



**UNIVERSITI PUTRA MALAYSIA**

***AGRONOMIC AND BIOCHEMICAL EXPRESSION OF ZINC,  
MANGANESE, AND PHOSPHORUS INTERACTION IN SWEET CORN  
PLANTS (ZEA MAYS L. VAR. SACCHARATA (STURTEV.) L. H. BAILEY)***

**AMIN SOLTANGHEISI**

**FP 2015 62**



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By

**AMIN SOLTANGHEISI**

**Thesis Submitted to the School of Graduate Studies,  
Universiti Putra Malaysia, in Fulfilment of the  
Requirement for the Degree of  
Doctor of Philosophy**

**February 2015**

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## DEDICATION

*I would like to dedicate this thesis to my beloved wife*

*Jasmine*

*for nursing me with affections and love*



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

**AGRONOMIC AND BIOCHEMICAL EXPRESSION OF ZINC, MANGANESE, AND PHOSPHORUS INTERACTIONS IN SWEET CORN PLANTS (*ZEA MAYS* L. VAR. SACCHARATA (STURTEV.) L. H. BAILEY)**

By

**AMIN SOLTANGHEISI**

**February 2015**

**Chairman: Professor Zaharah Abdul Rahman, PhD**

**Faculty: Agriculture**

Zinc and phosphorus have antagonistic effects on the absorption and translocation of each other in plants. Phosphorus-induced Zn deficiency is more common than Zn-induced P deficiency because growers commonly apply large amounts of P fertilizer as compared to Zn fertilizer. Manganese and Zn also interact with each other and this interaction can affect the yield of corn plants. This research was conducted to examine the effects of different levels of Zn, Mn, and P on the yield, Zn, Mn, and P concentrations and uptake, the ultrastructure of chloroplast, physiological characteristics, root growth parameters, and chlorophyll contents of sweet corn plants. Sweet corn was grown in nutrient culture containing all combinations of Zn as  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  at levels of 0.0, 5.0, 10.0, and 20.0  $\text{mg L}^{-1}$  and of P as  $\text{KH}_2\text{PO}_4$  at levels of 0.0, 20.0, 40.0, and 80.0  $\text{mg L}^{-1}$ . The treatment  $\text{Zn}_0\text{P}_{20}$  produced the highest yield and the yields decreased with P application in combination with Zn. The lowest dry weight of young corn plants was recorded under  $\text{Zn}_0\text{P}_{80}$  treatment at both harvesting times due to both Zn deficiency and P toxicity. Chlorophyll content decreased with high Zn and P applications and this can be attributed to the interactions of Zn and P with iron in the growth medium. The study has shown that Zn deficiency can enhance P uptake and translocation to such an extent that P may accumulate to toxic level in leaves. Sweet corn was grown in nutrient culture containing all combinations of P at levels of 0.0 and 80.0  $\text{mg L}^{-1}$  as  $\text{KH}_2\text{PO}_4$  and Zn at levels of 0.0 and 20.0  $\text{mg L}^{-1}$  as  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , and harvested at 14 and 28 days after transplanting. Phosphorus and Zn concentrations in leaves increased with increasing P and Zn concentration in nutrient solution. Zinc supply did not affect P concentration but Zn concentration reduced with increasing P supply in nutrient solution at both harvests. Carbonic anhydrase activity in leaves was enhanced with increasing Zn levels and decreased with increasing P levels at both harvest times. Carbonic anhydrase activity is a

