

**Improvement of aluminum tolerance through scavenging reactive
oxygen species and lipid peroxide-derived aldehydes**

A thesis

Submitted to the United Graduate School of Agricultural Sciences

Tottori University

In Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

Lina Yin

March 2010

活性酸素と過酸化脂質由来アルデヒド消去による
アルミニウム耐性の改善

鳥取大学大学院連合農学研究科

学位論文

課程博士

殷 俐娜

2010年3月

Contents

Summary	1
---------------	---

Chapter 1

General introduction

1.1 Al toxicity in the world	5
1.2 Al toxicity and oxidative stress	5
1.3 Antioxidant and antioxidant enzymes in plants	7
1.4 The toxicity of lipid peroxide-derived aldehydes induced by stresses in plants	8
1.5 Objectives	10

Chapter 2

Overexpression of dehydroascorbate reductase, but not monodehydroascorbate reductase, confers tolerance to aluminum stress in transgenic tobacco

2.1. Abstract	12
2.2. Introduction	15
2.3. Materials and methods	19
2.3.1. Plant materials and growth conditions	19
2.3.2. Western blot analysis	19
2.3.3. Effect of Al on root growth	20
2.3.4. Al distribution and accumulation in root tips.....	20
2.3.5. Visualization of lipid peroxidation and measurement of MDA content	21
2.3.6. Visualization of plasma membrane integrity	22
2.3.7. Oxidative DNA damage analysis	22
2.3.8. Assaying H ₂ O ₂ content	24

2.3.9. Enzyme analysis	24
2.3.10. Determination of AsA and GSH levels	25
2.3.11. Statistical analyses	26
2.4. Results	27
2.4.1. Effect of Al on root growth	27
2.4.2. Al distribution and accumulation in root tips	27
2.4.3. Lipid peroxidation and plasma membrane integrity	28
2.4.4. Oxidative DNA damage	33
2.4.5. Hydrogen peroxide content	33
2.4.6. Enzyme activities	36
2.4.7. AsA and GSH levels	36
2.5. Discussion	41

Chapter 3

Overexpression of glutathione reductase in Arabidopsis confers tolerance to aluminum stress

3.1. Abstract.....	48
3.2. Introduction	50
3.3. Materials and methods	53
3.3.1. Construction of plant expression vector using Gateway cloning technology....	53
3.3.2. Agrobacterium-mediated transformation of Arabidopsis	54
3.3.3. Plant materials and growth conditions	55
3.3.4. Western blot analysis and Al treatment	55
3.3.5. Al content.....	56
3.3.6. H ₂ O ₂ content, MDA and Enzyme activities determination	58
3.3.7 Determination of Asc and GSH level	60

3.3.8. Statistical analysisi	61
3.4. Results	62
3.4.1. Effect of Al on root elongation and Al accumulation in root tip.....	62
3.4.2. Effect of Al on lipid peroxidation and hydrogen peroxide generation	67
3.4.3. Effect of Al on GR activities	67
3.4.4. Effect of Al on other antioxidant enzyme activities.....	70
3.4.5. Contents of AsA and GSH.....	70
3.5. Discussion	73

Chapter 4

The involvement of lipid peroxide-derived aldehydes in aluminum toxicity of tobacco roots

4.1. Abstract	76
4.2. Introduction	78
4.3. Materials and methods	81
4.3.1. Plant materials and growth conditions, and Treatment with AlCl ₃	81
4.3.2. Al distribution and accumulation and H ₂ O ₂ detection and determination	82
4.3.3. Visualization of lipid peroxidation and cell death	83
4.3.4. Electrolyte leakage assay and Assessment of enzyme activity	84
4.3.5. Aldehyde identification and quantitation by HPLC	85
4.3.6. Effect of exogenous application of 2-alkenal on root growth	86
4.3.7. Effect of BHA on root elongation under Al stress	86
4.3.8. Statistical analyses	87
4.4. Results	88
4.4.1. AER-overexpressing plants show Al tolerance	88
4.4.2. AER-overexpressing plants accumulate Al and H ₂ O ₂	94

4.4.3. Differential aldehyde levels are correlated with differences in cell death	95
4.4.4. Specific aldehydes are suppressed in AER-overexpressing plants	99
4.4.5. 2-Alkenals inhibit root growth	106
4.4.6. BHA partly protects roots from Al injury	106
4.5. Discussion	111

Chapter 5

General discussion	123
---------------------------------	------------

Chaper 6

Conclusion	127
-------------------------	------------

References.....	128
------------------------	------------

Acknowledgement.....	139
-----------------------------	------------

和文要旨.....	141
-----------	-----

List of Publication	145
----------------------------------	------------