



شبكة المعلومات الجامعية  
التوثيق الإلكتروني والميكروفيلم

# بسم الله الرحمن الرحيم



**MONA MAGHRABY**



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# شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلم



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# جامعة عين شمس التوثيق الإلكتروني والميكروفيلم

## قسم

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# **Enhancement of broccoli's chemo-preventive agent sulforaphane production using plant tissue culture techniques**

*Presented By*

**May Ahmed Amer**

B.Sc. Botany, 2006. M.Sc. Genetics,  
Botany Department, Faculty of Science,  
Tanta University, 2013.

*A Thesis*

*Submitted in Partial Fulfillment of the Requirements for*

*Ph.D. Degree of Science*

*Genetics and Cytology*

*Botany Department, Faculty of Women For  
Arts, Science and Education, Ain Shams University*

**2021**



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## *Declaration*

This thesis has not been previously submitted for a degree or any other one and it is the original work of the author.

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## DEDICATION

*I would like to dedicate my thesis to my mother  
who could not see this thesis completed.*

*Without her endless love, encouragement and  
prays of day and night i would never have been  
able to complete my graduate studies. I love you  
until my last breath and i appreciate everything  
that you have done for me.*

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## Abstract

Broccoli (*Brassica oleracea* var. *italica*) belongs to the Brassicaceae family and is one of the most important cruciferous vegetables. It has gained popularity as it has distinct genotypes with high glucosinolate concentrations which have positive potential in cancer treatment. In this study, the effects of different concentrations of various elicitors; Zinc Sulfate ( $\text{ZnSO}_4$ ), Copper Sulfate ( $\text{CuSO}_4$ ), methyl jasmonate (MeJA) and salicylic acid (SA) on the production of sulforaphane in broccoli 7-days old seedlings and hairy roots has been studied. Real time PCR was used to quantify myrosinase gene expression associated with sulforaphane production. In addition, the anti-cancer activity of sulforaphane treatments on the breast cancer cell line MDA-MB-231 were also evaluated and the transcription levels of apoptosis associated genes were determined. The results showed that higher amounts of sulforaphane were produced in broccoli seedlings at 8  $\mu\text{M}$   $\text{ZnSO}_4$  and 16  $\mu\text{M}$   $\text{CuSO}_4$  after 8 hrs of elicitation and at 80  $\mu\text{M}$  SA and 40  $\mu\text{M}$  of MeJA after 24 hrs of elicitation. In treated hairy root cultures, sulforaphane scored the higher amounts at 16  $\mu\text{M}$   $\text{ZnSO}_4$  and 4  $\mu\text{M}$   $\text{CuSO}_4$  after 16 hrs of elicitation and 80  $\mu\text{M}$  SA and 40  $\mu\text{M}$  of MeJA after 24 hrs of elicitation. Increased production of sulforaphane was mostly associated with over-expression of the myrosinase (MY) gene. The results of the cytotoxicity showed that, sulforaphane extract obtained from broccoli seedlings treated with MeJA has higher inhibitory effect on the breast cancer cell line MDA-MB-231 than sulforaphane extract obtained from broccoli seedlings treated with other elicitors. The inhibitory effect increased by using purified sulforaphane. whereas sulforaphane extract obtained from broccoli hairy roots treated with  $\text{CuSO}_4$  showed higher inhibitory effect on the breast cancer cell line MDA-MB-231 than sulforaphane extract obtained from broccoli hairy roots treated with other elicitors. All sulforaphane treatments down-regulated the transcription of the antiapoptotic gene Bcl-2, while up-regulating the proapoptotic genes Bax, caspase-3, caspase-8 and caspase-9. In conclusion, increased sulforaphane production is possible with the used elicitors and an increase in its anti-cancer activity against the breast cancer cell line MDA-MB-231 is improved by its purifications in seedlings. Sulforaphane down-regulated the transcription of the antiapoptotic gene Bcl-2, while up-regulating the proapoptotic genes.

## **LIST OF ABBREVIATIONS**

<b>BAX</b>	: Bcl2 Associated X, Apoptosis Regulator
<b>BAP</b>	: Benzylaminopurine
<b>Bcl2</b>	: B-cell lymphoma 2
<b>B5</b>	: Gamborg B5 medium
<b>CuSO<sub>4</sub></b>	: Copper sulfate
<b>cDNA</b>	: Complementary DNA
<b>d. H<sub>2</sub>O</b>	: Distilled water
<b>dNTPs</b>	: Deoxyribonucleotide Triphosphates
<b>dw</b>	: Dry weight
<b>DMED</b>	: Dulbecco's Modified Eagle Medium
<b>DMSO</b>	: Dimethyl sulfoxide
<b>DNA</b>	: Deoxyribonucleic acid
<b>EDTA</b>	: Ethylene Diamine Tetraacetic Acid
<b>FBS</b>	: Fetal bovine serum
<b>g</b>	: Gram
<b>GAPDH</b>	: Glyceraldehyde 3-phosphate dehydrogenase
<b>H<sub>2</sub>O</b>	: Water
<b>HCl</b>	: Hydrochloric acid
<b>HPLC</b>	: High-pressure liquid chromatography
<b>hrs</b>	: Hours
<b>IC<sub>50</sub></b>	: The half maximal inhibitory concentration
<b>JA</b>	: Jasmonic acid
<b>KCl</b>	: Potassium chloride
<b>KH<sub>2</sub>PO<sub>4</sub></b>	: Potassium dihydrogen phosphate
<b>L</b>	: Liter
<b>LC-MS</b>	: Liquid Chromatography Mass Spectrometer
<b>LB</b>	: Luria-Bertani medium
<b>MDA-MB-231</b>	: M.D Anderson-Metastatic Breast Cancer
<b>MeJA</b>	: Methyl jasmonate
<b>mg/g</b>	: Milligram per gram
<b>mg/kg</b>	: Milligram per kilogram

<b>mg/l</b>	: Milligram per liter
<b>min</b>	: Minute
<b>ml</b>	: Milli liter
<b>μl</b>	: Micro liter
<b>ml/min</b>	: Milli per minute
<b>mM</b>	: Milli mol
<b>μm</b>	: Micro meter
<b>mRNA</b>	: Messenger RNA
<b>MS</b>	: Murashige and Skoog medium
<b>MY</b>	: Myrosinase
<b>NAA</b>	: Naphthaleneacetic acid
<b>NaCl</b>	: Sodium chloride
<b>NaOH</b>	: Sodium hydroxide
<b>Na<sub>2</sub>HPO<sub>4</sub></b>	: Di sodium hydrogen phosphate
<b>ng/g</b>	: Nanogram per gram
<b>nm</b>	: Nanometer
<b>qPCR</b>	: Quantitative polymerase chain reaction
<b>°C</b>	: Degree centigrade
<b>PCR</b>	: Polymerase chain reaction
<b>PBS</b>	: Phosphate-buffered saline
<b>ppm</b>	: Part per million
<b>RNA</b>	: Ribonucleic acid
<b>rpm</b>	: Revolutions per minute
<b>SA</b>	: Salicylic acid
<b>SH</b>	: Schenk and Hildebrandt medium
<b>V/V</b>	: Volume per volume
<b>W/V</b>	: Weight/volume
<b>ZnSO<sub>4</sub></b>	: Zinc sulfate
<b>%</b>	: Percent

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