better indicator of Zn nutritional status than Zn concentration alone. The ultrastructure of chloroplast was affected by P and Zn supply. Sweet corn was grown in nutrient culture containing all combinations of Zn and Mn at levels of 0.0, 0.1, 1.0, and 10.0 mg L<sup>-1</sup> as ZnSO<sub>4</sub>.7H<sub>2</sub>O and MnSO<sub>4</sub>.H<sub>2</sub>O, respectively and harvested at 28 days after transplanting. Manganese and Zn concentrations in roots and shoots increased with increasing Mn and Zn concentration in nutrient solution. Zinc concentration in both roots and shoots was enhanced with increasing Mn levels. Manganese concentration in shoots did not show any correlation with Zn concentration in nutrient solution but Mn concentration in roots decreased with increasing levels of Zn. The lowest dry weight of young corn plants was recorded under Zn<sub>10</sub>Mn<sub>0</sub> treatment due to Mn deficiency. Sweet corn grown in pot culture containing all combinations of Zn at levels of 0.0, 5.0, and 10.0 mg kg<sup>-1</sup> soil and P at levels of 0.0, 50.0, 100.0, and 200.0 mg kg<sup>-1</sup> soil as ZnSO<sub>4</sub>.7H<sub>2</sub>O and KH<sub>2</sub>PO<sub>4</sub>, respectively and harvested at 28 days after transplanting showed dry matter yield increased with P supply, while Zn application did not show any significant effect on this parameter. Zinc and P uptake by shoots increased with increasing Zn and P application to the soil. Zn concentration in shoots decreased with increasing P supply, but P concentration and uptake was enhanced. Phosphorus-induced Zn deficiency in this study is mostly related to the dilution effect. The percentage of P derived from fertilizer reduced with increasing Zn application, although P uptake by shoots was unchanged.

Abstrak tesis yang dikemukakan kepada Senati Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**EKSPRESI AGRONOMIK DAN BIOKIMIA TERHADAP INTERAKSI ZINK, MANGAN, DAN FOSFORUS DALAM TANAMAN JAGUNG MANIS (*ZEA MAYS L. VAR. SACCHARATA* (STURTEV.) L. H. BAILEY)**

Oleh

**AMIN SOLTANGHEISI**

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Zink dan fosforus mempunyai kesan bermusuhan ke atas penyerapan dan translokasi satu sama lain dalam tumbuhan. Kekurangan Zn disebabkan oleh berlebihan P adalah lebih biasa berlaku daripada kekurangan P yang disebabkan oleh berlebihan baja Zn. Ini adalah kerana penanam biasanya memberi jumlah baja P yang lebih tinggi berbanding dengan baja Zn. Mangan (Mn) dan zink (Zn) juga berinteraksi antara satu sama lain dan interaksi ini boleh memberi kesan pada hasil tanaman jagung. Kajian ini dijalankan untuk mengkaji kesan tahap berbeza unsur Zn, Mn, dan P pada hasil, kepekatan dan pengambilan Zn, Mn, dan P, ultrastruktur kloroplas, ciri-ciri fisiologi, parameter pertumbuhan akar, dan kandungan klorofil tanaman jagung manis. Jagung manis ditanam menggunakan larutan nutrien yang mengandungi semua kombinasi Zn diberi sebagai  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  pada tahap 0.0, 5.0, 10.0, dan 20.0  $\text{mg L}^{-1}$  dan P sebagai  $\text{KH}_2\text{PO}_4$  pada tahap 0.0, 20.0, 40.0, dan 80.0  $\text{mg L}^{-1}$ . Rawatan  $\text{Zn}_0\text{P}_{20}$  memberi hasil tertinggi dan hasil telah menurun dengan pemberian P ber kombinasi dengan Zn. Berat kering tanaman jagung muda yang paling rendah telah direkodkan di dalam rawatan  $\text{Zn}_0\text{P}_{80}$  pada kedua-dua penuaian disebabkan oleh kekurangan Zn dan ketoksikan P. Kandungan klorofil menurun dengan aplikasi Zn yang tinggi bersama P dan ini boleh dikaitkan dengan interaksi Zn dan P dengan Ferum dalam medium pertumbuhan. Kajian ini telah menunjukkan bahawa kekurangan Zn boleh meningkatkan pengambilan dan translokasi P, dimana P boleh terkumpul dalam daun ke tahap toksik. Jagung manis ditanam dalam larutan nutrien yang mengandungi semua kombinasi P pada tahap 0.0 dan 80.0  $\text{mg L}^{-1}$  diberi sebagai  $\text{KH}_2\text{PO}_4$  dan Zn pada tahap 0.0 dan 20.0  $\text{mg L}^{-1}$  diberi sebagai  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , dan dituai pada 14 dan 28 hari selepas pemindahan. Kepekatan P dan Zn dalam daun meningkat dengan peningkatan P dan Zn dalam larutan nutrien. Kepekatan Zn tidak mempengaruhi kandungan P tetapi kepekatan Zn berkurangan dengan

