

(Considered Single - Shared with others outside the specialization –

Published in International Journal).

## **Salinity Effects on Gene Expression, Morphological, Additionally, Physio-Biochemical Responses of *Stevia rebaudiana* Bertoni In Vitro.**

Plants. 10, 820. <https://doi.org/10.3390/plants10040820>

Clara R. Azzam <sup>1</sup>, Sudad K. Al-Taweel <sup>2</sup>, Ranya M. Abdel-Aziz <sup>3</sup>, Karim M. Rabea <sup>4</sup>, Alaa I. B. Abou-Sreca <sup>5</sup>,

Mostafa M. Rady <sup>6,\*</sup> and Esmat F. Ali <sup>7</sup>

<sup>1</sup> Cell Research Department, Field Crops Research Institute, Agricultural Research Center, Giza 12619, Egypt;

<sup>2</sup> Medical and Aromatic Plants Research Unit, College of Agriculture Engineering Sciences, University of Baghdad, Baghdad, Iraq

<sup>3</sup> Sugar Crops Research Institute, Agriculture Research Center, Ministry of Agriculture, Giza 12619, Egypt;

<sup>4</sup> Horticulture and Landscape Design Department, College of Agriculture Engineering Sciences, University of Baghdad, Al-Jadiriya, Baghdad 10071, Iraq

<sup>5</sup> Department of Horticulture, Faculty of Agriculture, Fayoum University, Fayoum 63513, Egypt;

<sup>6</sup> Botany Department, Faculty of Agriculture, Fayoum University, Fayoum 63514, Egypt

<sup>7</sup> Department of Biology, College of Science, Taif University, Taif 21944, Saudi Arabia;

Article status	Considered Single - Shared with another outside the specialization – Published in International	Impact Factor: 4.658
----------------	---	----------------------

### **Abstract**

*Stevia rebaudiana* Bertoni is a little bush, which is cultivated on a large scale in many countries for medicinal purposes and used as a natural sweetener in food products. The present work aims to conduct a protocol for stevia propagation in vitro to produce and introduce *Stevia rebaudiana* plants as a new sweetener crop to Egyptian agriculture. To efficiently maximize its propagation, it is important to study the influence of stress factors on the growth and development of *Stevia rebaudiana* grown in vitro. Two stevia varieties were investigated (Sugar High A3 and Spanti) against salt stress. Leaves were used as the source of explants for callus initiation, regeneration, multiplication and rooting. Some stress-related traits, i.e., photosynthetic pigments, proline contents, and enzyme activity for peroxidase (POD), polyphenol oxidase (PPO), and malate dehydrogenase (MDH) were studied. Murashig and Skoog (MS) medium was supplemented with four NaCl concentrations: 500, 1000, 2000, and 3000mgL<sup>-1</sup>, while a salt-free medium was used as the control. The data revealed that salinity negatively affected all studied characters: the number of surviving calli, regeneration%, shoot length, the number of multiple shoots, number of leaf plantlets<sup>-1</sup>, number of root plantlets<sup>-1</sup>, and root length. The data also revealed that Sugar High A3 is more tolerant than Spanti. The total chlorophyll content decreased gradually with increasing NaCl concentration. However, the opposite was true for proline content. Isozyme's fractionation exhibited high levels of variability among the two varieties. Various biochemical parameters associated with salt tolerance were detected in POD. Namely, POD4, POD6, POD 9 at an *Rf* of 0.34, 0.57, and 0.91 in the Sugar High A3 variety under high salt concentration conditions, as well as POD 10 at an *Rf* of 0.98 in both varieties under high salt concentrations. In addition, the overexpression of POD 5 and POD 10 at *Rf* 0.52 and 0.83 was found in both varieties at high NaCl concentrations. Biochemical parameters associated with salt tolerance were detected in PPO (PPO1, PPO2

and PPO4 at an *Rf* of 0.38, 0.42 and 0.62 in the Sugar High A3 variety under high salt concentrations) and MDH (MDH 3 at an *Rf* of 0.40 in both varieties at high salt concentrations). Therefore, these could be considered as important biochemical markers associated with salt tolerance and could be applied in stevia breeding programs (marker-assisted selection). This investigation recommends stevia variety SugarHigh A3 to be cultivated under salt conditions.