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REGULATION OF STEVIA ORGANOGENESIS UNDER STRESS AND MICRO-CLIMATIC CONDITIONS

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ABSTRACT

This study aimed to evaluate the performance of Stevia (var. Spanti) to *in vitro* salinity and drought stresses as well as micro climatic conditions. Nodal segments were cultivated on B5 medium under (22±2ºC) temperature and light intensity (2500 LUX). Different concentrations from NaCl (0, 1000, 2000, 3000, 4000 and 5000 ppm) and mannitol (0, 2, 4, 6, 8 and 10 bar) were used to study salinity and drought stresses. Moreover, two different temperature degrees (18 and 22°C) with three levels of light intensity (1000, 2000 and 3000 LUX) were used to study the influence of temperature and light intensity on stevia development. The growth parameters values for both salinity and drought treatments were recorded highest values using 1000 ppm and recorded lowest values using 10 bar, respectively. The results showed that stevia plants under 22°C and 2000 LUX treatments showed the highest values for each of growth parameters and organic and mineral contents.

Key words: *In vitro* salinity, drought stress, plant growth regulators, microclimate, *Stevia rebaudiana* Bertoni.

INTRODUCTION

Stevia rebaudiana Bertoni belonging to Asteraceae family, an important medicinal plant native from Northeastern Paraguay and Southern Brazil. This species is now being cultivated on a large scale in many other countries including Japan, Taiwan, Korea, Thailand and Indonesia for medicinal purposes and for use as a natural sweetener in food products **(Sreedhar *et al*., 2008)**. Germination and establishment from seeds is often poor and sometimes unsuccessful **(Shaffert and Chebotar, 1994)**. There were two options were studied by **Farrar and Davis (2000)** for multiplication using tissue culture technique and stem cutting for stevia. They found that tissue culture was the best option for multiplication. **Mubarak *et al.* (2012**) evaluated the growth of *Stevia rebaudiana* under stress conditions, they illustrated that plantlet growth characters decreased as salinity and drought stress level increased. Drought stress was more sever on plantlet growth than salinity stress. While, **Badran *et al.* (2013)** evaluated the differences of plant growth, as well as sweetener material of two types of *Stevia rebaudiana* under conditions of absence and presence of drought stress. They found that selection based on survival (%) would be more effective to improve stevioside content of stevia plants in drought stress conditions.

Zeng *et al*. (2013) indicates that *S. rebaudiana* is mildly tolerant to salt stress. So salt and drought stress may be effective for optimizing the steviol glycoside composition. Also, **Pandey and Chikara (2014)** reported that increasing NaCl and mannitol concentration in medium, decreased shoot number, shoot length, root number,

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root length, shoot/root ratio, leaf number, leaf fresh weight, stem dry weight, root dry weight, shoot dry weight and leaf dry weight of stevia. However, rate of proline accumulation increased and protein content was decreased by increasing salt concentration. In this respect, **Rathore *et al.* (2014)** investigated the effect of salinity on biochemical parameters in two Stevia genotypes. Chlorophyll amount was observed to be decreased as compared to sugars, proline and phenols with increased salt concentrations. **Ghaheri *et al.* (2017)** studied the effect of different concentrations of mannitol on some morphological aspects of stevia under *in vitro* conditions. They observed that by increasing the concentration of mannitol in medium, shoot length, internode length, fresh weight of plants and growth rate were decreased. Moreover, the results showed that osmotic stress significantly reduces the growth and yield components of *S. rebaudiana* Bertoni.

Concerning micro-climatic conditions, **Uddin and Baten (2006)** noticed that the temperature of *Stevia rebaudiana* incubated the culture room was maintained around 25°C. **Hossain *et al.* (2008)** pointed that all cultures of *Stevia rebaudiana* were grown in an air conditioned culture room illuminated by 40 W white fluorescent tubes with an intensity varied from 2000-3000 lux. The photoperiod was maintained as 16 hours light and 8 hours dark.So this study aimed to investigate the effects of plant growth regulators, media composition, *in vitro* salinity and drought stresses, as well as micro-climatic conditions on growth and development of stevia plants using tissue culture technique.

MATERIALS AND METHODS

Plant Material and Explants Source

Stevia *(Stevia rebaudiana)* explants were obtained from Sugar Crops Research Institute in sterilized jars (4 weeks old).

