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Callus induction and regeneration of *Stevia rebaudiana* Bertoni

M. Abd El-Motaleb, Asmaa R. Abd El-Hameid and Hoda M. H. Elnaggar, M. S. Abdel-Hady

Plant Biotechnology, Botany Department, National Research Centre, Egypt

Abstract: *Stevia rebaudiana* Bertoni is an important non-caloric sweetening herb.It has some kind of diterpenoidsteviol glycosides that had no negative effect on blood sugar level.In the present study, efficient plant regeneration *via* callus was established. Explants were cultured on MS supplemented with different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) alone and the combination with 1.0 mg/L BA were used to initiate callus. Maximum frequency of callus induction (89%)were observed on MS supplemented with 2.0 mg/L2,4-D.Multiple shoots were obtained on MS medium containing 0.5 mg/L BA and 1.0 mg/LNAA from friable, granule and healthy calli which obtained after 45 days.Regeneration percentage, number of shootlets/ callus part, length of shootlets, number of nodes and number ofleaveswere 86 %, 13.2, 4.36 cm, 4.0 and 8.0, respectively.
Key words: *Stevia rebaudiana*, *In-vitro*,Micropropagation, Murashige and Skoog's Medium (MS), Callus,Multiple shoots,shootlets, regeneration.

Introduction

Stevia rebaudiana Bertoni belongs to the family Asteraceae as a perennial herb native to Paraguay and Southern Brazil. The leaves of the plant contain diterpeneglycosides, which is 100 400 times sweeter than glucose and has chemical and pharmacological characteristics that make it suitable for using in human diet as a natural calorie-free agent[1].

Stevia is a small shrub by perennial growing up to 65 cm tall, with sessile, oppositely arranged lanceolate to oblanceolate leaves, serrated above the middle. It is originally a South American wild plant, but could be now all found growing in semi-arid habitat ranging from grassland to shrub forest to mountain terrain all over the world. The first report of commercial cultivation in Paraguay was in 1964 began a large effort aimed at establishing Stevia as a crop in Japan[2].

Stevia rebaudiana is non (toxic, calorie, plaque, fermentative, carcinogenic, addictive sweetness for children), flavor enhancing, and an intense sweetener compared to sucrose. Apart from this due to calorie free property it is absolutely safe for diabetics, phenyl ketonuria patients and slimming people[3].

In Egypt, the gap between sugar production (1.757 million tons) and consumption (2.6 milliontons) represents a serious problem, since it was estimated to be 0.843 million tons. Nowadays, attention is concentrated upon using Stevia in food industries, in order to close the gap between the production and consumption. Stevia cultivation indifferent places of the world; it is expected that in the Egyptian agricultural environment; one feddan of Stevia may produce up to 400 kg of Stevia sugar, annually. Taking the sweetening powder of the Stevia sugar into consideration; these 400 Kg of Steviasugar are equivalent to about 80,000 sweetening units [4].

Economically the plant has much in store for bakery, confectionary and beverage sectors. S. *rebaudiana* leaf tea offers excellent relief for an upset stomach. Like cucumber, a wet *Stevia* leaf bag provides a cooling effect to eyes and helps to reduce weight and blood sugar management. The addition of *Stevia* powder also helps in rejuvenating the pancreatic gland**[5].**

Callus is a mass of unorganized parenchyma cells derived from plant tissues. Callus cells are those cells that cover a plant wound. Callus formation is induced from plant tissues after plating onto *in vitro* special tissue culture medium. Plant growth regulators, such as auxins, are supplemented into the medium to initiate callus formation. 2,4-D is the most commonly auxin used and is extremely effective in most circumstances. Callus cells have the ability to regenerate the whole plant body under certain conditions. However, it has also been acknowledged that calli are very diverse and can be classified into subgroups based on their morphological characteristics. For example, calli with no apparent organ regeneration typically are called friable or compact callus. Other calli that display some degrees of organ regeneration are called rooty, shooty, or embryonic callus, depending on the organs they generate [6]**.**

Callus can be produced from a single differentiated cell, and many callus cells are totipotent, being able to regenerate the whole plant body[7].

The highest response of callus induction from leaf explants of *Stevia rebaudiana* was obtained on MS medium supplemented with 3.0 mg/L 2,4-D while nodal explants gave the best results for callogenesis on MS medium supplemented with 3.0 mg/L NAA and 1.0 mg/L benzyl adenine (BA) [8, 9].

