## **Table of contents**

## List of tables

List of figures

List of gene abbreviations

## Summary

1	Introd	uction	1
	1.1	Secondary growth in plant stems and the activity of vascular cambium	1
	1.2	KNOX (Knotted-1 Like homeobox) gene function in plant development.	2
	1.3	Molecular and genetic control of secondary growth, xylem differentiation and secondary cell wall deposition	4
	1.4	Working hypothesis.	6
	1.5	Objectives	7
2	Mater	ials and methods	8
	2.1	Plant material and growth conditions	8
	2.2	Identification and production of homozygous single and double <i>knox</i> mutants	8
	2.3	KNAT1-GR induction.	11
	2.4	RNA extraction and cDNA synthesis	11
	2.5	Quantitative Real time RT-PCR	13
	2.6	GUS assays	16
	2.7	Histologic analysis and quantitative measurements of secondary growth.	16
	2.8	Embedding and Toluidine Blue staining	17
	2.9	Light microscopy	18
	2.10	Identification of genes co-expressed with <i>STM</i> and <i>KNAT1</i>	18

3	Results		
	3.1	Isolation of insertional alleles as tool for functional analyses of <i>Arabidopsis KNOX</i> genes	19
	3.2	Defective secondary xylem development in <i>Arabidopsis</i> hypocotyls <i>stm</i> and <i>knat1</i> mutants	22
	3.3	STM and KNAT1 are specifically expressed in the cambial zone of Arabidopsis hypocotyls	27
	3.4	The development of secondary xylem is inhibited in <i>stm</i> and <i>knat1</i> mutants	28
	3.5	Phase II of secondary xylem development of <i>Arabidopsis</i> hypocotyl is inhibited in <i>stm</i> and <i>knat1</i> mutants	35
	3.6	Non-epistatic interaction between <i>STM</i> and <i>KNAT1</i> in <i>Arabidopsis</i> hypocotyls	37
	3.7	Secondary growth of <i>Arabidopsis</i> hypocotyls was reduced by overexpression of <i>KNAT1</i>	39
	3.8	Overexpression of <i>KNAT1</i> gene did not restore the <i>stm</i> phenotype in <i>stm-GK</i>	42
	3.9	STM and KNAT1 are required for ATHB-8::GUS expression in precursors of xylem fibers	45
	3.10	STM/KNAT1 are upstream of genes associated with vascular meristem differentiation, fiber development and secondary cell wall	46
4	Discussion.		
	4.1	Insertional mutagenesis	54
	4.2	Secondary growth in <i>Arabidopsis</i> hypocotyls <i>versus</i> inflorescence stems	55
	4.3	Pleiotropic effects of reduced STM and KNAT1 gene function on secondary growth	56
	4.4	Genetic interaction between <i>KNAT1</i> and <i>STM</i>	60

	4.5	Downstream factors	61		
	4.6	Overexpression controversy	63		
	4.7	Conflict with previously published data	64		
	4.8	Working model of action of <i>STM</i> and <i>KNAT1</i> during secondary xylem development	65		
5	References		67		
6	Supplemental data				
7	Appendixes				
	Publication (Sunaryo and Fischer, 2009)				
	Acknowledgements  Curriculum vitae.				