Explant Sterilization

Shoot tip and nodal segments (1.5-2.0 cm) were washed under running tap water with mild detergent for 5 minutes, followed by distilled water. Thereafter, in aseptic conditions, the explants were soaked in 5% (V/V) Clorox for 10 minutes after has been washed three times with sterile distilled water. Later, it was immersed in 0.15 % mercuric chloride ($Hg\ Cl_2$) solution for 1 minute and finally four washes with sterilized distilled water to remove all traces of ($Hg\ Cl_2$). All steps of the sterilization had been done under (Laminar air flow hood). The explants were cultured on B5 medium including vitamins **(Gamborg *et al*., 1968)** in jars containing 50 ml medium, supplemented with 3% (*W/V*) sucrose, 0.8% (*W/V*) agar and 0.1 g L^{-1} myo-inositol. Medium pH was adjusted to 5.7- 5.8, before gelling in all stages and the medium was boiled on the hot plate before autoclaving at 121ºC for 20 min. The explants (3 explants/ jar) were cultured on medium under complete aseptic conditions and after that the cultures were put in the light and incubated at 22±2ºC under a 16/8 hr. day/night photoperiod provided by cool white fluorescent lamps (light intensity 2500 lux).

In vitro Stress Tolerance Studies

Explants were cultivated under six levels of NaCl (0, 1000, 2000, 3000, 4000 and 5000 ppm) to study the response of stevia to *in vitro* salinity stress, while, for drought stress, six concentrations of mannitol (0, 15, 30, 45, 60 and 75 grams) were added to B5 basal medium to give (0, 2, 4, 6, 8 and 10 bar) per liter (W/V), respectively.

Micro-climatic Conditions

Explants were cultivated on B5 medium and grown under three levels of light intensity (1000, 2000 and 3000 LUX) in combination with two levels of temperature (18 and 22 °C) to study the effect of interaction between light intensity and temperature on stevia development.

Statistical Analysis

Experiments were set up in completely randomized design (CRD) with three replicates (three explants per replicate). Data were subjected to analysis of variance (ANOVA) and the statistical difference among the means was least significant difference (LSD) using statistical analysis system (SAS) program **(SAS, 1997)**.

RESULTS AND DISCUSSION

Effect of Salinity Treatments

Growth parameters

The effect of six different NaCl concentrations (0, 1000, 2000, 3000, 4000 and 5000 ppm) on number of shoots, number of leaves, shoot length (cm), number of roots, root length (cm), plant dry weight, plant fresh weight (g) and leaves fresh weight (g) are presented in Table 1. The growth parameters were significantly affected by different concentrations of NaCl, except plant dry weight. This finding was true in the five NaCl concentrations, as well as the control (B5 free).

According to measuring the effect of salinity on development parameters of *Stevia rebaudiana*, the control treatment (B5 free) recorded the highest values of most growth parameters (number of shoots, number of leaves, shoot length and root length), while the concentration of 5000 ppm recorded the lowest values of all growth parameters. The highest values of number of roots was obtained from 1000 ppm of NaCl treatments. The same results were found by **Savithri *et al.* (2001), Xiong *et al.* (2002), Abd El-Sadek (2003) and Taleie *et al.* (2012)**. They found that plant growth parameters were decreased by increasing the salinity levels.

Results presented in Table 2 indicate that the plant fresh and dry weights, as well as leaves fresh weight were significantly affected by different concentrations of NaCl. This finding was true in four NaCl concentrations, as well as the control treatment. Cultivating stevia plants in low concentration of NaCl leads to increasing fresh weight gain to (1.38 g). In contrast, increasing NaCl concentration to 5000 ppm recorded the lowest plant fresh weight (0.33 g). The influence of salinity stress furnished favorable conditions for decreasing the plant water content.

Obtained results in Table 2 showed that salinity stress exerted a significant effect on plant dry weight (g). Control traits (B5 free) gave the significantly highest value of dry weight (0.18 g). Results cleared that dry weight achieved by B5 free outyielded those obtained by both 5000 ppm and 4000 ppm of NaCl treatments by 66.6 %.

Lack of NaCl gave significantly highest value of leaves fresh weight (0.49 g). While, 3000 ppm NaCl gave the lowest value (0.09 g), these finding agreed with the results reported by **Zeng *et al.* (2013), Rathore *et al.* (2014)**, **Pandey and Chikara (2015) and Rameeh *et al.* (2017)**.

Organic and mineral contents

Results presented in Table 3 indicate that the percentages of nitrogen, phosphorus, proline and chlorophyll a+b were significantly affected by different concentrations of NaCl. This finding was true in the five NaCl concentrations, as well as the control (B5 free).

Cultivating stevia plants in control NaCl treatment recorded the highest nitrogen percent (0.27%), while, 4000 ppm NaCl recorded the lowest one (0.09%).

