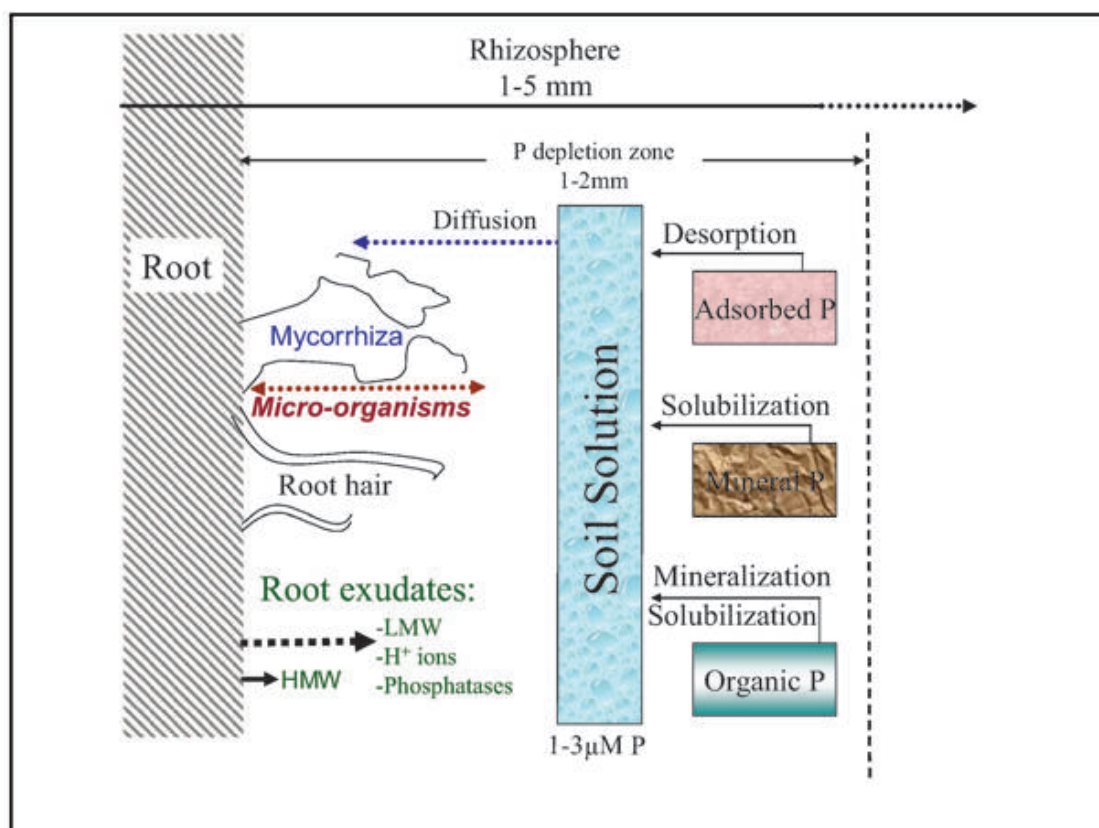


Identification by HPLC-MS of new detected compounds in sugar beet root exudates for soil P mobilization



Reza Khorassani

Identification by HPLC-MS of new detected compounds in sugar beet root exudates for soil P mobilization

Dissertation
To obtain the Ph.D. degree
In the Department of Crop Science and Plant Nutrition,
Faculty of Agriculture Sciences,
Georg-August-University Göttingen, Germany

Submitted by
Reza Khorassani
Born in Bojnourd, Iran

Göttingen, May 2008

Bibliografische Information der Deutschen Nationalbibliothek

Die Deutsche Nationalbibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet über <http://dnb.ddb.de> abrufbar.

1. Aufl. - Göttingen : Cuvillier, 2008
Zugl.: Göttingen, Univ., Diss., 2008

978-3-86727-611-5

Name of referee: Prof. Dr. Norbert Claassen

Name of co-referee: Prof. Dr. Rolf Rauber

Date of Dissertation: May 2008

© CUVILLIER VERLAG, Göttingen 2008

Nonnenstieg 8, 37075 Göttingen

Telefon: 0551-54724-0

Telefax: 0551-54724-21

www.cuvillier.de

Alle Rechte vorbehalten. Ohne ausdrückliche Genehmigung des Verlages ist es nicht gestattet, das Buch oder Teile daraus auf fotomechanischem Weg (Fotokopie, Mikrokopie) zu vervielfältigen.

