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IN VITRO RESPONSE GROWTH OF *STEVIA REBAUDIANA* UNDER SALINITY AND DROUGHT

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ABSTRACT

This work aimed to evaluate growth of *Stevia rebaudiana* under stress conditions. The plantlets were cultured on modified MS medium supplemented with different concentrations of NaCl at 0.0, 5000, 7500 and 10000 ppm for salinity stress, as well as mannitol at 0.0, 5, 7.5 and 10 bar. Results showed that the plantlet growth characters decreased as salinity and drought stress level increased, Plantlet growth traits significantly varied under deferent stress levels. Drought strees was more sever on plantlet growth than salinity stress. The highest values for plantlet growth traits were observed at 5000 ppm salinity level while the lowest values were observed at 10 bar drought stress. Some treatments were promised stevia tolerance under both of salinity and drought stress conditions.

Key words: *Stevia rebaudiana*, micropropagation, drought, salinity and stress.

INTRODUCTION

Stevia rebaudiana Bertoni is a small shrub of the Asteraceae (Composite) family, Stevia is an herb with an extensive root system and brittle stems producing small, elliptic leaves, the tiny white florets are perfect, borne in small corymbs of 2–6 florets. Corymbs are arranged in loose panicles**.**

Oddone (1997) considered that stevia is self-incompatible and insect pollinated. Additionally, he mentioned that clear seeds are infertile. Seeds are contained in slender achene's, about 3 mm in length. Each achene has about 20 persistent pappus bristles.The native occurrence of *Stevia rebaudiana* is between 22-24°S and 53-56°W in Paraguay and Brazil. The plant growth requires mild temperature between 15° and 38°C and relative humidity of about 80% (**Soejarto *et al.*, 1983).** The cultivation of such a plant could be an alternative for the new land reclamation projects to meet the sugar demands of the Egyptian markets and generate income for the growers.

(El-Zifzafi, 2003 and Ibrahim *et al.*, 2008).Stevia is helpful for hypoglycemia and diabetes because it nourishes pancreas and thereby helps to restore its normal function **(Soejarto *et al.*, 1983). Oviedo (1971)** reported that 35.2% fall in normal blood sugar levels whiten 6-8 hours following to the ingestion of stevia leaf extract **(Miyazaki *et al.*, 1978).** Also, Stevia leaves extract can be used as anti fungal and anti bacteria agent. Therefore, it lowers incidence of cold and flu. Poor seed germination is one of the factors limiting large scale cultivation.

In this concern, referred to **Shock (1982); Duke (1993) and Carneiro *et al.* (1997)** who reported that poor viable seeds were produced by stevia with very low germination percentage **(Felippe and Lucas, 1971; Latha and Usha, 2003).** Seed germination rate is often poor, less than 10% are common **(Miyazaki et *al.*, 1978; Goettemoeller and Ching, 1999**).

Stevia grows well in sandy loam soils with an ample supply of water. Stevia prefers acidic to neutral soil with a pH range of 6.5-7.5 for its best growth. Saline soils should be avoided as stevia plant is susceptible to water logged conditions. *Stevia rebaudiana* is best grown in sunny areas of the garden or in containers. Raised beds are the best choice for growing this herb if the soil is heavy or has high clay content. Ideal soil would be a friable garden loam high in organic matter. Soil pH levels range from acid to slightly alkaline.

Stevia is not a drought tolerant herb; the soil should be kept continuously moist, but not saturated **(Tucker and Thomas, 2009). Goettemoeller (2010)** and the Herb Society of America **(2010).** The present investigation aimed to evaluate *Stevia rebaudiana* for optimum propagation via tissue culture techniques both under drought and saintly stress conditions.

MATERIALS AND METHODS

This study was carried out in Plant Tissue Culture Laboratory, Faculty of Environmental Agricultural Sciences (FEAS) El-Arish, North Sinai, Suez Canal University (SCU). The present work was conducted to display in details the whole protocol for stevia propagation through tissue culture techniques to produce and introduce *Stevia rebaudiana* plants as a new sweetener crop to Egyptian agriculture.

In order to efficiently maximizing of plant propagation *via* direct organ-ogenesis, it is important to study the influence of stress factors on the growth and development of *Stevia rebaudiana* grown *in vitro*. Seedlings of *Stevia rebaudiana* var. Spanti were kindly obtained from Sugar Crops Res. Inst., Agri., Res. Cent., Ministry of Agri., Egypt, which were grown in the green-house of the Faculty of Environ. Agri. Sc. El-Arish. Actively growing shoots were used as the explants source during period from February to March. The terminal shoots were collected from growing plants which were 2-3 months age and were cut into 1-1.5 cm pieces**.**

Expellant sterilization.