meningkatnya bekal P dalam larutan nutrien di kedua-dua tuaian. Aktiviti karbonik enhidrasa dalam daun telah dipertingkatkan dengan meningkatnya tahap Zn, dan menurun dengan peningkatan aras P di kedua-dua tuaian. Aktiviti karbonik enhidrasa adalah petunjuk yang lebih baik terhadap status pemakanan Zn daripada kepekatan Zn sahaja. Ultrastruktur kloroplas dipengaruhi oleh bekal P dan Zn. Jagung manis ditanam dalam larutan nutrien yang mengandungi semua kombinasi Zn dan Mn pada tahap 0.0, 0.1, 1.0, dan 10.0 mg L<sup>-1</sup> sebagai ZnSO<sub>4</sub>·7H<sub>2</sub>O dan MnSO<sub>4</sub>·H<sub>2</sub>O, dan dituai 28 hari selepas pemindahan menunjukkan Mn dan Zn dalam akar dan pucuk meningkat dengan meningkatnya kepekatan Mn dan Zn dalam larutan nutrien. Kepekatan Zn dalam akar dan pucuk dipertingkatkan dengan meningkatkan tahap Mn. Kepekatan Mn dalam pucuk tidak menunjukkan sebarang korelasi dengan kepekatan Zn dalam larutan nutrien tetapi kepekatan Mn dalam akar menurun dengan peningkatan aras Zn. Berat kering tanaman jagung muda paling rendah telah direkodkan di rawatan Zn<sub>10</sub>Mn<sub>0</sub> disebabkan oleh kekurangan Mn. Jagung manis yang ditanam dalam kajian berpasu yang diberi semua kombinasi Zn pada tahap 0.0, 5.0, dan 10.0 mg kg<sup>-1</sup> tanah dan P pada tahap 0.0, 50.0, 100.0, 200.0 mg kg<sup>-1</sup> tanah sebagai ZnSO<sub>4</sub>·7H<sub>2</sub>O dan KH<sub>2</sub>PO<sub>4</sub>, dan dituai 28 hari selepas pemindahan mendapati hasil bahan kering meningkat dengan bekal P, manakala pemberian Zn tidak menunjukkan apa-apa kesan yang ketara ke atas parameter ini. Pengambilan Zn dan P dalam pucuk meningkat dengan meningkatnya pemberian Zn dan P kepada tanah. Kepekatan Zn dalam pucuk menurun dengan peningkatan bekal P, tetapi kepekatan dan pengambilan P dipertingkatkan. Kekurangan Zn disebabkan oleh kadar P tinggi dalam kajian ini disebabkan oleh kesan pencairan. Peratusan P yang diserap daripada baja dikurangkan dengan peningkatan kadar Zn diberi, walaupun pengambilan P dalam pucuk tidak berubah.