1. Auflage, 2008

Gedruckt auf säurefreiem Papier

978-3-86727-611-5

Dedicated to my wife and my daughter

Table of contents

TABLE OF CONTENTS	I
LIST OF TABLES	III
LIST OF FIGURES	IV
1. CHAPTER 1: GENERAL INTRODUCTION	1
1.1 PHOSPHOROUS.....	2
1.2 PHOSPHORUS AVAILABILITY IN SOIL.....	2
1.2.1 <i>Phosphorus chemical behaviors in soil</i>	3
1.2.2 <i>Phosphorus transport in soil</i>	6
1.3 PHOSPHORUS ACQUISITION BY PLANT	9
1.4 RHIZOSPHERE AND ROOT EXUDATES.....	12
1.5 ROOT EXUDATES AND P MOBILIZATION IN SOIL	15
1.6 HPLC-MS TECHNIQUE TO ANALYZE ROOT EXUDATES.....	19
1.7 OBJECTIVES OF STUDY AND OUTLINE OF THE THESIS	23
2 CHAPTER 2: PHOSPHORUS UPTAKE EFFICIENCY OF CROPS AS EFFECTED BY P INFLUX AND A MECHANISTIC MODEL EVALUATION TO ESTIMATE P CHEMICAL MOBILIZATION.....	25
2.1 INTRODUCTION.....	26
2.2 MATERIALS AND METHODS	28
2.2.1 <i>Pot Experiment</i>	28
2.2.2 <i>Soil and Plant analysis</i>	29
2.2.2.1 Soil solution P concentration.....	29
2.2.2.2 Available P by the CAL-P method.....	29
2.2.2.3 Phosphorus concentration in shoot, roots and seeds	30
2.2.3 <i>Root length</i>	30
2.2.4 <i>Calculation and model parameters</i>	31
2.2.4.1 Phosphorus influx and relative shoot growth rate	31
2.2.4.2 Model parameters	31
2.2.5 <i>Statistics</i>	33
2.3 RESULTS.....	34
2.3.1 <i>CAL- P and soil solution P</i>	34
2.3.2 <i>Shoot dry matter</i>	36
2.3.3 <i>Relative shoot dry matter yield</i>	37
2.3.4 <i>Relative growth rate (RGR)</i>	40
2.3.5 <i>Shoot P concentration</i>	41
2.3.6 <i>Phosphorus uptake</i>	42
2.3.7 <i>Root system</i>	44
2.3.7.1 Root length	44
2.3.7.2 Root-shoot ratio	45
2.3.8 <i>Phosphorus influx</i>	46

2.3.9	<i>Modeling of P influx</i>	48
2.4	DISCUSSION	58
2.4.1	<i>Phosphorus uptake efficiency</i>	59
2.4.2	<i>Phosphorous influx and model calculation</i>	61
3	CHAPTER 3: RESEARCH ARTICLE	67
	IDENTIFICATION OF SOIL P MOBILIZING COMPONENTS OF SUGAR BEET ROOT EXUDATES AFTER DIFFERENTIAL METABOLIC PROFILING BY HPLC-MS	67
3.1	ABSTRACT	68
3.2	INTRODUCTION.....	69
3.3	MATERIALS AND METHODS	72
3.3.1	<i>Hydroponic experiment</i>	72
3.3.2	<i>Root exudates collection</i>	72
3.3.3	<i>Preparation of samples and standards for HPLC-MS analysis</i>	73
3.3.4	<i>HPLC-MS instruments and analysis</i>	73
3.3.4.1	<i>Metabolic profiling of root exudates</i>	73
	<i>Targeted analysis and identity verification for selected metabolites</i>	74
3.3.4.2	<i>HPLC- MS data analysis</i>	75
3.3.5	<i>P solubility experiment</i>	75
3.4	RESULTS AND DISCUSSION.....	77
3.4.1	<i>Shoot dry weight and shoot P concentration</i>	77
3.4.2	<i>Exudation rate</i>	78
3.4.3	<i>HPLC-MS analysis of root exudates</i>	79
3.4.4	<i>Phosphorus Mobilization in soil</i>	80
3.4.5	<i>Identification of salicylic and citramalic acids in root exudates</i>	81
3.5	REFERENCES.....	85
4	CONCLUDING DISCUSSION	88
5	SUMMARY	92
6	REFERENCES OF CHAPTERS 1 AND 3	94
7	ACKNOWLEDGEMENTS	103
8	CURRICULUM VITA	104