Callus cultures were established from nodal and leaf explants. Maximum callus biomass was observed in MS medium supplemented with 2, 4-D 1.0 mg/L [10].

The present work aimed to establish a complete *in vitro* protocol for shoot multiplication of *Stevia rebaudiana* Bertoni from callus.

Materials and Methods

The present investigation was carried out at Biotechnology Research Group, Tissue Culture Laboratory, Botany Department, National Research Centre.

Collection ofplant materials

The plants of *Stevia rebaudiana* Bertoni were obtained from the Sugar Crops Research Institute, Agricultural Research Center, Ministry of Agriculture Egypt.

Explants preparation

Leaves and nodal segments were washed under running tap water to remove the traces of dust etc. followed by 70% ethanol for a minute. Then the explants were sterilized in 10% sodium hypochlorite with 2 drops of Tween-20 for 10 minutes, and finally washed 3-4 times with sterile double distilled water.

Growth conditions

The explants were transferred on the media and maintained at 25±2°C in a constant temperature growth room, under cool white fluorescent light using a 16 hour photoperiod provided by cool florescent light intensity of 2500 lux.

Callus induction

Two types of explants (leaves and nodal segments) were used to determine which explants are the better for callus induction. Eight treatments (0.5, 1.0, 2.0 and 4.0 mg/L of 2,4-D and the same 2,4-D concentration in combination with 1.0 mg/L BA) in addition to the control medium without plant growth regulators were used in this experiment. All cultures were examined after 45 days of incubation at 25±2ºC under 16 hr. light and 8 hr. dark provided by cool florescent light intensity of 2500 lux to record the following parameters, callus initiation percentage, callus intensity, callus nature, callus fresh & dry weight and time for explants response.

Plant Regeneration

Friable, granule and healthy calli was obtained after 45 days from leaf explants incubated on MS medium supplemented with 2.0 mg/L 2,4-D was used with four different concentrations of BA (0.5 & 1.0 mg/L) in combination with NAA (0.5 & 1.0 mg/L). All cultures were examined after 60 days of incubation at 25±2ºC under 16 hrs. / light (2500 lux) and the following criteria were scored, plant regeneration percentage, time of regeneration, number of shootlets per callus part, length of shootlets and number of nodes & leaves per produced shootlets.

Data analysis

The experiments were performed to completely randomized design. Variance analysis of data was carried out using Statistical Package for the Social Sciences (SPSS) program for statistical analysis. The differences among means for all treatments were tested for significance at 5% level by using Duncan´s multiple range tests. Means followed by the same letter are not significantly different at $P \leq 0.05$.

Results and Discussion

Effect of 2,4-D on Leaves and nodal segment explants.

Leaves and nodal segment explants cultured under aseptic conditions on MS basal salt medium supplemented with 3% sucrose and different concentrations of 2,4-D (0.0, 0.5, 1.0, 2.0 and 4.0 mg/L) data obtained from 5 replicates to each concentration, and the experiment repeated three times. Data were recorded after 60 days of incubation of cultures in 16/8 hr. light at 25^{o}C, while dry weight was obtained by drying of calli naturally for 45 days at room temperature.

Ratios of responded explants from total inoculated explants were presented in **Table(1)** appeared that the maximum responses (98 and 90%) was obtained from nodal segment explants with 2.0 mg/L 2,4-D and 1.0 mg/L 2,4-D respectively, followed by (80%) which given by act of 2.0 mg/L 2,4-D on leave explants. While poorest responses (11 and17 %) with leaves and nodal segments were recorded onMS basal salt medium without hormone respectively. This result indicated that percentage of callus initiation was increased with increasing of 2,4-D concentrations up to a certain limit. Higher concentration was not good for callus initiation. It could be concluded that the highest percentage of callus induction from leaves and nodal segments recorded on MS medium supplemented with 2.0 mg/L 2,4-D as compared with control. Similar results were obtained by [11] they studied leaf, nodal and internodal segments of Stevia as explants and they find that, the highest amount of callus was found in MS medium with 3.0 mg/L 2,4-D and MS medium with 5.0 mg/L 2,4-D given the poorest callus.

