings. The sub-factors were, Glass house, under the cover of perforated polythene, under the shade (2 feet above the soil surface) prepared by straw and under the open sky.

Then six trays containing six types of medium and cuttings were placed in glass house, six were under the polythene cover to prevent moisture loss, six were under the shade of straw and the last six were placed under open sky. Then these trays were kept under continuous observation.

Second experiment:

This experiment was set up directly in the field. A randomized complete block design was used with six treatments and three replications. Each block was 2.5 m wide and 4 m long and 1 m apart. Each plot was 1m2 (1m x 1m). It was two factorial experiment.

Factor A. Environment

Straw shade (2 feet above soil surface), polythene (2 feet over soil surface) cover and open sky

Factor B. Cutting.

With leaves and without leaves

Cuttings were taken as before. But in case of cuttings without leaves, just the leaves were removed. In each plot cuttings were planted 15 cm apart at each side. From this experiment the cuttings with leaves under straw shade showed distinctly superior performance compared to others. Considering the results of this experiment another experiment was set up.

Third Experiment:

Randomized complete block design was used with two treatments and three replications. In this experiment, only straw shade was used. Each block was 1 m wide and 2.5 m long and 1 m apart. Area of each plot was 1m2 (1m x 1m). Treatments were as follows:

A. Cuttings with leaves and
B. Cuttings without leaves.

Cuttings were collected in the same manner as done in the first experiment. The cuttings were planted 15cm apart from each other at both sides. Water was applied in such a manner so that the soil always remains wet (around field capacity).The recorded data were compiled and tabulated for statistical analysis. Analysis of variance was done with the help of statistical package Mstatc. The mean difference among the treatments was adjudged with Least Significant Difference test (Gomez and Gomez, 1984).

Results and discussion

To determine the proper method of propagation of stevia through vegetative means the cuttings were placed under different environments with different medium. The results are described below:

First experiment

Four environments (viz. glass house, polythene shade, straw shade and under the open sky) and six medium (viz. soil, sand, saw dust and their combinations) were selected and cuttings were placed. Under the open sky the cuttings of all medium were remain as it was placed, in the first day. But in the next day morning (10.00 am) they start to wither and at afternoon almost all were very close to death. In the third day of planting only one cutting of soil medium was alive.

In case of glass house the same results were obtained like open sky. This might be due to excess heat cause excess loss of moisture through transpiration. In the glass house finally only one of soil medium was survived. Under the polythene cover almost same results was obtained. Finally two cuttings of soil and soil+ sand were survived. The highest survivability was found from the straw shade. The third day three cuttings of soil were survived.

Second experiment

Based on the results of the above experiment another experiment was set up considering the survivability percent. During designing this experiment another point was taken under consideration, which is presence of leaves. From the results of the first experiment, it was assumed that removal of leaves may reduce the transpiration rate which might increase survivability percent. In this experiment three environments (viz. open sky, polythene shade and straw shade) were used with two types of cuttings (viz. with leaves and without leaves).

Effect of environments

From this experiment it was found that there was significant effect of environments on the final survivability of cuttings at 0.1% level of probability. The straw shade gave the highest percentage of survivability (67.17%) followed by polythene cove (11.83%) and under open sky (7.17%). The survivability of 1st, 2nd and 3rd day also showed the similar pattern of survivability which is presented in the Table 1.

Effect of presence of leaves: Presence of leaves was significantly influence the final survivability percent of cuttings. Presence of leaves has the significant effect on the final survivability percent at 0.1% level of probability. The maximum percent survivability (36.33%) was found from cuttings with leaves followed by 21.12 % from without leaves. The survivability of 2^{nd}, 3^{rd} and 4^{th} day was similar with the final survivability, which is presented in the Table 2.

Combined effect of survivability

The combined effect of environments and presence of leaves on the final survivability of stevia cuttings was found significant at 0.1% level of probability. The highest percent survivability (90.33%) was obtained from cuttings with leaves under the straw shade which was significantly different from all others combinations. Next to this cuttings without leaves under the straw shade produced

Table1: Effect of different environments and medium on the production seedlings through stem cuttings

