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Research Article

Diatomite alleviates the adverse effects of salinity stress on growth and yield of *Stevia rebaudiana*

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ABSTRACT

The present study was planned to investigate the role of diatomitein salt tolerance of *Stevia rebaudiana* using pot experimental. Diatomite increased the growth characters of plants, hence resulted in higher yields. In contrast, the seawater treatments alone significantly decreased these parameters in both seasons. While, treating *Stevia rebaudiana*plants with diatomite prior to salinity stress decreased the detrimental effectof salt stress on growth and yield.The Esterase isoenzymesprofiles has indicated that salinity, diatomite and their combinations has caused biochemical changes in *Steviar.* plants. On the other hand, saline water irrigation decreased Rebaudioside A concentration at the second harvest as estimated by HPLC analysis in both seasons .The effect of the interaction between salinity and diatomite was the mosteffective treatments for Rebaudioside A when *Stevia rebaudiana*plants treated by salt concentration 4000 ppm and 2.5 g/kgsoil diatomite.

Key words:- *Stevia rebaudiana*, Diatomite, Salinity, Esterase ,Rebaudioside A

1. INTRODUCTION

Stevia (*Stevia rebaudiana*Bertoni) is a perennial herb of Asteraceae (Compositae) family, which grows wild as a small shrub in parts of South America [27,21]. The economic part of the plant are Leaves [27], with a high concentration of steviol glycosides, possible substitutes of synthetic sweeteners [3,27,30]; which gives stevia a great importance as a natural food sweetener supplier crop. Stevia shows a high content of sweeteners, which are up to 150 times sweetener than sugar, but virtually with no calories [9]. Stevia can, apparently, be successfully grown under different conditions regarding climate and soils [15].

There is often a tendency for a relation between growth and yield of crops, and salinity, usually, the higher salinity level the less growth and yield of the crop [29,18,19,833]. Growth and yield reduction occurs when salts accumulate in the root zone to such an extent that the crop is no longer able to extract sufficient water from the salty soil solution, resulting in a water stress for a

significant period of time [4,23]. Stevia plant was sensitivity to salinity stress as stevia yield was reduced when electrical conductivity of the irrigation water was higher than 3.0 dS m−1 [29]. Many studies considered that, silicon is considered as one of the important beneficial nutrient for plant growth [22].

Diatomite de Mozambique (DDM) is a naturally occurring sedimentary rock primarily composed of fossilized remains of fresh water diatoms. It is chemically composed of SiO_2 (86-89%) in a soluble form available to plants and small amount of trace elements. However, recently, numerous studies have demonstrated that silicon is one of the important elements of plants and plays an important role in tolerance of plants to environmental, heavy metal and biotic stresses [14, 35].

Esterases exist in different isoenzymes in plant and animal tissues, their electrophoretic pattern was also analyzed. The relationship between esterase activity and salinity has been investigated in several plant species [10,16,38].

Plant species were differed in their sensitivity or tolerance to salt stress, since diatomite contain significant quantities of silica which demonstrated to be beneficial for alleviating both biotic and abiotic stress in plants, therefore, we herein report the effect of diatomite (DDM) on growth and quality of stevia plants under salt stress which might establish a possible correlation between salt tolerance and esterase activity (EST) as well as Rebaudiosid A containing.

2. MATERIALAND METHODS

In this study, the effect of diatomite was investigated as a Silica source to increase salt tolerance and Rebaudioside A yield of *Stevia rebaudiana*.

2.1 Plant Material

The plant material used in these experiments were kindly provided by Medicinal and Aromatic plants Department, Horticulture Research Institute, Agriculture Research Center (ARC), Giza, Egypt. The experiments were conducted during the two successive seasons for six months from March[1st] to September[1st]2012 and 2013 at the Faculty of Agriculture Experimental Station, Suez Canal University, Ismailia, Egypt.