Data in **Table (1)**show that the maximum callus intensity (3) obtained from leaf explants incubated on MS medium supplemented with 2.0 mg/L 2,4-D followed by (2) that's given by act of 1.0 mg/L 2,4-D. While the poorest Calli (1&0) obtained from 0.5, 4.0 mg/L 2,4-D and MS basal salt medium without hormone (control) respectively. Increasing auxin concentration was increased callus formation until certain concentration (2.0 mg/L), while the high concentration (4.0 mg/L) leads to decrease the callus intensity.

Yellow brownish, granular and friable callus resulted by act of 2.0 mg/L 2,4-D on leaves and nodal segments, while yellow brownish, granular and compact callus given from 0.5, 1.0 and 4.0 mg/L 2,4-D.

Fresh & dry weight are presented in **Table (2)** differed significantly according to 2,4-D concentration. Fresh & dry weight obtained from leave and nodal segment explants were increased by increasing 2,4-D concentration then decreased with highest concentrations. Nodal segment explants given the greatest fresh & dry weight (3.0 & 0.51 g) on MS medium supplemented with 2.0 mg/L 2,4-D followed by (2.89 & 0.60 g) that obtained by the same concentration on leave explants. However, the lightest fresh & dry weight was given by MS basal salt medium without hormone (1.09 & 0.276g) and (1.22 & 0.29g) with leaves and nodal segments respectively. At 2.0 mg/L 2,4-D concentration fresh weight of nodal segment explants increased by 3.8% higher than leaf explants. On the other hand the highest fresh weight at 2.0 mg/L 2,4-D concentration gave 165.1% followed by 150% for leave and nodal segment explants, respectively as compared with control. While dry weight of leaves and nodal segment explants was 114.3% and 77.1% at the same concentration respectively as compared with control.

The fastest response for caulls initiation (7 & 10 days) was obtained when nodal segment and leaf explants incubated at 16 /8 hr. light, 25°C with MS medium supplemented with 2.0 mg/L 2,4-D respectively. While the latest response (24 days) noticed from MS basal salt medium without hormone with leaves and nodal segment respectively.

Table.1: Means of callus percentage, callus intensity and callus nature of *S. rebaudiana*explants as affected by different 2,4- D concentrations.

Treatments Mg/L	Callus. Percentage		Callus Intensity		Callus Nature		
	Leaves	Nodal segments	Leaves	Nodal segments	Leaves & Nodal segments		
	Means ± SE	Means ± SE			Color	Surface	Rigidity
Control	11.00 ± 1.87 [a]	17.00 ± 3.00 [a]	0	0	White	Smooth	Friable
0.5 2,4-D	55.00 ±9.35 [b]	60.00 ± 10.00 [b]	1	1	Yellow brownish	Granule	Compact
1.0 2,4-D	75.00 ± 9.35 [b]	90.00 ± 6.12 [c]	2	2	Yellow brownish	Granule	Compact
2.0 2,4-D	80.00 ± 7.91 [b]	98.00 ± 2.00 [c]	3	2	Yellow brownish	Granule	Friable
4.0 2,4-D	55.00 ± 12.25 [c]	55.00 ± 12.25 [d]	1	1	Yellow brownish	Granule	Compact
F ratio	9.465	17.213					
P value	***	***					

Columns with similar letters are not significantly different according to LSD. NS = non-significant, *= significant at P < 0.05, ** = significant at P < 0. 01, *** = significant at P < 0.001.o = poor, 1= medium, 2= high, 3= very high of Callus Intensity.

Table.2: Means of callus fresh weight, callus dry weight and callus initiation time of *S. rebaudiana* explants as affected by different 2,4- D concentrations.