List of Tables

TABLE 1: CAL-P OF UNPLANTED SOIL AND PLANTED FOR MAIZE, SUGAR BEET AND GROUNDNUT AT THREE LEVELS OF P FERTILIZATION AT THE FIRST HARVEST AND THIRD HARVEST	34
TABLE 2: SOIL SOLUTION P CONCENTRATION OF UNPLANTED SOIL AND PLANTED TO MAIZE, SUGAR BEET AND GROUNDNUT AT THREE LEVELS OF P FERTILIZATION AT THE FIRST HARVEST AND THIRD HARVEST	35
TABLE 3: SHOOT DRY MATTER OF MAIZE, SUGAR BEET AND GROUNDNUT AT THREE LEVELS OF FERTILIZATION AND THREE HARVESTS	36
TABLE 4: RELATIVE GROWTH RATE (RGR) OF MAIZE, SUGAR BEET AND GROUNDNUT AT THREE LEVELS OF FERTILIZATION AND THREE HARVESTS.	40
TABLE 5: P CONCENTRATION OF MAIZE, SUGAR BEET AND GROUNDNUT AT THREE LEVELS OF FERTILIZATION AND THREE HARVESTS	42
TABLE 6: PHOSPHORUS UPTAKE OF MAIZE, SUGAR BEET AND GROUNDNUT AT THREE LEVELS OF FERTILIZATION AND THREE HARVESTS.	43
TABLE 7: ROOT LENGTH OF MAIZE, SUGAR BEET AND GROUNDNUT AT THREE LEVELS OF FERTILIZATION AND THREE HARVESTS.....	44
TABLE 8: ROOT-SHOOT RATIO OF MAIZE, SUGAR BEET AND GROUNDNUT AT THREE LEVELS OF FERTILIZATION AND THREE HARVESTS	46
TABLE 9: MODEL PARAMETERS OF MAIZE, SUGAR BEET AND GROUNDNUT IN TWO GROWING PERIODS.	48
TABLE 10: ROOT HAIRS DATA FOR MAIZE GROUNDNUT AND SUGAR BEET	49
TABLE 11: MEASURED AND CALCULATED P INFLUX OF THE ROOT CYLINDERS ALONE (RC) OR ROOT CYLINDER WITH ROOT HAIRS (RC+RH) AND THE CONTRIBUTION OF ROOT HAIRS TO THE CALCULATED P INFLUX (AS GIVEN BY THE OUT PUT OF THE MODEL CALCULATION) AND RATIO OF CALCULATED (C) TO MEASURED (M) INFLUX GIVEN THE PROPORTION OF MEASURED INFLUX EXPLAINED BY THE PROCESSES AND FACTORS INCLUDED IN THE MODEL.	50
TABLE 12: NUMBER OF HPLC-MS SIGNALS ENHANCED UNDER LOW P CONDITIONS.	79