2.2 Plant and Growth Conditions

Seedlings of *Stevia rebaudiana*(5 cm in length and carrying 2 pairs of leaves) were replanted in plastic pots (37.5cm in diameter and 27.5cm in depth) containing (25 kg) of a moist mixture of sand and petmoss (1:1, v:v) per pot. The soil in pots was evenly mixed with diatomite (DDM) one week before transplanting, at concentrations of 0.0, 2.5 and 5 g/kg soil in each pot respectively. Pots were arranged in a split plot design with two factors, diatomite concentration as the sub factor and saline water as main factor (control, 1000, 2000 and 4000 ppm) with 28 pots per treatment, each pot had one plant. Saline water was prepared by mixing tap water (Table1) with sea water brought from the Suez Canal, Ismailia, Egypt (35,000 ppm) to achieve salinity levels of (Control (tap water), 1000, 2000 and 4000 ppm) using electrical conductivity meter (EC Model 20234571). Plants were irrigated with salinity treatments after one month from transplantingto raise the soil water field capacity.The pH of salinity treatments was measuredusing pH meter (CRISON Basic 20). Other agricultural practices such as weeding and fertilization were carried out as recommended.

The diatomite are mined and ground up to render a powder that looks and feels like talcum powder. Diatomite is characterized by 89.00% silicon (SiO_2), 0.20% magnesium (MgO), 0.32% sodium (Na_2O), 0.88% iron (Fe_2O_3), 0.63% potassium (K_2O), 5.95% aluminum (Al_2O_3) and other trace minerals such as 0.29% titanium (TiO_2) and 0.10% carbonate (CaO).(Sekem company, Cairo, Egypt).

Table (1) Some chemical components of the experimental water

Salinity Level mg^{-1}	Cations				Anions				EC ppm	SAR
	Ca^{+2}	Mg^{+2}	Na^{+}	K^{+}	CO_3^{-2}	HCO_3^{-}	SO_4^{-2}	Cl^{-}		
(288)	1.13	1.34	1.77	0.27	n.d	2.65	0.47	1.4	288	1.68

2.3 Measurements were taken onVegetative growth characters

The first and second harvest occurred in Jun[1st] and September[1st]respectively. The following data were determined. At first harvest, total fresh and dry weights of vegetative growth (branch and leaves) were recorded. At the second harvest, Plant height, number of branches, root length, number of root and fresh and dry weight of herb (leaves and stalks) and root were recorded.

2.4 Mineral composition.

Plant Leaf samples from the second harvest were dried at 70 ºC for 24 h, the obtained dry matter was ground and digested according to (Piper, 1947) methods to determine chloride, sodium and potassium contents. Sodium and potassium were determined by using Atomic Absorption Flame Photometric method (3300). Chloride was measured with chloride meter according to Wilde etal. (1985) and Black etal. (1965) [40,7].

2.5 Isoenzyme Electrophoresis.

2.5.1 Isoenzyme Extraction.

Equal weights of fresh leaves samples (from the secound season second harvest) were crushed directly in an ice-cold (0-4c°)1M tris buffer**,** pH 7.8**,** containing 0.2% (W:V) sodium ascorbate**,** 1% (W:V) sodium tetraborate**,** 2% (W:V) sodium metabisulfite and 1% (W:V) polyvinylpyrrolidone 40.

The isoenzyme extraction buffer and procedures were applied according to Tanksley and Orton (1983).

A 400 µl lysate were transferred to a 1.5 ml Eppendorf microfuge tube containing 200 µl ice-cold extraction buffer**,** then centrifuged for 8-10 minutes at 8000 rpm. The clear supernatant was transferred to a new Eppendrof microfuge tube and all sample tubes were kept frozen till loading for electrophoresis .

2.5.2 Polyacrylamide Gel preparation.

For separating gels**,** a 15% discontinuous-dissociating polyacrylamide molds were used for screening isoesterase banding patterns. N**,**N**,**N**,**N-tetramethyl ethylenediamine**,** 0.03µl**,** and freshly-prepared 1.5% ammonium persulphate**,** 3ml were added to initiate polymerization of acrylamide monomer in a Tris-EDTA-Boric buffer (0.18 M Tris**,** 0.004 M EDTA and 0.1 M Boric acid) with pH 8.6. A total of 40µl (25µl of sample in the extraction buffer + 15µl of 10% sucrose in 0.002% bromophonal blue solution) was loaded separately into slots, vertical gel electrophoresis (Multigel - Long cat. ≠ 010-400, Biometra®). Electrophoresis was continued until the bromophenol blue dye front has traveled to the end of the run. About ten hours and thirty minutes were needed for run using constant voltage of 250 DC volts.

2.5.3 Staining Techniques.

For detection of esterases isozyme bands the procedures of Kahler and Allard [20] were applied with some modification suggested by Tanksley and Rick [37]. The gels were incubated in freshly prepared mixtured of α and β naphtly acetate and 0.1% fast blue RR salt. Then**,** gels were kept in the staining solution in a dark cabinet at 30°c**,** for 1-2 hrs**,** till the reddish bands appear in a dark background.