Phosphorus percent values of 2000 ppm NaCl were significantly decreased by about 60% compared to those of 3000 ppm NaCl. As well as, results cleared that the percentages of proline achieved by B5 free was not fair enough to gave the highest values compared to those obtained by 3000 ppm of NaCl treatment. Moreover, the 3000 ppm of NaCl gave the highest values of

Table 1. Effect of different NaCl concentrations on number of shoots, number of leaves, shoot length (cm), number of roots and root length (cm) of *Stevia rebaudiana*

NaCl concentration (ppm)	No. shoots	No. leaves	Shoot length (cm)	No. roots	Root length (cm)
Control	8.00±1.15[a]	28.78±1.16[a]	4.50±0.35[a]	6.33±1.45[a]	3.43±0.48[a]
1000	6.67±0.88[a]	18.22±1.18[b]	3.90±0.17[ab]	6.67±0.88[a]	1.07±0.19[b]
2000	4.00±0.58[b]	16.00±1.54[bc]	3.57±0.29[cb]	6.33±0.67[a]	0.93±0.29[b]
3000	3.67±0.33[b]	16.00±0.77[bc]	3.13±0.19[cd]	4.33±0.88[ab]	0.67±0.24[b]
4000	3.67±0.67[b]	13.78±0.79[dc]	2.87±0.20[cd]	4.33±0.88[ab]	0.63±0.25[b]
5000	3.33±0.33[b]	11.11±0.97[d]	2.70±0.17[d]	1.67±0.67[b]	0.60±0.27[b]

** Means having the same letter within each column are not significantly differed at 0.05 level, according to LSD test.

Table 2. Effect of different NaCl concentrations on plantlet fresh and dry weight (g), as well as leaves fresh weight (g) of *Stevia rebaudiana*

NaCl Concentration (ppm)	Plantlet fresh weight (g)	Plantlet dry weight (g)	fresh weight of leaves (g)
Control	1.38±1.38[a]	0.18±0.02[a]	0.49±0.15[a]
1000	0.70±0.27[b]	0.09±0.02[b]	0.15±0.06[b]
2000	0.55±0.27[b]	0.07±0.03[b]	0.16±0.07[b]
3000	0.51±0.02[b]	0.09±0.01[b]	0.09±0.02[b]
4000	0.34± 0.01[b]	0.06±0.00[b]	0.16±0.02[b]
5000	0.33±0.05[b]	0.06±0.01[b]	0.19±0.03[b]

** Means having the same letter within each column are not significantly differed at 0.05 level, according to LSD test.

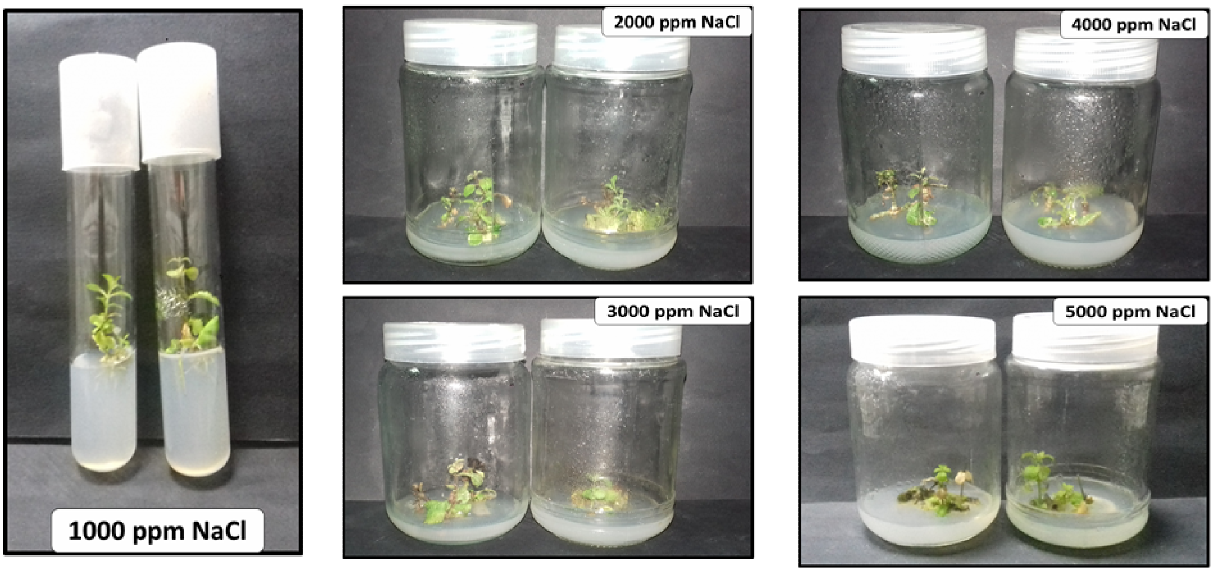

Fig. 1. Effect of NaCl concentrations on plantlets development parameters of *Stevia rebaudiana*