Treatments Mg/L	Callus fresh weight/g		Callus dry weight/g		Callusing time	
	Leaves	Nodal segments	Leaves	Nodal segments	Leaves	Nodal segments
	Means ± SE	Means ± SE	Means ± SE	Means ± SE	Means ± SE	Means ± SE
Control	1.09 ± 0.04 [a]	1.22 ± 0.08 [a]	0.29 ± 0.04 [a]	0.28 ± 0.06 [a]	24.80 ± 0.37 [a]	18.20 ± 1.7 [a]
0.5 2,4-D	1.25 ± 0.11 [ab]	1.55 ± 0.08 [ab]	0.30 ± 0.10 [b]	0.29 ± 0.05 [ab]	15.00 ± 0.71 [b]	10.60 ± 0.51 [b]
1.0 2,4-D	1.57 ± 1.80 [b]	1.79 ± 0.11 [b]	0.32 ± 0.04 [a]	0.32 ± 0.05 [b]	16.80 ± 0.37 [c]	12.00 ± 0.32 [b]
2.0 2,4-D	2.89 ± 0.16 [c]	3.00 ± 0.15 [c]	0.51 ± 0.02 [a]	0.60 ± 0.05 [c]	10.40 ± 0.51 [d]	7.40 ± 0.51 [c]
4.0 2,4-D	1.54 ± 0.17 [b]	1.63 ± 0.19 [b]	0.31 ± 0.02 [a]	0.031 ± 0.03 [b]	15.00 ± 0.84 [d]	16.40 ± 1.02 [a]
F ratio	79.483	44.664	3.596	7.634	79.483	21.208
P value	***	***	***	**	***	***

Columns with similar letters are not significantly different according to LSD. NS= non-significant, * = significant at P < 0.05, ** = significant at P < 0. 01, *** = significant at P < .001.

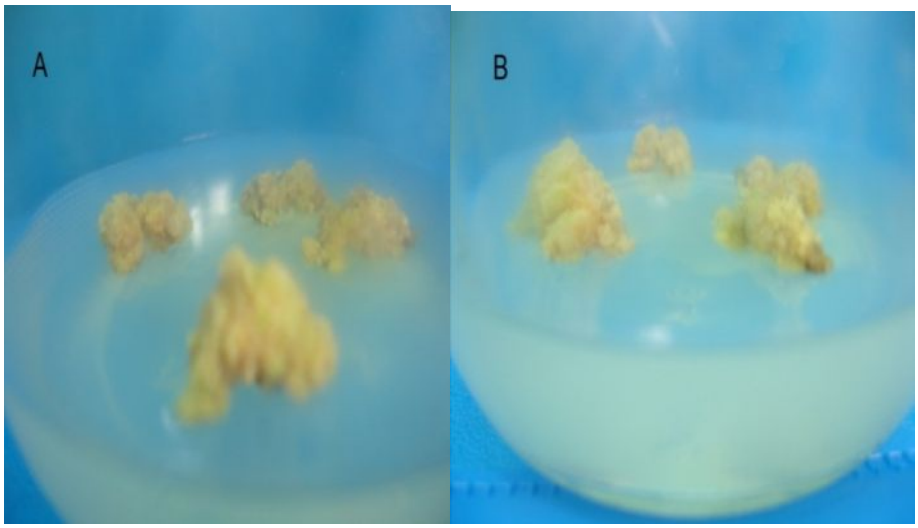

Figure.1: Effect of 2.0 mg/L 2,4-D on callus induction of nodal segment (A) and leaf (B)explants.

Effect of combination between 1.0 mg/L BA + 2,4-D (0.5, 1.0, 2.0 and 4.0 mg/L) concentrations on Leave and nodal segment explants.

Data in **Table (3)** appeared that 1.0 mg/L BA + 0.5 mg/L 2,4-D was given the higher percentage (80 %) with leaf and nodal segment explants. At the same time 1.0 mg/L BA + 2.0 mg/L 2,4-D also given 80% with nodal segment explants. While poorest responses recorded at MS basal salt medium without hormone (control) that given (11 & 17 %) with leaves and nodal segment respectively, precede by (40 and 45%) were recorded on medium supplemented with 1.0 mg/L BA + 4.0 mg/L 2,4-D.

The maximum callus intensity (3) seen on MS medium supplemented with 1.0 mg/L BA + 2.0 mg/L 2,4-D, followed by (2) that's given by act of 1.0 mg/L BA + 1.0 mg/L 2,4-D. While, the poorest Calli (1&0) obtained from 1.0 mg/L BA + 0.5 mg/L 2,4-D, 1.0 mg/L BA + 4.0 mg/L 2,4-D and MS basal salt medium without hormone (control).