2.6 HPLC method.

2.6.1 Instrumentation

Chromatographic experiments were performed with HPLC Thermo - Dionex® HPLC Model Ultimate 3000. Separation and quantitation were made on a 150×4.6 mm (i.d) 5 µm ODS column (Phenosphere-Next ,Phenomenex®, USA). The

detector was set at λ 210 nm. Data acquisition was performed on Chromeleon software.

2.6.2 Material and reagents

The Authentic standard of Rebaudioside A was supplied by Sigma-Aldrich Co. (St. Louis, MO, USA). Methanol and water were HPLC grade (Fisher, Leis LE 11 5 RG UK).

2.6.3 HPLC conditions

Separation and quantitation were made on a 150×4.6 mm (i.d) 5 µm ODS column (Phenosphere-Next ,Phenomenex®, USA). The mobile phase was prepared by mixing methanol and water in a ratio 80:20 v/v.

The flow rate was set to 0.9 ml/min, injection volume: 10µL. All determinations were performed at ambient temperature (at 25 °C). The mobile phase was filtered using 0.45 µm membrane filter (Millipore, Milford, MA) and degassed by vacuum prior to use. The samples were also filtered using 0.45 µm disposable filters.

2.6.4 Standard solutions and calibration

Stock standard solutions were prepared by dissolving 5 mg Rebaudioside A in 5 ml of mobile phase. The standard solutions were prepared by dilution of the stock standard solution with the mobile phase to reach concentration ranges of 20–100 to be injected in Triplicates of 20 µl for each concentration; chromatographed under the specified conditions described above. The peak area values were plotted against corresponding concentrations to obtain the calibration graph.

2.6.5 Sample preparation

The extracts from (leaves grain from second harvest on both seasons) each treatment were prepared from the dried samples by extraction with methanol. Different identified weights of the dried samples were soaked in the extraction solvent to be replaced every day for five consequent days to insure complete extraction before drying under vacuum. Complete extraction was confirmed by thin layer chromatography. For the HPLC analysis, 5 mg of each sample was dissolved in 5 ml of the mobile phase in a volumetric flask. The content of each flask was shaken vigorously for 10 min, sonicated for 15 min before filtration through 0.45 µm disposable filters. The samples were injected with 25 µL Hamilton analytical syringe. A sample of 20 µl was then injected in a triplicate manner using the general procedures described under calibration and the concentrations of Rebaudioside A was calculated.

2.7 Statistical analysis

The experiment design was split plot with 28 replicates. Salinity, treatments represented the main plots, while diatomite treatments were considered as sub-plots. Data were statistically analyzed using ANOVA\MANOVA of Statistica 6 software, StatSoft company [32], the significance of differences among means was carried out using the Least Significant Test (L.S.D) at $p = 0.05$.

3. RESULTS

3.1Growth responses

The Fresh and dry weight of the herb (leaves and stalks) in the first cut under salt stress with and without diatomite treatments were observed and presented in Table (2). The results indicated that diatomite treatments alone increased these parameters compared to control plants in both seasons. Maximum fresh and dry weight of herbs was observed for diatomite concentration (2.5 g/kg soil) (Table 2). In contrast, the seawater treatments alone significantly decreased these parameters in both seasons (Table 2). The interaction between salinity and diatomite concentration caused significant increase in the fresh and dry weight of herbs under salinity treatment compared to control treatment in the two seasons (Table 2). Maximum fresh and dry weight of herb under salinity level (1000 ppm) were determined under diatomite concentration (2.5 g/kg soil), on contrast maximum fresh and

dry weight of herb under salinity level (2000 and 4000 ppm) were determined under diatomite concentration (5 g/kg soil) Table (2).

Data cited in Tables (3) shows the increase in the Plant height, branches number and length of root in the second cut of *Stevia rebaudiana*Bertoni as a result of the diatomite treatments. The Diatomite concentration alleviated the salinity effects on these parameters; on contrast the salinity levels decreased these parameters (Table 3). Data presented in Table (4) shows that the fresh and dry weight of the whole herbs and roots of *Stevia* significantly increased at the second cutting in both seasons as a result to diatomite application. For the dry weight of root the increase was not significant in the second season. It is apparent that these characters significantly decreased with increasing salinity levels (Table 4). Diatomite applications has showed significant stimulation effect on the fresh and dry weight of herb and root under 1000, 2000 and 4000 ppm salinity stress in both seasons compared with the control treatments (Table 4).