Table 3. Effect of different NaCl concentrations on nitrogen, phosphorus, proline percentages as well as chlorophyll a and b contents (mgl^{-1}) of *Stevia rebaudiana*

NaCl Concentration (ppm)	N (%)	P (%)	Proline (%)	Chl. a	Chl. b
Control	0.27±0.04^{a}	0.03±0.00^{b}	5.39±0.09	1.70±0.18^{a}	3.26±0.43^{a}
1000	0.25±0.05^{a}	0.03±0.00^{ab}	5.47±0.02	1.29±0.12^{ab}	2.59±0.17^{ab}
2000	0.13±0.00^{cb}	0.02±0.00^{b}	5.50±0.05	1.37±0.20^{ab}	2.49±0.34^{ab}
3000	0.22±0.03^{ab}	0.05±0.01^{a}	6.15±0.04	1.45±0.51^{ab}	2.69±0.99^{ab}
4000	0.09±0.01^{c}	0.03±0.00^{b}	5.96±0.09	1.28±0.11^{ab}	2.34±0.17^{ab}
5000	0.12±0.01^{c}	0.03±0.00^{b}	5.48±0.03	0.75±0.18^{b}	1.36±0.31^{b}

** Means having the same letter within each column are not significantly differed at 0.05 level, according to LSD test.

proline percentage (6.15%), while, 0.0 NaCl (B5 free) gave the lowest one (5.39%) in this order.

B5 free (0.0 NaCl) gave the highest values of chlorophyll (a) and chlorophyll (b) (1.70, 3.26 mgl^{-1}). However, increasing NaCl concentrations to 5000 ppm gave the lowest values for both chlorophyll a and b content (0.75, 1.36 mgl^{-1}). These results are in agreement with those confirmed by **Zeng *et al.* (2013), Rathore *et al.* (2014)** and **Rameeh *et al.* (2017).**

Effect of Drought Treatments

Growth parameters

The results of six different mannitol concentrations (0, 2, 4, 6, 8 and 10 bar) on number of shoots, number of leaves, shoot length (cm), number of roots, root length (cm), plant fresh weight (g), plant dry weight and fresh weight of leaves (g) are presented in Tables 4 and 5 which indicated clearly that the growth parameters were significantly affected by different concentrations of mannitol. This finding was true in four mannitol concentrations, as well as the control.

Increasing mannitol concentration reduced most of growth parameters. The highest number of shoots (8.00) and number of leaves (28.78) were obtained without mannitol, while, the lowest number of shoots (3.67) and number of leaves (16.45) were recorded by the highest concentration of mannitol (10 bar). Correlation between drought and the increase in number of leaves could be attributed to the favorable effect of drought on growth parameters of stevia plants which accounts much for increase in the amount of metabolites synthesized by plant. This increased also the proportion of assimilates migrated to leaves and resulted in a conspicuous increase in number of leaves.

The highest values of shoot length (4.50 cm) and number of roots (6.33 cm) were recorded for B5 free (control), while, the lowest values of shoot length (2.73 cm) and number of roots (0.67) were recorded for 8 bar of mannitol. The obtained results showed that root length was effected by increasing mannitol concentrations. Therefore, the highest values of root length was recorded for 2 bar (3.67 cm), however, the lowest values of root length (0.27 cm) was obtained from 10 bar of mannitol. These results agreed with the results concluded by: **Mubarak *et al.* (2012), Pandey and Chikara (2014), Pandey and Chikara (2015)** and **Ghaheri *et al.* (2017)**.

Table 4. Effect of different mannitol concentrations on number of shoots, number of leaves, shoot length (cm), number of roots and root length (cm) of *Stevia rebaudiana*

Mannitol concentration (bar)	No. shoots	No. leaves	Shoot length (cm)	No. roots	Root length (cm)
Control	8.00±1.15^{a}	28.78±1.16^{a}	4.50±0.35^{a}	6.33±1.45^{a}	3.43±0.48^{a}
2	7.33±0.88^{a}	27.33±1.02^{a}	3.93±0.23^{ab}	3.33±0.67^{b}	3.67±0.29^{a}
4	6.00±0.58^{ab}	19.78±1.18^{bc}	3.87±0.19^{ab}	3.33±0.88^{b}	0.93±0.18^{b}
6	5.33±1.45^{ab}	21.33±1.54^{b}	4.03±0.18^{a}	0.33±0.33^{c}	0.60±0.60^{b}
8	3.67±0.67^{b}	17.11±1.60^{c}	2.73±0.38^{c}	0.67±0.67^{c}	0.50±0.50^{b}
10	3.67±0.67^{b}	16.45±1.18^{c}	3.03±0.43^{cb}	0.67±0.67^{c}	0.27±0.27^{b}

** Means having the same letter within each column are not significantly differed at 0.05 level, according to Least Significant Difference test.