Callus intensity increased with increasing of 2,4-D concentrations till 2.0 mg/L then decreased with higher concentration of 2,4-D (4.0 mg/L). Shiny green, smooth and compact callus obtained from MS media supplemented with 1.0 mg/L BA + 0.5 mg/L 2,4-D and 1.0 mg/L BA + 1.0 mg/L 2,4-D. While, shiny white greenish, smooth and compact callus resulted by adding of 1.0 mg/L BA + 2.0 mg/L 2,4-D and 1.0 mg/L BA + 4.0 mg/L 2,4-D. At the same time, white, smooth and friable callus obtained from MS basal salt medium without hormones (control).

The greatest fresh and dry weight (3.5 & 0.51 g) was obtained from the interaction between nodal segment explants and 1.0 mg/L BA + 2.0 mg/L 2,4-D concentration.

On the other hand, MS medium supplemented with 1.0 mg/L BA + 4.0 mg/L 2,4-D gave the lightest fresh and dry weight (1.698 & 0.277 g) with leaves explants.

Results in **Table(4)** showed that, the fastest response (11 & 11.2 days) was obtained when explants cultured on MS medium supplemented with 1.0 mg/L BA + 0.5 mg/L 2,4-D respectively, followed by (11.6 days) that was recorded with nodal segment explants at 1.0 mg/L BA + 1.0 mg/L 2,4-D.

While the latest response (24 and 18.2 days) was recorded from MS basal salt medium free hormone (control) on leave and nodal segment explants respectively.

Table.3: Means of callus percentage, callus intensity and callus nature of *S. rebaudiana*explants as affected by different 2, 4- D concentrations in combination with 1.0 mg/L BA.

Treatments Mg/L	Callus percentage		Callus Intensity		Callus Nature		
	Leaves	Nodal segments	Leaves	Nodal segments	Leaves & Nodal segments		
					Color	Surface	Rigidity
	Means ± SE	Means ± SE					
Control	11.00 ± 1.87 [a]	17.00 ± 3.00 [a]	0	0	White	Smooth	Friable
1.0 BA + 0.5 2,4-D	80.00 ± 5.00 [b]	80.00 ± 9.35 [b]	1	1	shiny green	Smooth	Compact
1.0 BA + 1.0 2,4-D	65.00 ± 6.12 [b]	75.00 ± 5.00 [b]	2	2	shiny green	Smooth	Compact
1.0 BA + 2.0 2,4-D	70.00 ± 7.90 [b]	80.00 ± 9.35 [b]	3	3	shiny white greenish	Smooth	Compact
1.0 BA + 4.0 2,4-D	40.00 ± 6.12 [c]	45.00 ± 5.00 [c]	1	1	shiny white greenish	Smooth	Compact
F ratio	24.720	15.818					
P value	***	***					

Columns with similar letters are not significantly different according to LSD.NS= non-significant
* = significant at P < 0.05,** = significant at P < 0. 01,*** = significant at P < .001. o = poor, 1= medium, 2= high, 3= very high of Callus Intensity.

Table.4: Means of callus fresh weight, callus dry weight and callus initiation time of *S. rebaudiana* explants as affected by different 2, 4- D concentrations in combination with1.0 mg/L BA.

Treatments Mg/L	Callus fresh weight/g.		Callus dry weight/g.		Callusing time.	
	Leaves	Nodal segments	Leaves	Nodal segments	Leaves	Nodal segments
	Means ± SE	Means ± SE	Means ± SE	Means ± SE	Means ± SE	Means ± SE
Control	1.09 ± 0.04 [a]	1.22 ± 0.09 [a]	0.22 ± 0.06 [a]	0.26 ± 0.04 [a]	24.80 ± 0.36 [a]	18.40 ± 1.68 [a]
1.0 BA + 0.5 2,4-D	2.77 ± 0.24 [b]	2.42 ± 0.25 [b]	0.30 ± 0.05 [b]	0.40 ± 0.07 [b]	11.20 ± 0.58 [b]	9.60 ± 0.51 [b]
1.0 BA + 1.0 2,4-D	2.86 ± 0.11 [b]	3.04 ± 0.16 [c]	0.39 ± 0.03 [c]	0.42 ± 0.05 [b]	15.00 ± 0.32 [c]	11.60 ± 0.68 [b]
1.0 BA + 2.0 2,4-D	3.20 ± 0.15 [b]	3.54 ± 0.10 [c]	0.50 ± 0.04 [d]	0.51 ± 0.06 [c]	20.40 ± 0.51 [d]	18.20 ± 0.51 [a]
1.0 BA + 4.0 2,4-D	1.69 ± 0.14 [c]	1.78 ± 0.17 [a]	0.28 ± 0.06 [a]	0.31 ± 0.02 [a]	18.80 ± 1.7 [d]	17.00 ± 0.71 [a]
F ratio	17.383	7.042	14.915	2.331	34.753	19.245
P value	***	*	***	NS	***	***

