بسم الله الرحمن الرحيم

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ملاحظات: لا يوجد

INDUCE GENETIC VARIABILITY AND SELECTION FOR SALT TOLERANCE IN *STEVIA* PLANT (*Stevia rebaudiana*, Bertoni)

By

DOAA OMER SAYED MOHAMED

B.Sc. Agric. Sci. (Plant Breeding and Propagation Technology), Fac. Agric., Cairo Univ., 2009

THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of

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In

Agricultural Sciences

(Agronomy)

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APPROVAL SHEET

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Title of Thesis: Induce Genetic Variability and Selection for Salt Tolerance in *Stevia* Plant (*Stevia rebaudiana*, Bertoni)

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ABSTRACT

Tissue culture technique became one of the most important tools in plant breeding and *in vitro* culture could be used to enhance selection process for salt stresses. Callus is important for rapid mass multiplication, generation of variability, cell suspension culture, preservation of cell line culture and production of secondary metabolites. Therefore, this study was carried out on *Stevia* plant for establishing efficient methods of formation and regeneration of callus as well as *in vitro* evaluation of salt stress effects on callus induction, regeneration and biochemical constituents. Three different explants (shoot tips, leaves and nodal segments) and MS medium with different combinations of plant growth regulators (PGRs) as well as five levels of salt stresses (0.0, 2000, 3000, 4000, 5000 ppm of NaCl) were studied. Results showed that all studied media induced callus for all explants, but MSc_4 (MS+1.0 mgl^{-1} 2,4-D +0.75 mgl^{-1} NAA) gave the highest values of callus fresh weight. Only, calli obtained from MSc_4 callus induction medium gave the best response to regenerate a sufficient number of shoots. Half strength MS medium with 1 mgl^{-1}IBA was found to be the optimum medium for root formation. Adding NaCl salt to the media significantly decreased fresh and dry weights of callus, but it significantly increased callus production of Stevioside, Rebaudioside A and total SGs (Steviol Glycosides). There was no regeneration with salt concentration of 4000 and 5000 ppm. Adding 2000 and 3000 ppm had no effect on the regeneration and multiplication process, but plantlet growth parameters in terms of shoots number, shoots length, nodes number, leaves number, leaves fresh weight, leaves dry weight, roots number, roots length and survival rate% were decreased significantly with raising NaCl level up to 3000 ppm. Increase of salinity level up to 3000 ppm significantly decreased chlorophyll a, b and total chlorophyll contents, but significantly increased proline contents. Genetic variability in induced callus under salt concentrations was studied through Peroxidase isozyme and Poly phenyl oxidase banding patterns and the results indicated a difference between the various saline stress factors.

Key words: *Stevia rebaudiana*, Explants, Plant growth regulators, Callus formation, Plant regeneration. Salt stresses, Stevioside, Isozymes electrophoresis.

DEDICATION

I dedicate this work to whom my heartfelt thanks: to my father (Rhamo Alha), mother, brothers, sister and my husband for all the support and encouragement they continually offered along the period of my post-graduation. Also, I dedicate this work to my precious daughter.

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LIST OF ABBREVIATIONS AND INITIALS

µM	Micro Moll
2, 4-D	2,4-dichlorophenoxy acetic acid
B_5	Gamborg, miller and ojima basal medium
BA, BAP	Benzyl adenine or N^6-Benzyl amino purine
DW	Dry weight
FW	Fresh weight
GA_3	Gibberellic acid
HPLC	High performance liquid chromatography
IAA	Indole acetic acid
IBA	Indole butyric acid
KN, Kin	Kinetin , N^6-furfuryladenine
mM	Milli Moll
MS	Murashige and skoog's basal medium
NAA	Naphthalene acetic acid
Native-PAGE	Native–polyacrylamide gel electrophoresis
PGRs	Plant growth regulators
PPO	Poly phenyl oxidase
Px	Peroxidase
R-A	Rebaudioside A
SGs	Steviol glycosides
ST	Stevioside