The effect of six different mannitol concentrations (0, 2, 4, 6, 8 and 10 bar), as well as the control treatment (B5 free) on plantlet dry and fresh weight (g), as well as fresh weight of leaves (g) of *Stevia rebaudiana* indicated in Table 5. Decreasing mannitol concentrations down to 2 bar led to increase plantlet fresh weight to (1.45 g) and dry weight to (0.21 g). In contrast, increasing mannitol concentration up to 10 bar recorded the lowest plantlet fresh weight (0.43 g) and dry weight to (0.09 g).

The effect of drought stress on fresh weight of leaves (g) was without a constant trend. More or less, lack of mannitol gave the significantly highest values of leaves fresh weight (0.49 g). However, increasing mannitol concentration to 4 and 6 bar gave the lowest values (0.14 g). In this case, **Zeng *et al.* (2013)** illustrated that the total dry weight and chlorophyll contents decreased and indicates that *S. rebaudiana* is mildly tolerant to salt stress. So salt and drought stress may be effective for optimizing the steviol glycoside composition. They concluded that mannitol is a six carbon sugar alcohol that often used in laboratories as a medium for inducing osmotic stress in plant and tissue cultures. In addition of mannitol to nutrient solution, over a period of 3-4 weeks that can be effect on gene expression, morphological and physiological characteristics and biochemical content in plants.

Organic and mineral contents

Results presented in Table 6 indicate that the percentages of nitrogen, phosphorus and proline, as well as chlorophyll a and b were significantly affected by different concentrations of mannitol. This finding was true in the five mannitol concentrations as well as the control (B5 free).Cultivating stevia plants in lack of mannitol led to increase the percentages of nitrogen gain to (0.27%). In contrast, increasing mannitol concentrations to 6 bar recorded the lowest percentages of nitrogen (0.06%).

Obtained results in Table 6 show that drought stress exerted a significant effect on phosphorus percentages. The highest values of phosphorus (0.03) was obtained from B5 free and 8 bar mannitol while, the lowest values (0.02) was obtained from 2 bar mannitol.

The effect of drought stress on chlorophyll a and b contents was without a constant trend, increasing mannitol concentrations to

Table 5. Effect of different mannitol concentrations on plantlet fresh and dry weight (g) as well as fresh weight of leaves (g) of *Stevia rebaudiana*.

Mannitol concentration (bar)	Plantlet fresh weight (g)	Plantlet dry weight (g)	Fresh weight of leaves (g)
Control	1.38±0.17^{a}	0.18±0.02	0.49±0.15^{a}
2	1.45±0.36^{a}	0.21±0.05	0.36±0.09^{ab}
4	0.89±0.16^{ab}	0.15±0.03	0.14±0.01^{b}
6	0.61±0.23^{b}	0.42±0.27	0.14±0.03^{b}
8	0.53±0.23^{b}	0.11±0.04	0.15±0.04^{b}
10	0.43±0.09^{b}	0.09±0.02	0.20±0.06^{b}

** Means having the same letter within each column are not significantly differed at 0.05 level, according to LSD test.

Table 6. Effect of different mannitol concentrations on nitrogen, phosphorus, proline percentages as well as chlorophyll a and b contents (mgl) of *Stevia rebaudiana*

Treatment	N (%)	P (%)	Proline (%)	Chl. a	Chl. b
Control	0.27±0.04^{a}	0.03±0.00^{a}	5.39±0.86	1.70±0.18^{a}	3.26±0.43^{ab}
2 bar	0.09±0.01^{bc}	0.02±0.00^{d}	7.00±0.49	1.48±0.08^{ab}	2.68±0.15^{cb}
4 bar	0.16±0.03^{b}	0.02±0.00^{b}	5.63±0.29	1.25±0.14^{cb}	2.40±0.33^{cd}
6 bar	0.06±0.02^{c}	0.02±0.00^{c}	6.16±0.28	1.07±0.02^{c}	1.79±0.04^{d}
8 bar	0.10±0.01^{bc}	0.03±0.00^{a}	5.77±0.52	1.27±0.10^{cb}	2.19±0.20^{cd}
10 bar	0.16±0.02^{b}	0.02±0.00^{c}	6.06±1.15	1.77±0.10^{a}	3.56±0.27^{a}