Columns with similar letters are not significantly different according to LSD. NS= non-significant,* = significant at P < 0.05, ** = significant at P < 0. 01*** = significant at P < 0.001.

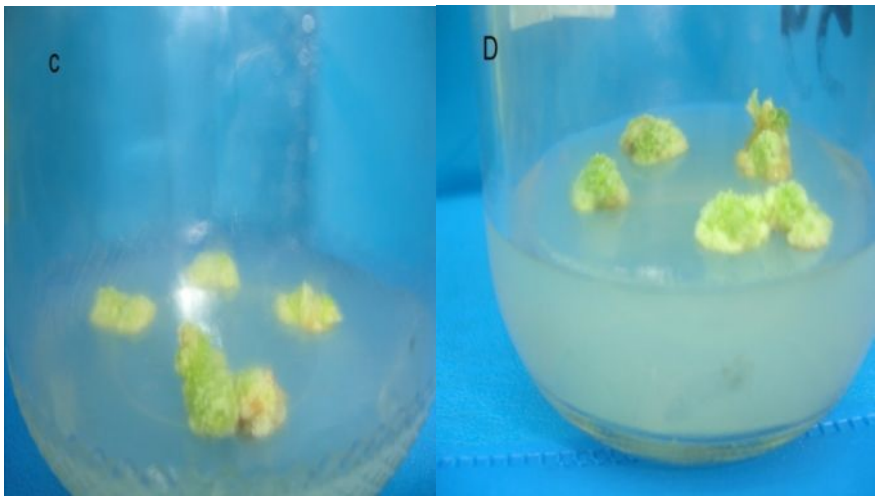

Figure-2: Effect of 1.0 mg/L BA + 2.0 mg/L 2,4-D on callus induction of leaf (C) and nodal segment (D) explants.

Comparison between leaves and nodal segments on callus induction of Stevia

Data showed that nodal segment explants were better than leaf explants in callusing percentage on fresh, dry weight and callus time per day.

While, leaf explants were better than nodal segment explants in callus intensity at all treated treatments.

Differentiation based on exogenous auxin ratio. This may be due to: (i) the degree of cell sensitivity towards growth regulators due to the origin of the explant, (ii) the endogenous levels of active growth regulator molecules, (iii) their uptake, (iv) their degree of glycosylation and hydrolysis, (v) the type of auxin and cytokinin used, (vi) their mode of action or (vii) the activity of auxin and cytokinin oxidases [12].

Comparison between 2,4-D alone and 2,4-D in combination with 1.0 mg/L BA on callus induction of Stevia.

Data showed that adding of 1.0 mg/L BA to medium supplemented with 2.0 mg/L 2,4-D increased the callus fresh weight by 12.5%.

On the other hand, adding of 1.0 mg/L BA to medium supplemented with 0.5 mg/L 2,4-D leaded to decreasing the percentage of callusing by 10.11% and increased the time for callusing by 14.4% and decreased the callus dry weight by 8.9%.

Table.5: Illustration of the differences between 2,4-D alone and 2,4-D in combination with 1.0 mg/L BA on callus induction of Stevia.

Parameter	2,4-D	2,4-D + 1.0 mg/L BA	Differences
Callusing percentage	89%	80%	10.11 for 2,4-D
Callusing time	8.9 days	10.4 days	14.4% for 2,4-D
Callus dry weight	0.56 g	0.51 g	8.9% for 2,4-D
Callus fresh weight	2.95 g	3.37 g	12.5 % for 2,4-D + 1.0 mg/L BA

Observationally there were differences between the nature of calli that's obtained by 2,4-D alone and that obtained by 2,4-D in combination with 1.0 mg/L BA. Adding of 1.0 mg/L BA to 2,4-D concentrations differ color from yellow brownish to white greenish and differ surface of calli from granule to smooth as in **Figure(3)**. Majority of plant tissues growing in vitro require exogenous hormones in the nutrient medium. The reaction of isolated tissues to auxins depends upon their endogenous auxin level at the time of excision and their genetic capacity for its synthesis. Those tissues which do not require an external supply meet their auxin requirement endogenously by biosynthesis. 2,4-D is very effective for the induction and growth of callus. 2,4-D is also an important factor for the induction of somatic embryogenesis [13].