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I certify that a Thesis Examination Committee has met on 9 February 2015 to conduct the final examination of Amin Soltangheisi on his thesis entitled "Agronomic and Biochemical Expression of Zinc, Manganese and Phosphorus Interactions in Sweet Corn Plants (*Zea Mays* L. var. *Saccharata* (sturtev.) L. H. Bailey" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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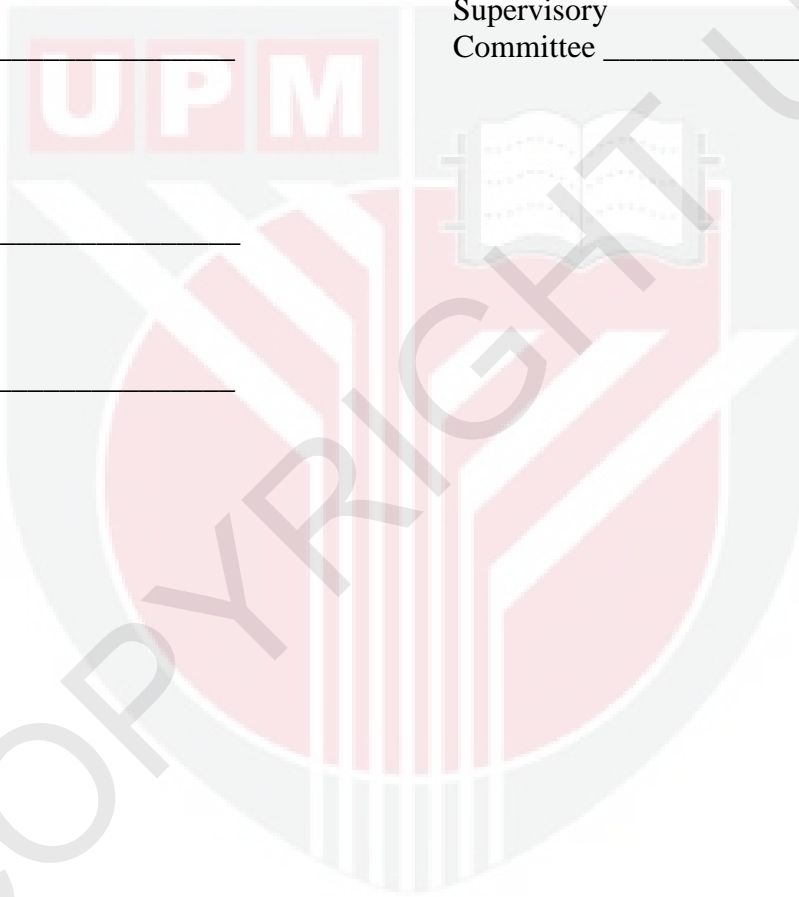
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## TABLE OF CONTENTS

		<b>Page</b>
	<b>ABSTRACT</b>	i
	<b>ABSTRAKT</b>	iii
	<b>ACKNOWLEDGMENTS</b>	v
	<b>APPROVAL</b>	vi
	<b>DECLARATION</b>	viii
	<b>LIST OF TABLES</b>	xiii
	<b>LIST OF FIGURES</b>	xv
	<b>LIST OF ABBREVIATIONS</b>	xviii
	<b>CHAPTER</b>	
1	<b>INTRODUCTION</b>	1
	1.1 General introduction	1
	1.2 Justification	2
	1.3 Objectives	2
2	<b>LITERATURE REVIEW</b>	4
	2.1 Zinc	4
	2.1.1 Zinc cycle in soil-plant system	4
	2.1.2 Physiological function of zinc in plants	5
	2.1.3 Zinc deficiency in soils and plants	16
	2.2 Phosphorus	22
	2.2.1 Phosphorus cycle in soil-plant system	22
	2.2.2 Physiological function of phosphorus in plants	27
	2.2.3 Phosphorus deficiency in soils and plants	31
	2.3 Manganese	33
	2.3.1 Manganese cycle in soil-plant system	33
	2.3.2 Physiological function of manganese in plants	34
	2.3.3 Manganese toxicity and deficiency in soils and plants	39
3	<b>GENERAL MATERIALS AND METHODS</b>	41
4	<b>PHOSPHORUS, ZINC AND MANGANESE UPTAKE AND THEIR INTERACTION EFFECT ON DRY MATTER AND CHLOROPHYLL CONTENT OF SWEET CORN (<i>ZEA MAYS</i> VAR. <i>SACCHARATA</i>)</b>	44
	4.1 Introduction	44
	4.2 Materials and methods	46
	4.2.1 Experimental design	46