** Means having the same letter within each column are not significantly differed at 0.05 level, according to LSD test.

10 bar gave the significantly the highest value for each of chlorophyll a content (1.77%) and chlorophyll b (3.56%). However, 6 bar gave the lowest values for both chlorophyll a and b (1.07%, 1.79%). These results are in agreement with the findings of **Rathore *et al.* (2014)** who investigated the effect of salinity on biochemical characters in two Stevia genotypes. Two node microcuttings were subjected to MS media supplemented with different NaCl concentrations (0, 25, 50, 75, 100, 125 mM). Chlorophyll amount was observed to be decreased as compared to sugars, proline and phenols with increased salt concentrations.

Effect of Temperature and Light Intensity

Growth development parameters

Cultivating stevia plants under 22°C and 2000 LUX gave the highest values of all growth development parameters (Table 7) and fig (3) . However the lowest values (2.00, 13.11, 2.97, and 2.00) of number of shoots, number of leaves, shoot length and greening rate, respectively, were obtained from interaction between 18°C and 1000 LUX.

Necrosis rate results weren't on the constant trend. The interaction between 18°C and 1000 LUX produced the highest

Table 7. Effect of interaction between light intensity and temperature on number of shoots, number of leaves, shoot length (cm), greening and necrosis of *Stevia rebaudiana*

Temp (°C)	Light (LUX)	No. shoots	No. leaves	Shoot length	Greening	Necrosis
	1000	2.00±0.58^{b}	13.11±0.80^{d}	2.97±0.33^{c}	2.00±0.58^{b}	3.00±0.58^{a}
18	2000	3.00±0.58^{ab}	15.56±0.80^{bc}	4.00±0.40^{cab}	4.00±0.58^{a}	1.00±0.58^{b}
	3000	2.67±0.33^{b}	13.56±0.59^{dc}	3.17±0.27^{cb}	3.67±0.33^{a}	1.33±0.33^{b}
	1000	3.00±0.58^{ab}	16.00±0.39^{b}	3.77±0.50^{cab}	3.67±0.33^{a}	1.33±0.33^{b}
22	2000	5.00±1.15^{a}	19.78±0.79^{a}	5.03±0.61^{a}	4.67±0.33^{a}	0.33±0.33^{b}
	3000	3.33±0.67^{ab}	16.44±0.80^{b}	4.50±0.40^{ab}	4.00±0.58^{a}	1.00±0.58^{b}

** Means having the same letter within each column are not significantly differed at 0.05 level, according to LSD test.

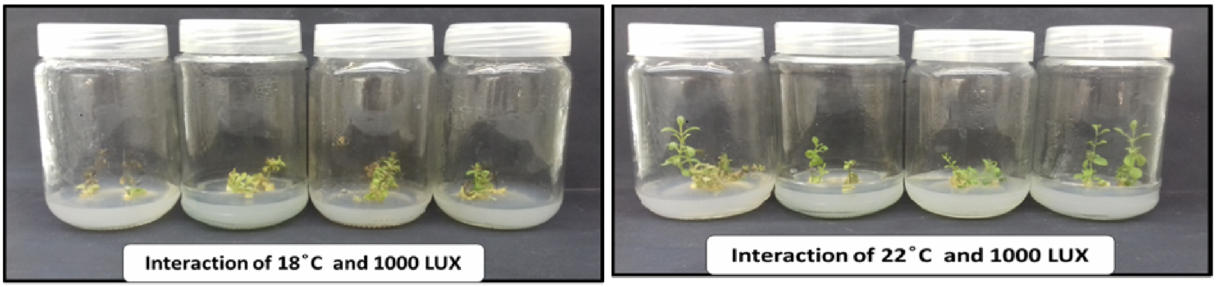

Fig. 2. Effect of interaction between low light intensity (1000 LUX) and temperature on plantlets development parameters of *Stevia rebaudiana*