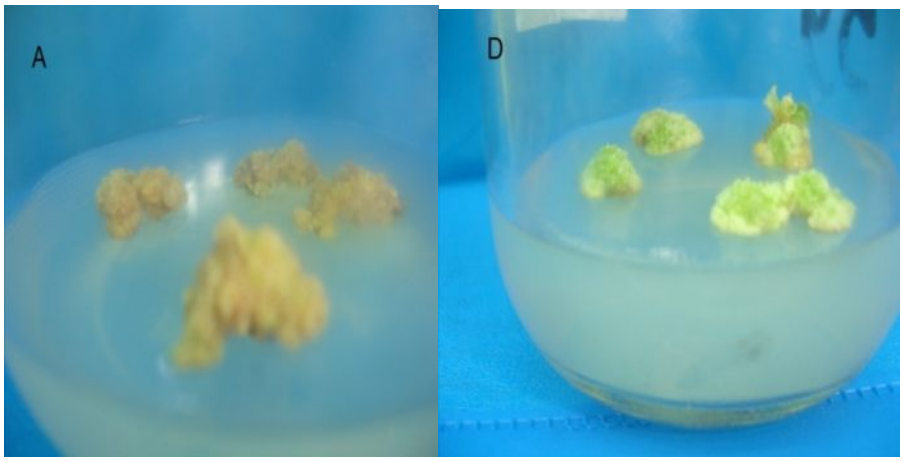

Figure-3: Effect of 2,4-D alone (A) and 2,4-D in combination with 1.0 mg/L BA (D) on callus induction.

Indirect organogenesis of *Stevia rebaudiana* Bertoni.

Friable, granule and healthy calli obtained after 45 days from leave explants incubated on MS medium supplemented with 2.0 mg/L 2,4-D used with four different concentrations of BA (0.5 & 1.0 mg/L) in combination with NAA (0.5 & 1.0 mg/L).

Results in **Table (6)** indicated that the highest percentage of regenerated calli (86%) was recorded from MS medium supplemented with 0.5 mg/L BA + 1.0 mg/L NAA. Followed with 75% that recorded from MS medium supplemented with 0.5 mg/L BA + 0.5 mg/L NAA. While the lowest percentage of regenerated calli 50% was obtained from medium supplemented with 1.0 mg/L BA + 1.0 mg/L NAA, preceded by 70% that recorded from MS salt basal medium (control).

The fastest response (13.4 days) was recorded from MS medium supplemented with 1.0 mg/L BA + 0.5 mg/L NAA, followed with 14.6 days that was recorded from medium supplemented with 0.5 mg/L BA +1.0 mg/L NAA.While, the lowest response (22.6 days) was obtained from MS basal salt medium without hormone (control).

MS medium supplemented with 0.5 mg/L BA + 1.0 mg/L NAA given the highest number of shoots (13.2 shoot), followed with MS medium supplemented with 0.5 mg/L BA + 0.5 mg/L NAA that given (11.8 shoots). While the lowest number of shoots (5.4 shoots) was obtained from MS basal salt medium (control).

MS medium supplemented with 0.5 mg/L BA + 1.0 mg/L NAA given144.4% as compared with the control, while MS medium supplemented with 1.0 mg/L BA + 1.0 mg/L NAA was better than the control by 18.5%.

Obviously, it was mentioned that increasing the concentration of the studied auxin (NAA) positively increased the values of shoot numbers, while increasing the concentration of the studied cytokinin (BA) decreased the values of shoot numbers.

Results showed that the highest shoot length (4.36 cm.) obtained from MS medium supplemented with 0.5 mg/L BA + 1.0 mg/L NAA, followed by 3.08 cm. which was recorded from MS medium supplemented with 0.5 mg/L BA + 0.5 mg/L NAA. However, the lowest shoots length (2.65 cm.) was achieved by 1.0 mg/L BA + 1.0 mg/L NAA treatment and MS basal salt medium without hormone (control). The highest shoot length (3.12 cm) were observed with MS medium supplemented with 1.0 mg /LBA+0.05 mg /L NAA [14].