	4.2.2	Measurements	46
	4.2.3	Calculations and statistical analysis	47
4.3		Results and discussion	47
4.4		Conclusion	56
<b>5</b>		<b>EFFECT OF ZINC AND PHOSPHORUS SUPPLY ON THE ACTIVITY OF CARBONIC ANHYDRASE AND THE ULTRASTRUCTURE OF CHLOROPLAST IN SWEET CORN (<i>ZEA MAYS VAR. SACCHARATA</i>)</b>	<b>57</b>
	5.1	Introduction	57
	5.2	Materials and methods	58
	5.2.1	Experimental design	58
	5.2.2	Measurements	58
	5.2.3	Calculations and statistical analysis	60
	5.3	Results and discussion	60
	5.4	Conclusion	69
<b>6</b>		<b>INTERACTION EFFECTS OF ZINC, MANGANESE AND IRON ON GROWTH, UPTAKE RESPONSE, AND CHLOROPHYLL CONTENT OF SWEET CORN (<i>ZEA MAYS VAR. SACCHARATA</i>)</b>	<b>68</b>
	6.1	Introduction	70
	6.2	Materials and methods	71
	6.2.1	Experimental design	71
	6.2.2	Measurements	72
	6.2.3	Calculations and statistical analysis	72
	6.3	Results and discussion	72
	6.4	Conclusion	86
<b>7</b>		<b>INTERACTION EFFECTS OF PHOSPHORUS AND ZINC ON THEIR UPTAKE AND <sup>32</sup>P ABSORPTION AND TRANSLOCATION IN SWEET CORN (<i>ZEA MAYS VAR. SACCHARATA</i>)</b>	<b>87</b>
	7.1	Introduction	87
	7.2	Materials and methods	88
	7.2.1	Experimental design	88
	7.2.2	Measurements	89
	7.2.3	Calculations and statistical analysis	89
	7.3	Results and discussion	90
	7.4	Conclusion	100
<b>8</b>		<b>SUMMARY/ GENERAL CONCLUSION/ FUTURE RECOMMENDATION</b>	<b>101</b>
		<b>REFERENCES</b>	<b>103</b>
		<b>APPENDIX</b>	<b>134</b>
		<b>BIODATA OF STUDENT</b>	<b>141</b>





## LIST OF TABLES

Table		Page
4.1	Dry weight ( $\text{mg plant}^{-1}$ ) and total chlorophyll content ( $\text{mg g}^{-1}$ fresh weight) in sweet corn plants in nutrient solution with different P and Zn levels at 7 and 14 DAT	49
4.2	Leaf P/Zn concentration ratio and root/shoot Zn uptake ratio in sweet corn plants in nutrient solution with different P and Zn levels at 7 and 14 DAT	50
4.3	Zn uptake ( $\mu\text{g plant}^{-1}$ ) and P uptake ( $\mu\text{g plant}^{-1}$ ) by shoots in sweet corn plants in nutrient solution with different P and Zn levels at 7 and 14 DAT	51
4.4	Zn uptake ( $\mu\text{g plant}^{-1}$ ) and P uptake ( $\mu\text{g plant}^{-1}$ ) by roots in sweet corn plants in nutrient solution with different P and Zn levels at 7 and 14 DAT	52
5.1	Phosphorus (%) and zinc ( $\mu\text{g g}^{-1}$ ) concentration and P/Zn ratio in leaves of sweet corn plants in nutrient solution with different Zn and P levels	62
5.2	Carbonic anhydrase activity ( $\text{EU g}^{-1}$ fresh tissue) and total chlorophyll content ( $\text{mg g}^{-1}$ fresh tissue) in leaves and average root diameter (mm) of sweet corn plants in nutrient solution with different Zn and P levels	64
6.1	Dry weight ( $\text{mg plant}^{-1}$ ) and total chlorophyll content ( $\text{mg g}^{-1}$ fresh weight) of sweet corn plants in nutrient solution with different Zn and Mn levels	76
6.2	Mn and Zn concentration ( $\mu\text{g g}^{-1}$ ) in leaves and roots, Mn/Zn ratio in leaves, root/shoot Mn uptake ratio, and root/shoot Zn uptake ratio of sweet corn plants in nutrient solution with different Zn and Mn levels	80