3.2 Some mineral element contents

Results in (Table 5) showed that all salinity treatments significantly increased the Cl^- and Na^+ content and decreased the K^+ content in the leaves of*Stevia rebaudiana* as compared to the control plants in both seasons, by contrast diatomite concentrations decreased the Cl^- and Na^+ content and increased K^+ content in the leaves of *Stevia rebaudiana* as compared to control plant in both seasons. The interaction between salinity and diatomite concentration caused increased of K^+ contain under salinity levels table (5).

3.3 Biochmical markers.

3.3.1 Esterase isozyme polymorphism.

In total, we have detected 8 Esterase isoenzymes (EST1 – EST8) activities (Fig1). We have grouped these isoenzymes into 4 groups. The first group, which includes EST1, EST2, and EST 5, were constitutively active regardless of salinity condition.

However, these isoenzymes were not active in the presence of 5g/Kg soil diatomite. In the presence of 2.5g diatomite /Kg soil , these enzymes showed differential activity profiles at different salinity levels. The second group, which includes EST4 and EST6, were activated at both diatomite concentration (2.5 and 5 g/Kg soil) with differential activity profiles at different salinity levels. The third group, which includes EST7 and EST 8, was deactivated by 5g diatomite /Kg soil treatment. However, these isoenzymes showed differential activity profiles depending on the salinity levels, in the absence or presence of 2.5g diatomite /Kg soil. The fourth group, is unique to the EST3, which activity was recorded at 5g/Kg diatomite concentration and 2000ppm salinity

3.4 HPLC analysis

For HPLC analysis, similar retention time of Rebaudioside A was observed in all the tested samples as compared with the standard and there was a good separation of Rebaudioside A from other components of the extract. The standard peak showed retention time of 3.657 min as shown in fig. 2. The linearity was determined at a concentration range of 10 to 80 µg/ml. The highest Rebaudioside A concentration of 3.5736 µg/10 µg extract was observed in the 2000 ppm salinity + 5g/kg soil diatomite treatment for the first season as shown in table 6. While in the second season, the highest concentration was 5.5719 µg/10 µg extract to be observed in response to 4000 ppm salinity + 2.5 g/Kg soil diatomite treatment.

4. DISCUSSION

The analysis of the yield of *Stevia rebaudiana* response to the diatomite, irrigation water salinity and their interactions were done using pot experimental. The yield reduction due to the salinity increase in the first and second cutting and there were two harvests, even with the saline

water irrigation, salinity levels cause reduction on all parameters under this study. These results are in agreement with those obtained by [27,11,13,12,24,29].

Results obtained from this study have shown that Diatomite is an effective amendment to improve plant growth and yield of *Stevia rebaudiana*, these results are in agreement with those obtained by [5,2,39]. Increasing diatomite in mixture increased meso, micro and ultra pores, which are important for plant water requirements and also increased macro pores which are desired for optimum plant growth and root distribution as well as nutrient absorption by root system [1,5]. Diatomite addition significantly correct the negative effects of salinity on *Stevia rebaudiana*plant. Na^+ and Cl^- uptake was higher in plants grown under salinity, however diatomite enhances salt tolerance by reduced Na^+ and Cl^- uptake resulting in a significant increase in K^+ contain in *Stevia rebaudiana*leaves [5,35]. K^+ has role in improving plant water status and mitigating the toxic effects of Na^+.

The response of plants to salinity and other treatments were based on the action of many defnse proteins/enzymes [31]. Plant ios-esterases have been related to heavy metal and pesticide toxicity, pathogenesis, morphogenesis and embryogenesis potential [25]. It is clear on our study that salinity stress alone or with diatomite treatments led to increased most esterase activities in the leaves of *Stevia rebaudiana*(Fig1). The Esterase isoenzymesprofiles has indicated that salinity, diatomite and their combinations has caused biochemical changes in the Stevia plants. This resulted with abrove with [17, 6,10].

The present investigation reports for the first time a comparative analysis for the occurrence and amount of Rebaudioside A in *Stevia rebaudiana* in response to different salinity and diatomite concentrations application. The HPLC analysis of different extracts revealed that Rebaudioside A content was higher in the plants when treated with 4000 ppm salinity and 2.5 (g/kg soil) of diatomite as compared to control plants.