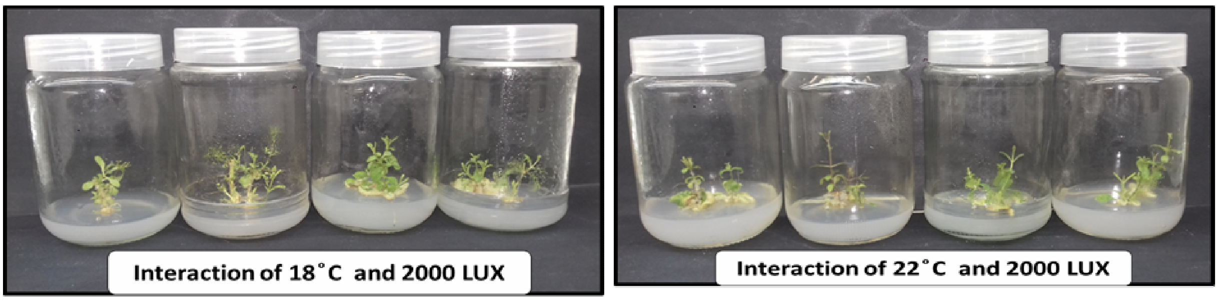

Fig. 3. Effect of interaction between medium light intensity (2000 LUX) and temperature on plantlets development parameters of *Stevia rebaudiana*

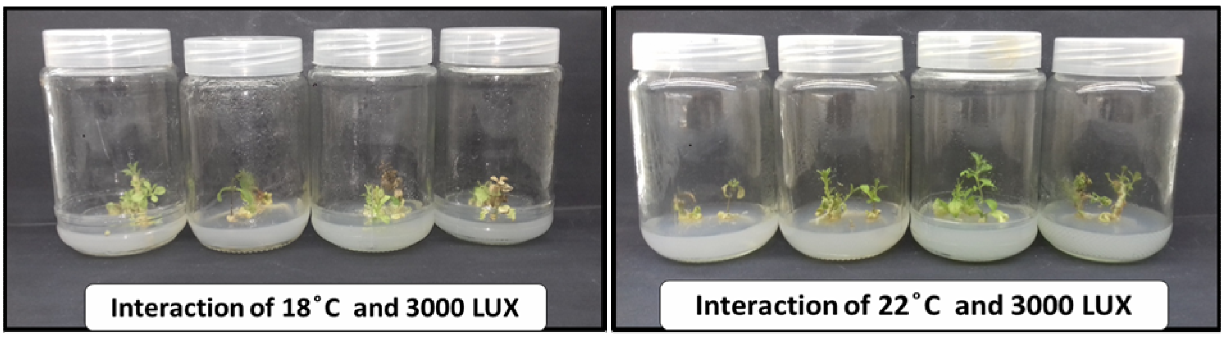

Fig. 4. Effect of interaction between high light intensity (3000 LUX) and temperature on plantlets development parameters of *Stevia rebaudiana*

values (3.00) of necrosis rate. Similar results were also reported by **Uddin *et al.* (2006)**, **Hossain *et al.* (2008)** and **Mubarak *et al.* (2008)**.

According to the results in Table 8, the highest values of plantlet fresh weight (1.55), dry weight (0.18) and leaves fresh weight (0.43) were obtained from the interaction between 18°C and 2000 LUX, while, the lowest values of both plantlet fresh and dry weight were obtained from the interaction between 18°C and 1000 LUX, but the lowest values of leaves fresh weight were obtained from the interaction between 22°C and 1000 LUX. Similar results were also reported by **Savithri *et al.* (2001), Morini *et al.* (2003), Uddin *et al.* (2006)**, **Dheeranupattana *et al.* (2007)** and **Hossain *et al.* (2008).** In the concern, **Mubarak *et al.* (2008)** reported that micropropagation of stevia by shoot tip, gave the highest number of plantlets than nodal segments. The multiplied shoots were cultured on modified MS medium supplemented with different concentrations of BA at 0, 0.5, 1.0 and 1.5 mgl^{-1}. The best result of shoots number were obtained at 25°C under light intensity 3000 lux and BA at 0.5 mgl^{-1}.

Organic and mineral contents

Organic and mineral contents were significantly influenced by the interaction between light intensity and temperature (Table 9).

Results presented in Table 9 indicate that the percentages of nitrogen and phosphorus, as well as the contents of chlorophyll A and B were significantly affected by the interaction between light intensity and temperature.

Mediate level of light intensity was recorded significantly highest percentage of nitrogen (0.19%) under low temperature conditions. Meanwhile, increasing light intensity under conditions of high temperature recorded the lowest value of nitrogen percentage (0.10%). The highest value of phosphorus (0.04) was obtained from the interaction between 22°C and both 1000 and 3000 LUX, while, the lowest value was obtained from the interaction between 18°C and 2000 LUX.