Results in **Table (6)** showed that no significant differences among treatments for leaves and nodes number of *Stevia rebaudiana* regenerated Shoots. In the present work it has been possible to affect organogenesis or differentiation of whole plants from the calli. Control of differentiation has been based on hypothesis of root and shoot differentiation is a function of interaction between two plant growth regulators auxin and cytokinin. A relatively high auxin and low cytokinin causes root formation while the reverse favors shoot formation. This is true in the case of many herbaceous angiosperms though it is not universally accepted [15].

Table 6: Effect of BA concentrations in combination with NAA on calli derived from leaves.

Treatments Mg/L	Reg. percentage	Reg. time	No. of shootlets	Length of shoots	No. of leaves	No. of Nodes
	Means ± SE	Means ± SE	Means ± SE	Means ± SE	Means ± SE	Means ± SE
Control	70.00 ± 9.35 [a]	22.60 ± 2.50 [a]	5.40 ± 0.51 [a]	2.65 ± 0.26 [a]	4.80 ± 0.80	2.40 ± 0.40
0.5 BA + 0.5 NAA	75.00 ± 7.91 [a]	14.80 ± 1.77 [b]	11.80 ± 0.97 [b]	3.08 ± 0.43 [a]	6.00 ± 0.89	3.00 ± 0.45
1.0 BA + 0.5 NAA	73.00 ± 6.44 [a]	13.40 ± 1.89 [b]	8.60 ± 0.93 [c]	2.88 ± 0.32 [a]	5.20 ± 0.80	2.60 ± 0.40
0.5 BA + 1.0 NAA	86.00 ± 5.79 [b]	14.60 ± 0.93 [b]	13.20 ± 1.07 [b]	4.36 ± 0.23 [b]	8.00 ± 0.63	4.00 ± 0.32
1.0 BA + 1.0 NAA	50.00 ± 7.91 [c]	20.00 ± 3.30 [a]	6.40 ± 0.51 [ac]	2.65 ± 0.37 [a]	4.80 ± 1.02	2.40 ± 0.51
F ratio	2.986	3.231	16.376	4.698	2.568	2.568

Columns with similar letters are not significantly different according to LSD. NS= non-significant,
* = significant at P < 0.05, ** = significant at P < 0. 01, *** = significant at P< 0. 001

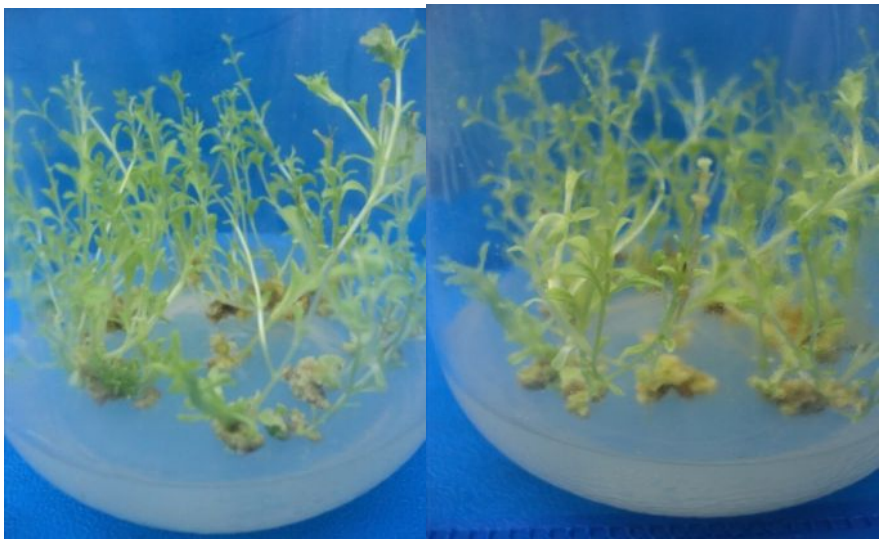

Figure.11: Effect of 0.5 mg/L BA + 1.0 mg/L NAA on calli derived from leaves.

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