Treatment combinations		Survivability (no.)				
		No. of total cuttings planted	One day after planting	Two day after planting	Three day after planting	Final survivability
Glass House	Soil	16	16	2	1	1
	Sand	16	15	1	0	0
	Saw dust	16	12	0	0	0
	Soil+Sand	16	15	0	0	0
	Soil+Saw dust	16	14	0	0	0
	Sand+Saw dust	16	13	1	0	0
Polythene Cover	Soil	16	16	2	1	1
	Sand	16	15	0	0	0
	Saw dust	16	10	1	1	1
	Soil+Sand	16	12	1	0	0
	Soil+Saw dust	16	14	1	0	0
	Sand+Saw dust	16	13	0	0	0
Straw Shade	Soil	16	16	4	3	3
	Sand	16	14	0	0	0
	Saw dust	16	15	1	0	0
	Soil+Sand	16	14	2	1	1
	Soil+Saw dust	16	12	0	0	0
	Sand+Saw dust	16	13	0	0	0
Open sky	Soil	16	16	1	1	1
	Sand	16	14	0	0	0
	Saw dust	16	10	0	0	0
	Soil+Sand	16	12	1	0	0
	Soil+Saw dust	16	11	0	0	0
	Sand+Saw dust	16	14	0	0	0

Table 2. Effect of different environments and presence of leaves on the survivability percentage of cuttings of stevia

Treatments	**Survivability %**				
	No. of total cuttings planted	One day after planting	Two days after planting	Three days after planting	Final survivability
With leaves	100	96.67 a	44.56 a	38.22 a	36.33 a
Without leaves	100	95.78 b	29.56 b	23.56 b	21.12 b
Level of significance		*	***	***	***
C.V. (%)		0.73	8.12	6.27	7.07
$LSD_{0.05}$		0.73	3.16	2.04	2.13

Table 3. Effect of presence of leaves on the survivability of cuttings

Treatments	Survivability %				
	No. of total cuttings planted	one day after planting	Two day after planting	Three day after planting	Final survivability
With leaves	100	97.00	96.67 a	95.00 a	93.67 a
Without leaves	100	90.33	44.67 b	43.67 b	38.67 b
Level of Significance		NS	**	**	**
C.V.		4.29	3.47	5.13	7.71
$LSD_{0.05}$		14.13	8.61	12.50	17.91

second highest percent survivability (44.00%). The lowest survivability was obtained from cuttings without leaves under the open sky which is presented in the Table 3.

Third experiment

Considering the results of above two experiments the third experiment was designed. From the above two experiments it is clear that the straw shade is the best for the stem cutting of stevia. But to observe the effect of presence of leaves on the percent survivability of cuttings this experiment was designed. In this experiment only straw shade was used as environment. Two types of cuttings were used. One was with leaves and another was without leaves. Presence of leaves significantly influence the survivability of cuttings at 1% level of probability. The cuttings with leaves produced highest percent (93.67%) final survivability followed by 38.67% from cuttings without leaves. The survivability percent at other days were similar to the final survivability which is presented in the Table 4.

From the above experiments it is clear that the cuttings with leaves under straw shade give the highest performance for propagation.

Method of propagation of stevia through vegetative means

From the above experiments it is found that the propagation of stevia is possible through stem cutting which is suitable with the agro-climatic condition and socio-economic situation of the farmers of our country. The method of propagation of stevia through stem cutting is described below.

1. At first a stem of 20-30 days old is to be selected. The stem must be of free from diseases and also from flowers or flower buds. Then 6-8 cm long apical part of the stem to be cut out with the help of a sharp knife. Then the lower leaves of the stem are to be removed and a slanting cut is to be given to increase the contact surface of the stem with soil.
2. There is no specialization about the preparation of bed for planting the cuttings except, the bed is to be raised for about 4-6 inches to avoid water stagnation. The bed is to be free from weeds and stubbles and the soil is to be more or less friable. A shade should to be given with the help of bamboo and straw on the bed, about 2.5-3ft above the soil level. The straw shade will keep the overall environment cool and will prevent water loss from the cuttings.
3. Then the cuttings are to be planted in the soil smoothly so that the basal portion of the stem remains unaffected. Immediately after planting the cuttings water is to be applied to make the environment including soil moist but not wet. It is better to apply water to the shade to make the shade wet.
4. The cuttings may wither slightly at the next day noon but those will recover this at afternoon if water is applied properly. Water is to be applied daily at morning to make the environment moist. Thus the environment needs to be kept moist for first three days. After three days water also to be applied but it is not as crucial as the first three days is. From three to ten days after planting the cuttings will remain as it was planted. After 10-12 days, the cuttings will start to give new leaves. After 21-28 days after planting, the stem will be healthy with sufficient number of leaves (15-20). And after one month the stems will be suitable for planting in the main field.

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