The effect of the interaction of temperature and light intensity on chlorophyll a and b contents was without a constant trend; mediate light intensity (2000 LUX) gave significantly the highest value of chlorophyll a (1.76) and chlorophyll b (3.03) under high temperature. Similar results were also reported by **Savithri *et al.* (2001), Morini *et al.* (2003), Uddin *et al.* (2006)**, **Dheeranupattana *et al.* (2007)** and **Mubarak *et al.* (2008).** In the concern, **Hossain *et al.* (2008)** pointed that all cultures of *Stevia rebaudiana* were grown in an air conditioned culture room illuminated by 40 W white fluorescent tubes with an intensity varied from 2000-3000 lux. The photoperiod was maintained as 16 hours light and 8 hours dark.

Table 8. Effect of interaction between light intensity and temperature on plantlet fresh and dry weigh (g) as well as leaves fresh weigh (g) of *Stevia rebaudiana*

Temp. (°C)	Light (LUX)	Plantlet fresh weight (g)	Plantlet dry weight (g)	leaves fresh weight (g)
18	1000	0.07 ± 0.02^{b}	0.09 ± 0.02^{b}	0.23 ± 0.05^{ab}
	2000	1.55 ± 0.14^{a}	0.18 ± 0.02^{a}	0.43 ± 0.12^{a}
	3000	1.13 ± 0.37^{a}	0.13 ± 0.04^{ab}	0.16 ± 0.07^{b}
22	1000	1.20 ± 0.33^{a}	0.13 ± 0.03^{ab}	0.09 ± 0.03^{b}
	2000	1.50 ± 0.23^{a}	0.18 ± 0.02^{ab}	0.29 ± 0.07^{ab}
	3000	0.93 ± 0.20^{a}	0.12 ± 0.02^{ab}	0.19 ± 0.06^{b}

** Means having the same letter within each column are not significantly differed at 0.05 level, according to LS D test.

Table 9. Effect of interaction between light intensity and temperature on nitrogen, phosphorus percentages as well as chlorophyll a and b contents of *Stevia rebaudiana*

Temp. (°C)	Light (LUX)	N (%)	P (%)	Ch. a	Ch. b
18	1000	0.15±0.01[ab]	0.04±0.00[b]	0.93±0.19[c]	1.61±0.39[b]
	2000	0.19±0.03[a]	0.03±0.00[e]	1.59±0.09[ab]	3.00±0.23[a]
	3000	0.18±0.05[ab]	0.03±0.00[d]	1.22±0.22[cb]	2.19±0.43[ab]
22	1000	0.14±0.00[ab]	0.04±0.00[a]	0.79±0.05[c]	1.29±0.06[b]
	2000	0.12±0.00[ab]	0.03±0.00[c]	1.76±0.17[a]	3.03±0.28[a]
	3000	0.10±0.01[b]	0.04±0.00[a]	1.62±0.13[ab]	2.68±0.27[a]

** Means having the same letter within each column are not significantly differed at 0.05 level, according to LSD test..

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الملخص العربي

تنظيم التكشف العضوي للإستيفيا تحت ظروف الإجهاد والمناخ الدقيق

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تهدف هذه الدراسة الي تقييم أداء نبات الاستيفيا (صنف: اسبانتا) بالنسبة لبعض عوامل الإجهاد من ملوحة وجفاف وبعض عوامل المناخ الدقيق من درجات حرارة ومستويات إضاءة، حيث استعملت تركيزات مختلفة من كلوريد الصوديوم (صفر، ١٠٠٠، ٢٠٠٠، ٣٠٠٠، ٤٠٠٠، ٥٠٠٠ جزء في المليون) لدراسة تأثير الملوحة، وتركيزات من المانيتول لدراسة تأثير الجفاف (صفر، ٢، ٤، ٦، ٨، ١٠ بار)، وتم إنماء هذه النباتات تحت اثنين من درجات الحرارة (١٨، ٢٢ درجة مئوية) وثلاث مستويات مختلفة من شدة الإضاءة (١٠٠٠، ٢٠٠٠، ٣٠٠٠ شمعة) لدراسة تأثير الحرارة وشدة الإضاءة علي نمو وتطور النبات. سجل تركيز ١٠٠٠ جزء في المليون أعلي معدل نمو للنباتات، بينما أظهر تركيز ١٠ بار أقل معدل لنمو النباتات، وأظهرت النباتات التي نمت تحت ظروف المناخ الدقيق من درجة حرارة عالية (٢٢ درجة مئوية) وشدة اضاءة متوسطة (٢٠٠٠ شمعة) أعلي قيم لنمو النبات ومحتواه العضوي والمعدني.

الكلمات الإسترشادية: معاملات الملوحة معملياً، إجهاد الجفاف، منظمات النمو النباتية، المناخ الدقيق، الإستيفيا.

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