6.3	Fe concentration ( $\mu\text{g g}^{-1}$ ) in leaves and roots of sweet corn plants in nutrient solution with different Zn and Mn levels	84
7.1	Selected physic-chemical properties of the soil	90
7.2	Dry weight ( $\text{mg plant}^{-1}$ ), chlorophyll a/b ratio, P (%) and Zn ( $\mu\text{g g}^{-1}$ ) concentration in leaves, and P ( $\text{mg plant}^{-1}$ ) and Zn ( $\mu\text{g plant}^{-1}$ ) uptake of sweet corn plants in soil with different P and Zn levels	93



## LIST OF FIGURES

Figure		Page
2.1	Relationships between yield and nutrient concentration in plant tissue. (a) generalized relationship frequently found in plants as nutrient supply increases from deficient to toxic; (b) the C-shaped or Piper-Steenbjerg effect	9
2.2	Relationship between plant growth and nutrient concentration in the plant for diagnosis or predicting nutrient deficiency and toxicity	19
2.3	The phosphorus cycle in soils	24
2.4	The cycle of the oxidation states of manganese found in nature	35
4.1	The effect of Zn supply on Mn concentration in shoots of sweet corn plants	53
4.2	Relationship between Zn supplies and Mn concentration in roots of sweet corn plants at (a) 7 days and (b) 14 days after transplanting	54
4.3	Effect of P and Zn supplies on Mn concentration in roots of sweet corn plants at (a) 7 days and (b) 14 days after transplanting	55
5.1	Chloroplast from full-nutrient plant	66
5.2	Chloroplast from $Zn_0P_{80}$ treatment	66
5.3	Chloroplast from $Zn_{20}P_0$ treatment	67
5.4	Chloroplast from $Zn_{20}P_{80}$ treatment	68

5.5	Chloroplast from Zn <sub>0</sub> P <sub>0</sub> treatment	69
6.1	Relationship between Mn supplies and Mn concentration in (a) roots and (b) shoots of sweet corn plants	73
6.2	Relationship between Zn supplies and Zn concentration in (a) roots and (b) shoots of sweet corn plants	74
6.3	Relationship between Mn supplies and Zn concentration in (a) roots and (b) shoots of sweet corn plants	75
6.4	Relationship between Zn supplies and Mn concentration in (a) roots and (b) shoots of sweet corn plants	77
6.5	Relationship between Zn supplies and root/shoot (a) Mn uptake ratio and (b) Zn uptake ratio of sweet corn plants	79
6.6	Relationship between Mn supplies and Fe concentration in shoots of sweet corn plants	81
6.7	Relationship between Zn supplies and Fe concentration in roots of sweet corn plants	82
7.1	Relationship between P supplies and dry weight in shoots of sweet corn plants	91
7.2	Relationship between P supplies and (a) Zn uptake and (b) Zn concentration in shoots of sweet corn plants	94
7.3	Relationship between P supplies and P uptake by shoots of sweet corn plants	95
7.4	Relationship between Zn supplies and (a) Zn uptake and (b) Zn concentration in shoots of sweet corn plants	96

7.5	Relationship between P supplies and chlorophyll a/b ratio of sweet corn plants	97
7.6	Relationship between P supplies and %Pdff in shoots of sweet corn plants	98
7.7	Relationship between Zn supplies and %Pdff in shoots of sweet corn plants	99
7.8	Foliar symptoms of Zn deficiency	100