List of Figures

FIGURE 1: DIAGRAM OF PROCESSES AFFECTING P AVAILABILITY IN THE RHIZOSPHERE...	4
FIGURE 2: A SCHEMATIC REPRESENTATION OF TWO DIFFERENT WAYS TO INCREASE P CONCENTRATION GRADIENT TOWARD THE ROOT.	9
FIGURE 3: BLOCK DIAGRAM OF THE HPLC COMPONENT	21
FIGURE 4: BLOCK DIAGRAM OF MASS SPECTROMETER	23
FIGURE 5: RELATIVE YIELD OF MAIZE, SUGAR BEET AND GROUNDNUT AT THREE LEVELS OF FERTILIZATION AND THREE HARVESTS.	37
FIGURE 6: PHOSPHORUS CONTENT IN SEEDS AND WHOLE PLANTS FOR SUGAR BEET (SB), MAIZE (M) AND GROUNDNUT (G) AT THE FIRST HARVEST	38
FIGURE 7: PHOSPHORUS INFLUX OF MAIZE (M), SUGAR BEET (SB) AND GROUNDNUT (G) AT THREE LEVELS OF P AND AT TWO PERIODS.	47
FIGURE 8: SENSITIVITY ANALYSIS OF I_{MAX} (MAXIMUM NET INFLUX), K_M (MICHAELIS CONSTANT), C_{LMIN} (MINIMUM CONCENTRATION WHERE NET INFLUX EQUAL TO ZERO), F (IMPEDANCE FACTOR), B (SOIL BUFFER POWER AND C_{LI} (INITIAL CONCENTRATION OF P IN SOIL SOLUTION) IN SUGAR BEET AT P30.	51
FIGURE 9: SENSITIVITY ANALYSIS OF I_{MAX} (MAXIMUM NET INFLUX), K_M (MICHAELIS CONSTANT), C_{LMIN} (MINIMUM CONCENTRATION WHERE NET INFLUX EQUAL TO ZERO), F (IMPEDANCE FACTOR), B (SOIL BUFFER POWER), AND C_{LI} (INITIAL CONCENTRATION OF P IN SOIL SOLUTION) IN MAIZE AT P30.	52
FIGURE 10: SENSITIVITY ANALYSIS OF I_{MAX} (MAXIMUM NET INFLUX), K_M (MICHAELIS CONSTANT), C_{LMIN} (MINIMUM CONCENTRATION WHERE NET INFLUX EQUAL TO ZERO), F (IMPEDANCE FACTOR), B (SOIL BUFFER POWER), AND C_{LI} (INITIAL CONCENTRATION OF P IN SOIL SOLUTION) IN GROUNDNUT AT P30.	52
FIGURE 11: CALCULATED P CONCENTRATION PROFILES IN THE RHIZOSPHERE OF SUGAR BEET, MAIZE AND GROUNDNUT AT P30 AFTER 14 DAYS OF UPTAKE	54
FIGURE 12: CALCULATED P CONCENTRATION PROFILES WITHOUT ROOT HAIR IN THE RHIZOSPHERE OF SUGAR BEET AT P30 AFTER 14 DAYS OF UPTAKE	55
FIGURE 13: CALCULATED P CONCENTRATION PROFILES WITHOUT ROOT HAIR IN THE RHIZOSPHERE OF MAIZE AT P30 AFTER 14 DAYS OF UPTAKE	55
FIGURE 14: CALCULATED P CONCENTRATION PROFILES IN THE RHIZOSPHERE IN DIFFERENT I_{MAX} OF MAIZE AT P30 AFTER 14 DAYS OF UPTAKE	56
FIGURE 15: CALCULATED P CONCENTRATION PROFILES AT RHIZOSPHERE OF SUGAR BEET IN DIFFERENT TIME AT P30 IN THE SECOND GROWTH PERIOD INDICATING ROOT COMPETITION	57
FIGURE 16: SHOOT DRY MATTER AND P CONCENTRATION OF SUGAR BEET GROWN IN NUTRIENT SOLUTION AT 2 (LOW P) AND 500 (HIGH P) μM P CONCENTRATION AT THREE HARVESTS.	77
FIGURE 17: RATE OF ROOT EXUDATION OF SUGAR BEET GROWN AT LOW (2 μM) AND HIGH (500 μM) P AT THREE CONSECUTIVE HARVESTS.	78
FIGURE 18: EFFECT OF DIFFERENT SUBSTANCES ON P CONCENTRATION IN SOLUTION AFTER 5H OF INCUBATION.	81
FIGURE 19: EXTRACTED ION CHROMATOGRAM FROM LC-MSMS ANALYSIS OF CITRAMALIC ACID STANDARD (100 NG/ML) (SOLID LINE), POOLED ROOT EXUDATES	

GENERATED UNDER LOW P CONDITIONS (DASHED LINE) AND POOLED ROOT EXUDATES GENERATED UNDER HIGH P CONDITIONS (DOTTED LINE).....	82
FIGURE 20: EXTRACTED ION CHROMATOGRAM FROM LC-MSMS ANALYSIS OF SALICYLIC ACID STANDARD (100 NG/ML) (SOLID LINE), POOLED ROOT EXUDATES GENERATED UNDER LOW P CONDITIONS (DASHED LINE) AND POOLED ROOT EXUDATES (DOTTED LINE) GENERATED UNDER HIGH P CONDITIONS.....	83
FIGURE 21: MOLECULAR STRUCTURE OF SALICYLIC AND CITRAMALIC ACID	84

1.Chapter 1: General Introduction