Shoot tip explants of stevia ranging in size from 0.5 to 1 cm were rinsed under running tap water with soap for 5 minutes to remove all the remaining detergent, then washed with sterilized distilled water. The explants were soaked for 10 minutes in 20% clorox concentration for explants surface sterilization, followed by 1.5 gl^{-1} ($HgCl_2$) Mercuric chloride for one minute, then washed with sterilized distilled water for 3-4 times to remove all traces.

All steps of the sterilization had been done under aseptic conditions inside the culture cabinet (Laminar air flow hood) when ten explants were cultured in each jar to containing 50 ml medium.Medium for all cultures contained 1.1 gl^{-1} MS **(Murashige and Skoog, 1962**) inorganic salts supplemented with 0.1 gl^{-1} myo-inositol, 30.0 gl^{-1} sucrose and 6.0 gl^{-1} Agar.

The pH of the medium was adjusted to 5.7 and autoclaved at 1.2 kgcm-2 and 121 ºC for 20 minutes. For salinity experiment MS medium was supplemented with different concentration of NaCl 0, 5000, 7500 and 10000 ppm. While, for drought experiment, MS Medium was supplemented with different concentrations of mannitol 0, 5, 7.5 and 10 bar. The amounts of mannitol needed to produce these osmotic potentials were 0, 37.31, 55.83 and 74.34 gl^{-1} (*W/V*).The concentrations of mannitol were

calculated according to formula given by **Helmerick and Pferifer (1954).**

(P = g R T/mV).

Where:-

P = osmotic potential in atmosphere

g = grams of mannitol

R = 0.0825 liter atmospheres per degree per mole

T = absolute temperature

m = molecular weight of mannitol

V = volume in liters.

The jars for each experiment (salinity and drought stress) were incubated at 25 C under 16/8 light/dark photoperiod regime with intensity about 3000 lux.

Statistical analysis:

Data were statistically analyzed by using a randomized complete design (RCD). Mean separations were done by using SPSS computer program and to compare between means least significant differences (**Duncan, 1955)** was used. Data were recorded every six weeks every treatment had four replicates.

RESULTS AND DISCUSSION

At multiplication stage, treatments included different concentrations of Na Cl and mannitol to study their effect on some vegetative characteristics. Data in Table (1) shows the effect of salinity and drought caused by NaCl and mannitol, respectively on stevia growth after six weeks of treatments. Control treatment was the best for all traits.

Shoot number and length:

The highest shoot number under stress was 16 with 5000 ppm salinity stress, while the smallest shoot number value was (9.6) with 10000 ppm salinity stress, Generally the number of shoots were significantly decreased (16, 11 and 9.6) as the salinity levels increased (5000,7500 and10000 ppm, respectively).

While the shoot length was 1.9, 1.8 and 1.6 under the same stress levels, respectively and there were no significantly differences between both 5000 and 7500 ppm concentrations.

Concerning the shoot number and length, the control treatment gave the greatest values (56 and 5.46, respectively). On the other hand, the shoot number values under drought stress decreased(8,4, 7.0 3.4) as the drought level increased, they ranged between 8.4 and 3.4 shoots with 5 and 10 bar treatments, respectively. While the shoot length values were 0.82, 0.54 and 0.38 cm with 5, 7.5 and 10 bar mannitol levels, respectively.

Node and leaf number:

For both node and leaf number traits, the values of these traits decreased as the stress type levels increased and there were slight differences between stress treatments. It seems that both salinity and drought stress gave the same effect on both treats. The highest values were 2.2 and 4.2 for both traits (node and leaf number) with5000 ppm salinity stress, while they were 2.2 and 4.4 with 5 bar of drought stress.

Root number and root length.

For root number of stevia, all tested treatments showed similar trend. The least value of root length were recorded with 5bar treatment while the highest value was observed with other treatments. There were no roots observed in both 7.5 and 10 bar drought stress treatments, while the highest root number value 15.2 was obtained roots with 5000 ppm salinity stress.

The highest root length value 29 cm was obtained with 5000 ppm salinity stress. The highest survival rate percentage value was 92% was observed with 5000 ppm salinity stress, while the smallest survival rate percentage value was 22% with 10 bar drought stress.

Table (1): Effect of different concentrations of NaCl and mannitol stress on growth characters of cultured *Stevia rebaudiana* after six weeks of treatments.