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0	0	0	0	2.5	2.5	2.5	2.5	5.0	5.0	5.0	5.0	Diatomite (g/Kg soil)
0	1	2	4	0	1	2	4	0	1	2	4	Salinity (x1000 ppm)

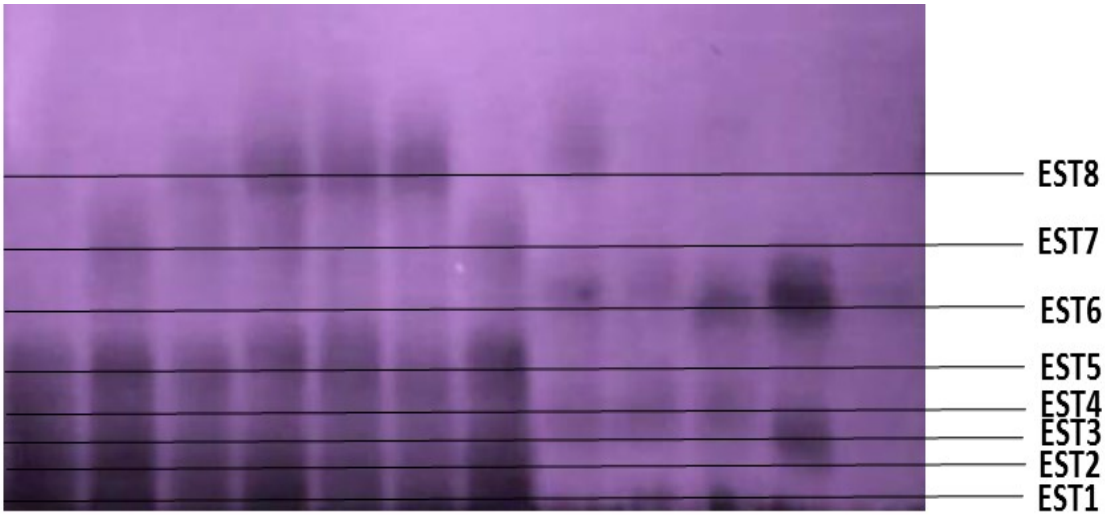

Fig (1): Polyacrylamid gels stained for esterase isozymes of*Stevia rebaudiana*leaves

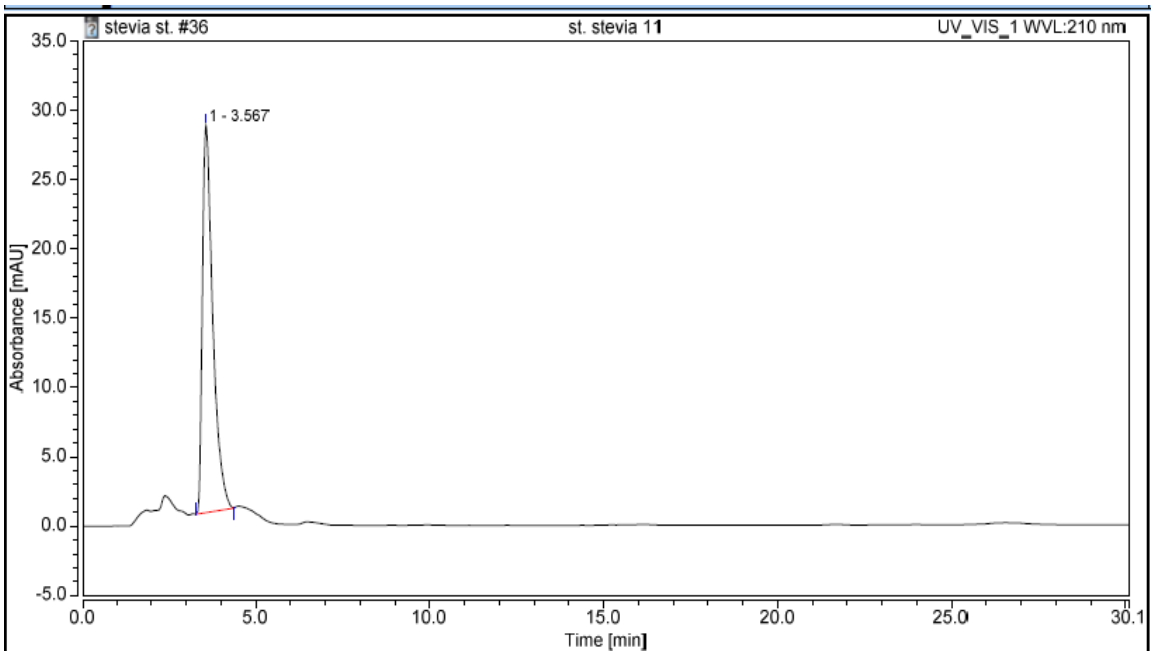

Fig. (2):- HPLC chromatogram of organic extract of *Stevia rebaudiana* showing Rebaudioside A