## LIST OF ABBREVIATIONS

°C	Degree Celsius
%	Percent
ALA	Aminulevulinic Acid
ATA	Ammonia Tri-acetic Acid
ATP	Adenosine Triphosphate
CA	Carbonic Anhydrase
CEC	Cation Exchange Capacity
CIPR	Christmas Island Phosphate Rock
DAS	Days After Sowing
DAT	Days After Transplanting
DNA	Deoxyribonucleic Acid
DRIS	Diagnostic and Recommendation Integrated System
DTPA	Diethylene Triamine Pentaacetic Acid
e.g.	example gratia
EDTA	Ethylene Diamine Tetra-acetic Acid
EU	Enzyme Unit
fw	fresh weight
g	gram
h	hour
ICP	Inductively Coupled Plasma
kBq	kilobecquerel
kDa	kilodalton
kg	kilogram
L	Liter
M	Molar
mg	milligram
mL	milliliter
mm	millimeter
mM	millimolar
mRNA	Messenger Ribonucleic Acid

nm	nanometer
OES	Optical Emission Spectrometry
OM	Organic Matter
Pdff	Phosphorus Derived From Fertilizer
PR	Phosphate Rock
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
SA	Specific Activity
SOD	Superoxide Dismutase
SOM	Soil Organic Matter
TEM	Transmission Electron Microscopy
UPM	Universiti Putra Malaysia
YML	Youngest Mature Leaf
μg	microgram
μM	micromolar

## ABBREVIATIONS OF CHEMICAL MATERIALS

$\text{CaCl}_2$	Calcium chloride
$\text{Ca}(\text{NO}_3)_2$	Calcium nitrate
$\text{CO}_2$	Carbon dioxide
$\text{CuSO}_4$	Copper(II) sulfate
DMSO	Dimethyl Sulfoxide
EDTAF <sub>e</sub>	Iron(III) Ethylene Diamine Tetra-acetic Acid
$\text{FePO}_4$	Iron(III) phosphate
$\text{H}_3\text{BO}_3$	Boric acid
HCl	Hydrogen chloride
$\text{HNO}_3$	Nitric acid
$\text{K}_2\text{SO}_4$	Potassium sulfate
$\text{KH}_2\text{PO}_4$	Monopotassium phosphate
KCl	Potassium chloride
KOH	Potassium hydroxide
$\text{MgCl}_2$	Magnesium chloride
$\text{MgSO}_4$	Magnesium sulfate
$\text{MnO}_2$	Manganese dioxide
$\text{MnSO}_4$	Manganese(II) sulfate
$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$	Ammonium heptamolybdate
$\text{P}_2\text{O}_5$	Phosphorus pentoxide
$\text{Zn}_3(\text{PO}_4)_2$	Zinc phosphate
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	Zinc sulfate heptahydrate



## ABBREVIATIONS OF STATISTICAL ANALYSIS

P	Probability
SAS	Statistical Analysis Software



## CHAPTER 1

### INTRODUCTION

#### 1.1. General introduction

Macro and micronutrients are required for normal growth, health, and reproduction of plants. Among the macronutrients, nitrogen, phosphorus, and potassium are consumed in large quantities. Micronutrients are needed in small quantities but they play an important role in plant's growth and health. Two of these essential micronutrients are zinc and manganese.

Practitioners are always searching for ways to achieve the optimum level of nutrition for horticultural crops but finding that is complicated because at least 12 essential nutrients are involved. Many factors influence the availability and uptake of these nutrients including environment, genetic differences, and nutrients interactions (May and Pritts, 1993).

Balanced supply of essential nutrients is one of the most important factors in increasing crop yields. In crop plants, the nutrient interactions are generally measured in terms of growth response and change in concentration of nutrients. Upon addition of two nutrients, an increase in crop yield that is more than adding only one, shows a positive interaction (synergistic). Similarly, if adding the two nutrients together produced less yield as compared to individual ones, the interactions are negative (antagonistic). When there is no change, there is no interaction. All the three interactions among essential plant nutrients have been reported. However, most interactions are complex. A nutrient has interaction simultaneously with more than one nutrient. This may induce deficiencies, toxicities, modified growth responses, and/or modified nutrient composition (Fageria, 2001).

