SOME PHYSIOLOGICAL STUDIES ON STEVIA (*Stevia rebaudiana*)

By

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B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., Egypt, 2001.
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APPROVAL SHEET

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Date: 27 / 8 / 2019

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Title of Thesis: Some physiological studies on Stevia (*Stevia rebaudiana*)

Supervisors: Prof. Dr. Mohamed K. Khalil,
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Department: Agricultural Botany **Branch:** Plant Physiology **Approval:** 27 / 8 / 2019

ABSTRACT

An investigation was consummated at the Agricultural Botany, Plant Physiology Section, Faculty of Agriculture, Cairo University. Plant tissue culture experiments were carried out in Plant Physiology Research Laboratory, Agric. Bot. Dept., Fac. Agric., Cairo Univ., Giza Egypt. Acclimatization experiment was carried out in the GreenHouse of Olive Dept., Horticultural Institute, Agric. Res. Cent., Giza, Egypt. Anatomical studies were carried out in Research Park, Fac. Agric., Cairo Univ. Giza Egypt.

The current project was conducted during 2014 - 2018 to study the effect of Flurprimidol (Flur.), Paclobutrazol (PBZ) and thidiazuron (TDZ) on plantlets growth and acclimatization of *Stevia rebaudiana* Spanti and China-1 cultiv. Stem nodal segments containing axillary buds were used as an explant and cultured on MS medium containing 3% (w/v) sucrose and 0.8% (w/v) agar supplemented with various concentrations of Flur., PBZ and TDZ. In Spanti cultivar maximum number of branches (6.4) and (6.52) were obtained in MS medium supplemented with 0.12 ppm Flur and 0.2 ppm TDZ, respectively with an average of 56.4 and 36.66 leaves / plantlet, having an average shoot length of 4.18 and 3.22 cm, respectively. The best *in vitro* root induction (100%) was achieved on MS medium with 0.16 ppm Flur. with an average of 10 roots /plantlet and root length of 4.82 cm (Spanti cultivar). Furthermore, in China-1 cultivar MS medium supplemented with 0.16ppm Flur.induced the best morphological characteristics. As a result of anatomy, all studied growth regulators significantly enhanced the anatomical characters of stevia vars. leaf and stem. Flur.at 0.16mg\l surpassed, for instance, midvein and lamina thickness, length and width of leaf vascular bundle as well as stem diameter, xylem and phloem thickness. The rooted plantlets from explant planted on (MS) medium supplemented with 0.16ppm Flur. were successfully established in soil and grown to maturity at the survival rate of 100% in the greenhouse in (Spanti) and (China-1) cultivar Pot experiment was conducted in greenhouse on stevia plant to study the effect of both NH_4NO_3 and KNO_3 under the treatments of BA, Kin, and TDZ. NH_4NO_3 excelled KNO_3 fertilization on all growth characters except plant height. BA treatment tended to increase total fresh weight/ plant as compared to those of Kin and TDZ in some cases.

Molecular studies showed that Spanti and China-1 vars. are closely related with some slight differences. The protein bands which present in the treatments and absent in control might be responsible of increase the total chlorophyll a, chlorophyll b and total carotenoid as expressions of hormone affects, whereas Flur. and TDZ produced more greener plantlets in both vars. compared to PBZ (Yellowish plant). TDZ treatments induced the most of unique bands which may be explain the greatest number of branches and the highest percentage of callus which produced by using TDZ. The results indicated that treatments were successful in inducing further characterized for morphological and chemical composition traits.

Leaf chemical composition of N, P, K and protein as well as chlorophyll% were determined during 2017 and 2018 seasons. K concentration was higher under potassium nitrate fertilization when compared to ammonium nitrate fertilization. Potassium nitrate addition resulted in slight increase in chlorophyll a & b and carotenoids over ammonium nitrate addition.

Key words: Asteraceae, *Stevia rebaudiana*, Stevia, Tissue culture, Flurprimidol, PBZ, TDZ, Acclimatization, Ammonium nitrate, Potassium nitrate, Electrophoresis.

DEDICATION

I dedicate this work to whom my heartfelt thanks; to my late father's soul, my mother, my sisters and my brother for all the support they lovely offered to me along the period of my post-graduat studies and preparation of thesis desertation.

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Thanks and all praise be to Allah. I thank God who expanded my heart easied for me dask.

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God bless them all

ABBREVIATIONS

BA	**: benzyl adenine**
Bp	**: Base pair**
Car.	**: Carotenoids**
Chl.	**: Chlorophyll**
Chl.a	**: Chlorophyll a**
Chl.b	**: Chlorophyll b**
DAS	**: Days After Sowing**
Flur.	**: Flurprimidol**
FW	**: Fresh weight**
HPLC	**: High Performance Liquid Chromatography**
ISSR	**: Inter-Simple Sequence Repeat**
KDa	**: Kilodalton**
Kin.	**: Kinetin**
KNO_3	**: Potassium nitrate**
L	**: liter**
mg	**: milligram**
Min	**: minute**
MS media	**: Murashige and Skoog**
NH_4NO_3	**: Ammonium nitrate**
PBZ.	**: Paclobutrazol**
PPO	**: Polyphenol oxidases**
PX	**: Peroxidase**
TEMED	**: Tetramethylethylenediamine**
TDZ	**: Thidiazuran**
TLC	**: Thin Layer Chromatography**
TRT	**: Treatment**

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