Table (2) Effect of salinity, diatomite concentration and their interaction on total fresh and dry weight of herb(leaves and stalks) of *Steviarebaudiana*Bertoniplant during 2012 and 2013on the first harvest.

Treatments		Fresh weight of herb(g)		Dry weight of herb (g)	
Seawater concentration (ppm)	Diatomite concentrations (g/kg soil)	Season 2012	Season 2013	Season 2012	Season 2013
		(A) Effect of diatomite			
0.0	0.0	26.72 a*	25.39 a	4.25 a	5.00 a
0.0	2.5	30.93 a	29.53 a	5.38 a	5.25 a
0.0	5	28.39 a	26.87 a	5.05 a	5.14 a
		(B)Effect of salinity			
0.0	0.0	45.47 a	35.42 a	7.17 a	8.75 a
1000	0.0	33.44 b	32.25 a	4.91 b	5.12 b
2000	0.0	22.13 c	21.33 b	4.64 b	3.64 c
4000	0.0	10.61 d	15.77 b	2.39 c	1.63 d
		© Effect of interaction between salinity and diatomite			
0.0	0.0	42.36 ab	33.72 ab	6.71 ab	7.42 ab
0.0	2.5	51.70 a	35.49 ab	9.15 a	10.68 a
0.0	5	42.35 ab	37.05 a	5.76 bcd	8.15 ab
1000	0.0	29.97 bcd	25.51 abc	3.32 def	4.60 bcd
1000	2.5	39.65 abc	37.71 a	6.31 bc	5.57 bc
1000	5	30.69 bcd	33.54 ab	5.09 bcd	5.19 bc
2000	0.0	16.71 de	15.65 cd	3.94 cdef	2.55 cd
2000	2.5	23.42 cd	30.04 abc	4.42 bcde	4.27 bcd
2000	5	25.81 bcd	31.32 abc	5.80 bcd	5.20 bc
4000	0.0	13.40 de	6.81 d	1.22 f	0.79 d
4000	2.5	14.69 de	7.57 d	1.71 ef	2.19 cd
4000	5	16.8 e	21.24 bcd	3.63 cdef	2.30 cd

*Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test

Table (3) Effect of salinity, diatomite concentration and their interaction on plant height ,branch number , root length and number of root of *Stevia rebaudiana*Bertoni plant during 2012 and 2013on the second harvest.

Treatments		Plant height (cm)		Branch number (n)		Root length (cm)		Root number (n)	
Seawater concentration(ppm)	Diatomite concentrations(g /kg soil)	Season 2012	Season 2013	Season 2012	Season 2013	Season 2012	Season 2013	Season 2012	Season 2013
(A)Effect of diatomite									
0.0	0.0	38.63 ab*	32.13 a	13.17 a	12.25 a	13.41 b	17.62 a	32.13 a	23.12 a
0.0	2.5	43.33 a	38.54 a	16.18 a	12.30 a	16.81 a	19.48 a	33.75 a	31.78 a
0.0	5	35.61 b	34.73 a	18.25 a	13.58 a	15.75 ab	18.90 a	51.31 a	44.44
(B)Effect of salinity									
0.0	0.0	44.33 a	40.50 a	29.67 a	16.11 a	20.50 a	25.00 a	54.17 a	47.17 a
1000	0.0	40.44 a	35.23 b	15.67 b	14.44 a	16.25 b	22.61 a	40.67 ab	34.89 ab
2000	0.0	37.44 a	33.33 b	9.80 c	10.10 a	17.00 ab	17.09 ab	32.50 b	31.43 ab
4000	0.0	32.57 a	29.80 b	7.00 c	8.67 a	7.54 c	9.96 b	28.92 b	18.97 b
		© Effect of interaction between salinity and diatomite							
0.0	0.0	35.33 abc	37.67 ab	23.67 bc	15.00 abc	14.00 de	23.50 abc	18.50 cd	38.00 abc
0.0	2.5	47.00 ab	45.00 a	36.00 a	17.00 ab	18.00 bcd	27.50 a	75.00 a	52.50 ab
0.0	5	39.00 abc	38.83 ab	29.33 ab	16.33 abc	16.75 bcd	24.00 abc	69.00 ab	51.00 ab
1000	0.0	33.00 bc	30.67 bc	9.33 de	10.33 abc	19.50 abc	20.33 abc	16.00 d	33.50 abc
1000	2.5	41.33 abc	32.33 bc	17.33 cd	19.67 a	21.00 ab	22.33 abc	60.50 abc	46.7 c
1000	5	38.00 abc	37.00 ab	20.33 bc	13.33 abc	21.22 ab	25.17 ab	51.50	66.50 a

