DOCTORAL THESIS

Functional characterization of arabidopsis DXO, a5'-3' RNA exonuclease

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Abstract

RNA decay plays an essential role in the regulation of gene expression during plant development and response to environmental stimuli. The protein DXO is a 5' to 3' exonuclease that functions in RNA degradation and RNA quality control that has been studied in animals. It has not yet been identified in plants. The gene locus At4g17620 in *Arabidopsis thaliana* encodes a protein homolog of the mammalian DXO, termed *AtDXO*. Recombinantly expressed AtDXO possesses a 5'-3' RNA exonuclease activity *in vitro*. Loss-of-function of *AtDXO* in *Arabidopsis* generates multiple growth defects, including curled and yellowish leaves, growth retardation and limited fertility, whereas overexpression show no obvious growth phenotype. The development defect of *atdxo* might be attributed to aberrant RNAs, which are not degraded when AtDXO is dysfunctioning. From the RNA-Seq analysis, the transcriptome pattern of *atdxo* mutants shows significant disparity from wild-type. Among the differences, the defense response genes are elevated in *atdxo* while photosynthesis-related and plastid genesis-related genes are downregulated. The constitutive expression of defense response genes causes the autoimmune phenotypes of *atdxo*. This could be modulated by temperature and is partially dependent on the master immunity regulators *EDS1* or *NPR1*. Reactive oxygen species (ROS) accumulation was also detected in the *atdxo* mutant, and *atdxo* showed insensitivity to oxidative stress imposed by paraquat. Moreover, the

atdxo mutant is hypersensitive to salt stress but not sensitive to general osmotic stress. In *Arabidopsis*, the 5’-3’ RNA decay pathway could act as a repressor of endogenous post-transcriptional gene silencing (PTGS), which is regulated by small RNAs (sRNA). The mutation of AtDXO caused productions of 24- and 25-nucleotide endogenous sRNAs. The growth defect phenotype of *atdxo* could not be repressed by dysfunction of the RDR6 (RNA-DEPENDENT RNA POLYMERASE 6)-dependent sRNA biogenesis pathway. These findings demonstrate that AtDXO functions as a 5'-3' exoribonuclease both *in vitro* and *in vivo* to regulate plant development and to mediate the response to environmental stresses.

Table of Contents

CHAPTER ONE - INTRODUCTION 1

1.1 RNA DECAY IN PLANTS 1

1.1.1 RNA deadenylation 1

1.1.2 3' to 5' RNA degradation 3

1.1.3 5' to 3' RNA degradation 4

1.1.4 Nonsense-mediated mRNA decay 5

1.1.5 RNA decay and PTGS pathway 6

1.2 THE RELATIONSHIP BETWEEN RNA DECAY AND STRESS RESPONSES 7

1.2.1 Abiotic stress 7

1.2.2 Biotic stress 9

1.3 DXO/RAI1 FAMILY PROTEIN 10

1.3.1 5'-3' exoribonuclease 10

1.3.2 Decapping activity 11

1.4 PERSPECTIVE AND TECHNICAL SCHEME 12

1.4.1 Perspective 12

1.4.2 Technical scheme 14

CHAPTER TWO - MATERIALS AND METHODS 15

2.1 PLANT MATERIALS AND GROWTH CONDITIONS 15

2.2 BACTERIAL STRAINS AND VECTORS 16

2.3 OLIGONUCLEOTIDE PRIMERS 17

2.4 TRANSFORMATION OF *ARABIDOPSIS* 22

2.4.1 Competent cell preparation and transformation of Agrobacterium 22

2.4.2 Floral dip transformation of Arabidopsis 23

2.5 TRANSGENIC LINES GENERATION AND SELECTION 24

2.6 EXORIBONUCLEASE ENZYME ACTIVITY ASSAY 24

2.7 EXTRACTION AND PURIFICATION OF GENOMIC DNA 25

2.8 EXTRACTION OF RNAS 26

2.9 CDNA SYNTHESIS 26

2.10 RNA-SEQ ANALYSIS 27

2.11 REAL-TIME QUANTITATIVE PCR ANALYSIS 28

2.12 B-GLUCURONIDASE (GUS) HISTOCHEMICAL STAINING ASSAY 28

2.13 3,3'-DIAMINOBENZIDINE (DAB) STAINING ASSAY 29

CHAPTER THREE - RESULTS 30

3.1 ATDXO IS AN RNA EXONUCLEASE 30

3.2 PLANT GROWTH AND DEVELOPMENT ARE SEVERELY ALTERED BY LOSS-OF-FUNCTION OF ATDXO 32

3.2.1 Phenotype characterization of AtDXO loss-of-function mutants 32

3.2.2 Plants overexpressing AtDXO showed no obvious phenotype under normal conditions 39

3.2.3 The chlorophyll content of dxo mutants is lower and independent on exogenous sucrose supply 41

3.3 EXPRESSION PATTERNS OF *ATDXO* 43

3.4 TRANSCRIPTOME PROFILING AND PCR VERIFICATION 45

3.4.1 RNA-Seq analysis showed that the dxo mutant transcriptome is extremely different compared to wild-type..... 45

3.4.2 qPCR verification corresponded with the RNA-Seq results and showed upregulation of defense-related genes and downregulation of photosynthesis-related genes 54

3.5. *ATDXO* MUTANTS SHOW ENHANCED IMMUNITY RESPONSE 60

3.6 *ATDXO* IS RESISTANT TO OXIDATIVE STRESS AND SENSITIVE TO SALT STRESS 66

3.7 SUPPRESSION OF GENES IN PTGS PATHWAYS DID NOT RESCUE THE *ATDXO* PHENOTYPE 69

CHAPTER FOUR - DISCUSSION 72

5.1 ATDXO IS A NEWLY IDENTIFIED RNA EXONUCLEASE IN PLANT 72

5.2 *ATDXO* NEGATIVELY REGULATES PLANT DEFENSE RESPONSES 73

5.3 *ATDXO* SUPPRESSED ENDOGENOUS SRNA BIOSYNTHESIS BUT DID NOT DEPEND ON THE *RDR6* PATHWAY..... 74

CHAPTER FIVE - CONCLUSION..... 76

LIST OF REFERENCES 77

CURRICULUM VITAE 91