Treatments	Shoots number	Shoot length (cm)	Nodes number	leaves number	Roots number	Roots Length (cm)	Survival rate %
Control	56[a]	5.46[a]	4.4[a]	8.8[a]	45.8[a]	110[a]	100[a]
5000 ppm NaCl	16[b]	1.9[b]	2.2[b]	4.2[bc]	15.2[b]	29[b]	92[b]
7500 ppm NaCl	11[c]	1.8[b]	1.6[bc]	3.2[c]	12.2[c]	23[c]	78[c]
10000 ppm NaCl	9.6[c]	1.6[c]	2[b]	4.6[b]	4.8[d]	8.6[d]	68[d]
5 bar mannitol	8.4[cd]	0.82[d]	2.2[b]	4.4[b]	4.6[d]	6.2[d]	56[e]
7.5 bar mannitol	7[d]	0.54[de]	2[b]	4[bc]	0.00	0.00	42[f]
10 bar mannitol	3.4[e]	0.38[e]	2[b]	4[bc]	0.00	0.00	22[g]

Survival rate:

Using NaCl induced a bad effects on all parameters under study up to the lethal concentration which caused by 5000 ppm NaCl under Salinity stress which caused almost death of the *in vitro* plantlets.

Increasing mannitol concentration in the medium, resulted in a significant for vegetative characters**.** Salt or mannitol tolerant cell lines selected by this way had smaller cells than unselected cell lines when cultured in the presence of salt or mannitol.

Cell lines selected for tolerance to one agent (sodium salt, potassium salt or mannitol) showed minimal tolerance to another agent. However, when plants were regenerated from salt- or mannitol-tolerant callus and new cultures derived from them, the new cultures showed tolerance to all of the salts and mannitol experiments.

Plant regeneration from the new cultures was not achieved under the conditions that led to the regeneration of the parent plants from callus. Plant cells contain large vessels known as vacuoles. These structures serve a number of functions, including water storage. Well-hydrated vacuoles push against the thick cell walls making the cell rigid, or turgid. When all cells are turgid, the plant itself is firm and crisp. When a plant loses water, the vacuole contracts and cannot maintain this pressure.

The cells become flaccid and the plant wilts.**(Epstein *et al.*, 1980, Francois *et al*., 1986, Sharp *et al*., 1990).**When water is scarce, aging foliage becomes a liability to the plant.

Deciduous plants may undergo early senescence, in which leaves go through the natural shedding process ahead of schedule. Other plants may simply shed wilted leaves. Symptoms of drought-

induced defoliation include curling, rolling, folding and eventual shedding of the leaves.(**Munns and Termaat, 1986; Munns, 1993; Beltras *et al*., 1997).** These results are in agreement with those obtained by **(Ochatt and Power 1989); Zidan *et al.,* 1990; Hussain and Rahman, 1995; Andria *et al.,* 1997; Tedeshi *et al.,* 1997; Escobar *et al.* (1998); Ledbetter *et al* (1998); EL-Midaoui *et al.,* 1999; Abdel-Sadek, 2003; El-Zifzafi, 2003; Hossain *et al.,* 2008; Pratibha *et al.* 2010)** they found that plant response to salt stress varies at different developmental stages.

Increasing salinity during plant development would delay germination, vegetate growth reduction and formation of thinner roots. Stalinization can inhibit both cell division and cell expansion in growing tissues of roots, stems and leaves.

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Fig. (1): Effect of drought and salinity stress on *Stevia rebaudiana in vitro.*

الملخص العربي

استجابة الاستيفيا لإجهاد الملوحة والجفاف معمليًا

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أجريت هذه الدراسة في معمل زراعة الأنسجة النباتية في كلية العلوم الزراعية البيئية العريش، شمال سيناء، جامعة قناة السويس، مصر وتهدف هذه الدراسة إلى تقيم نبات الاستيفيا ريبوديانا تحت ظروف الإجهاد، النُبيتات زرعت في بيئة الزراعة المعدلة والمضاف إليها تركيزات مختلفة من كلوريد الصوديوم هي صفر ٥٠٠٠- ٧٥٠٠-١٠٠٠٠ جزء في المليون كظروف للإجهاد. وكذلك المانتيول بتركيزات صفر ٥- ٧,٥-١٠ بار وقد أظهرت النتائج إن نمو النباتات يقل عند زيادة كلا من مستويات الملوحة والجفاف، وكانت صفات نمو النُبيتات معنوية تحت مستويات الإجهاد المختلفة. وكان اجهاد الجفاف أكثر تأثيراً من إجهاد الملوحة علي كل الصفات. وقد ظهر أعلي معدل لصفات النمو للنُبيتات عند تركيز ٥٠٠٠ جزء في المليون من الملوحة بينما أقل معدل ظهر عند تركيز ١٠ بار من الإجهاد، أظهرت بعض المعاملات أنها تبشر بتحمل الاستيفيا للملوحة والجفاف تحت ظروف الإجهاد.

الكلمات الاسترشادية: الاستيفيا، الإجهاد، الملوحة، الجفاف، الإكثار الخضري.

المحكمون:

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