								abcd	
2000	0.0	37.78 abc	37.00 ab	7.75 de	9.67 abc	11.00 e	14.44 cd	28.50 bcd	24.17 abc
2000	2.5	50.75 a	40.67 ab	8.33 de	13.33 abc	23.50 a	19.83 abc	31.00 bcd	27.13 abc
2000	5	42.33 abc	38.00 ab	14.00 cd	10.50 abc	16.50 bcd	17.00 bcd	38.00 abcd	43.00 abc
4000	0.0	26.00 c	14.00 d	2.00 e	2.67 c	3.63 f	6.88 d	18.50 cd	11.17 bc
4000	2.5	33.50 abc	21.17 cd	8.50 de	5.50 bc	4.75 f	8.25 d	21.50 cd	15.50 bc
4000	5	36.33 abc	40.33 ab	9.33 de	12.67 abc	14.25 cde	14.75 cd	46.75 abcd	30.25 abc

*Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test

Table (4) Effect of salinity, diatomite concentration and their interaction on total fresh and dry weight of herb(leaves and stalks) and root of *Stevia rebaudiana*Bertoniplant during 2012 and 2013on the second harvest.

Treatments		**Fresh weight of herb (g)**		**Dry weight of herb (g)**		**Fresh weight of root (g)**		**Dry weight of root (g)**	
Seawater concentration (ppm)	Diatomite concentrations (g/kg soil)	Season 2012	Season 2013	Season 2012	Season 2013	Season 2012	Season 2013	Season 2012	Season 2013
(A) Effect of diatomite									
0.0	0.0	8.88 b*	7.30 b	2.33 b	2.37 b	11.23 a	8.13 b	3.28 b	4.00 a
0.0	2.5	10.15 ab	8.96 ab	3.00 a	2.40 b	13.73 a	10.90b	3.78 ab	4.04 a
0.0	5	10.92 a	10.81 a	3.41 a	3.45 a	21.92 a	26.50 a	6.54 a	7.05 a
(B)Effect of salinity									
0.0	0.0	12.63 a	11.36 a	4.05 a	3.25 a	21.25 a	19.22 a	5.86 a	6.68 a
1000	0.0	12.23 a	10.84 a	3.07 ab	3.01 a	18.29 ab	17.40 a	5.28 a	6.39 a
2000	0.0	7.97 ab	8.40 ab	2.64 bc	2.63 ab	12.63 bc	12.10 a	4.13 a	4.40 ab
4000	0.0	6.33 b	4.78 b	1.72 c	1.88 b	10.33 c	11.97 a	2.87 a	2.67 b
© Effect of interaction between salinity and diatomite									
0.0	0.0	9.87 bcde	9.90 b	3.52 bc	2.60 bcde	17.71 abc	7.12 bc	4.93 b	2.68 cd
0.0	2.5	15.16 a	11.11 ab	3.55 bc	2.69 bcd	23.81 ab	20.09 bc	6.08 ab	8.13 abc
0.0	5	14.26 ab	11.98 ab	5.35 a	4.76 a	22.24 abc	25.00 b	6.59 ab	8.35 ab
1000	0.0	9.80 bcde	9.50 bc	2.31 de	1.46 f	6.60 bc	4.85 c	1.45 b	1.54 d
1000	2.5	11.50 abcd	9.60bc	2.59 cd	2.14 cdef	17.75 abc	8.02 bc	4.0 b	6.38 bcd
1000	5	16.60 a	16.00 a	4.55 ab	4.86 a	30.53 a	44.79 a	13.07 a	12.12 a
2000	0.0	4.49 f	6.10 bcd	1.69 de	2.04 def	11.57 abc	11.76 bc	3.71 b	4.04 bcd
2000	2.5	13.60 abc	11.40 ab	4.10 b	3.73 ab	13.80 abc	12.07 bc	4.75 b	4.16 bcd
2000	5	5.83 ef	7.71 bcd	2.13 de	3.26 bc	12.53 abc	12.49 bc	3.92 b	5.00 bcd
4000	0.0	3.03 f	2.48 d	1.33 e	1.60 ef	5.68 bc	5.31 c	4.13 b	2.80 bcd
4000	2.5	7.63 def	4.30 cd	1.76 de	1.88 def	2.95 c	6.91 bc	1.87 b	2.48 d
4000	5	8.33 cdef	7.58 bcd	2.06 de	2.17 cdef	22.38 abc	23.71 bc	2.61 b	2.74 cd

*Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test

Table (5) Effect of salinity, diatomite concentration and their interaction on (Cl, Na, K%) contents on the leaves of *Stevia rebaudiana*Bertoniplant during 2012 and 2013on the second harvest.

Treatments		Cl%		Na%		K%	
Seawater concentration (ppm)	Diatomite concentrations(g /kg soil)	Season 2012	Season 2013	Season 2012	Season 2013	Season 2012	Season 2013
(A) Effect of diatomite							
0.0	0.0	0.288 a*	0.291 a	0.679 a	0.559 a	1.337 c	1.424 b
0.0	2.5	0.250 b	0.261 b	0.307 b	0.503 ab	1.752 a	1.623 a
0.0	5	0.255 ab	0.265 b	0.579 a	0.461 b	1.611 b	1.555 a
(B)Effect of salinity							
0.0	0.0	0.241 c	0.251 b	0.418 bc	0.300 d	1.767 a	1.719 a
1000	0.0	0.257 bc	0.260 b	0.312 c	0.417 c	1.479 bc	1.392 c
2000	0.0	0.278 ab	0.321 a	0.478 b	0.741 a	1.588 b	1.568 b
4000	0.0	0.284 a	0.259 b	0.877 a	0.572 b	1.433 c	1.457 bc
© Effect of interaction between salinity and diatomite							
0.0	0.0	0.234 cd	0.248 d	0.617 cd	0.530 c	1.831 a	1.514 bc
0.0	2.5	0.284 bc	0.249 d	0.215 fg	0.079 e	1.215 e	1.420 c
0.0	5	0.253 cd	0.280 c	0.423 cdef	0.291 d	1.392 d	1.437 c
1000	0.0	0.223 d	0.333 ab	0.337 defg	0.374 d	1.880 a	1.677 ab
1000	2.5	0.231 cd	0.195 e	0.104 g	0.313 d	1.908 a	1.958 d
1000	5	0.373 a	0.249 d	0.496 cde	0.565 c	1.514 cd	1.540 abc
2000	0.0	0.249 cd	0.361 a	0.386 def	0.788 b	1.544 cd	1.507 bc
2000	2.5	0.244 cd	0.300 c	0.658 c	1.078 a	1.517 cd	1.590 abc
2000	5	0.223 cd	0.293 c	0.457 cdef	0.361 d	1.792 ab	1.685 ab
4000	0.0	0.335 ab	0.280 c	1.368 a	0.584 c	1.595 bc	1.692 a
4000	2.5	0.240 cd	0.249 d	0.252 efg	0.540 c	1.104 e	1.730 a
4000	5	0.277 bcd	0.224 de	1.011 b	0.591 c	1.601 bc	1.737 a

*Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test

Table (6) Effect of salinity, diatomite concentration and their interaction on (Rebaudioside A) contents on the leaves of *Stevia rebaudiana*Bertoni on the second harvest.

Treatments		Rebaudioside AConcentration (µg/10µg extract)	
Seawater concentration (ppm)	Diatomite concentrations(g /kg soil)	Season 2012	Season2013
0.0	0.0	3.2216	4.7174
0.0	2.5	3.1379	4.922
0.0	5	1.9662	1.7047
1000	0.0	1.7554	3.8672
1000	2.5	2.8122	4.0761
1000	5	2.8122	4.5956
2000	0.0	2.9086	2.4433
2000	2.5	2.4873	1.4188
2000	5	3.5736	5.1438
4000	0.0	2.1354	1.676
4000	2.5	2.8968	5.5719
4000	5	2.3621	2.6667