Interaction between nutrients in crop plants occurs when the supply of one nutrient affects the absorption and utilization of other nutrients. This type of interaction is most common when one nutrient is in excess concentration in the growth medium. Nutrient interactions can occur at the root surface or within the plant and can be classified into two major categories. In the first category are interactions which occur between ions because the ions are able to form a chemical bond. Interactions in this case are due to the formation of precipitates or complexes. For example, this type of interaction occurs where the liming of acid soils decrease the concentration of almost all micronutrients except molybdenum. But this decrease varies from nutrient to nutrient. For example, Cu is more strongly complexed by soluble organic matter than Zn, and effects of increasing soil pH are more marked on Zn uptake than Cu uptake by plants. The second form of interaction is between ions whose chemical properties are sufficiently similar that they compete for site of adsorption, absorption, transport, and function on plant root surfaces or within plant tissues. Such interactions are more common between nutrients of similar

size, charge, geometry of coordination, and electronic configuration. This type of interaction is common among  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ , and  $\text{Na}^+$  (Fageria, 2001).

Hiatt and Leggett (1974) suggested that cation-cation and anion-anion interactions occur mostly at the membrane level and are primarily of a competitive nature. Cation-anion interactions occur at both the membrane and in cellular processes after absorption. These cellular interactions are less understood. Epstein (1972) pointed out that the cation content of plant material is dependent on both the availability of the particular cation and the presence or absence of other cations in the growth medium. Generally an excess of one cation in the nutrient medium reduces the net uptake of other cations, whereas the sum of cations in the plant tissue often remains nearly constant. This phenomenon is called cation antagonism.

## 1.2. Justification

While Malaysian farmers apply N, P and K fertilizers widely, it is found that the application of micronutrients, such as Zn is not a usual practice (Liew, 2010). Zinc deficiency is found in crops due to intensive cropping, loss of fertile topsoil and losses of nutrients through leaching. Low levels of trace elements are expected in the oldest landscapes in zones of high rainfall and temperature, and where trace element concentrations in parent materials are originally low thus Malaysian soils are Zn deficient (White and Zasoski, 1999). The presence of Zn deficiency renders it impossible for the plant to gain maximum benefit from NPK fertilizer applications. Zn has been categorized as essential and plants cannot complete their life cycle in the absence of Zn (Marschner, 1996). The application of solely NPK fertilizers is no longer practical due to the continuous removal of micronutrients after harvesting, as well as losses due to leaching or surface runoffs. As Zn and P can act antagonistically with one another, the amount of P application is important because excessive P can cause Zn deficiency in plant tissue. Information on the photosynthetic response of corn plants to Zn deficiency or to P-induced Zn deficiency is absent and the mechanism which Zn and P interaction affects chlorophyll content is unknown. The response of roots to Zn and P interaction is also unknown. The best amount of P and Zn fertilizer in corn production is also investigated in this research. Manganese is one of the most important elements in Malaysian soils. The interaction of different levels of Mn and Zn are also investigated because Mn can directly affect the Zn concentration in plants and Malaysian soils have variable amount of Mn in soils from place to place.

## 1.3. Objectives

Four major experiments were carried out for this thesis with the overall objectives of:

- 1) To investigate the effects of Zn-P interaction on the chlorophyll content, nutrients uptake, chloroplast structure, carbonic anhydrase activity, and root characteristics at different vegetative growth stages of sweet corn plants.

- 2) To investigate the mechanism of P-induced Zn deficiency in sweet corn plants.
- 3) To evaluate the effect of P-Zn interaction on dry matter yield and  $^{32}\text{P}$  absorption of sweet corn plants in acid soils.
- 4) To investigate the effects of Zn-Mn interaction on the chlorophyll content, nutrients uptake, and root characteristics at different vegetative stages in sweet corn plants.



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