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# Molecular studies of genes controlling cambial cell differentiation and secondary cell wall formation

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## Introduction

During secondary growth, cambial daughter cells develop and specialize to xylem cells. Xylem cells undergo progressive stages of differentiation; (1) elongation/ enlargement, (2) secondary cell wall deposition, and (3) programmed cell death before being mature xylem (Turner et al. 2008). The major compounds of secondary cell walls are cellulose, hemicelluloses and lignin, an important source of fixed carbon used for woody materials and industrial purposes such as timber, pulp, furniture, fibers, and also as energy source or for other products (films, adhesives, etc). Although abundant data has been collected to address the genetic control of cambial activity and differentiation including the downstream processes like secondary cell wall formation, the mechanism behind is still little known.

Here, We reported the role of *KNOX* genes (Knotted1-like genes ,which comprise a small gene family with eight members in *Arabidopsis thaliana* e.g. *KNAT1*, *KNAT2*, *STM*) on cambial cell differentiation and secondary cell wall formation in the Arabidopsis hypocotyl.

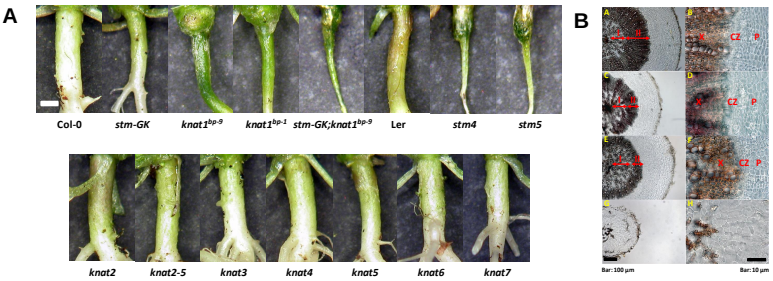


**Figure 1**. A. Hypocotyls of *knox* mutants and wild-type. Bar: 1 mm, B. Phase II of secondary xylem development was reduced in *stm* and *knat1* mutants compared to wild-type. Wild-type/Col-0 (A, B), *stm-GK* (C, D), *knat1*$^{bp-9}$ (E, F), and *stm-GK;knat1*$^{bp-9}$ (G, H). Stained using phloroglucinol-HCl. Bar for A, C, E, G: 100 µm and bar for B, D, F, H: 10 µm.

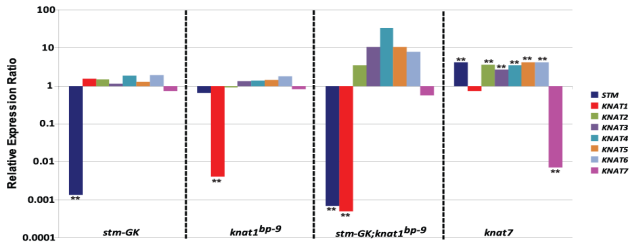


**Figure 2.** *KNOX* expression in hypocotyls of 6 weeks old *stm-GK*, *knat1*$^{bp-9}$, *stm-GK;knat1*$^{bp-9}$ and *knat7* mutants relative to wild-type. Data were analyzed from 3 biological and 3 technical replicates and normalized to the expression of *ACTIN2*. (**) Significant p≤ 0.01, t-test, compared to wild-type.

## Materials and Methods

T-DNA insertion mutants for all *Arabidopsis KNOX* genes were isolated. qRT-PCR and co-expression analysis were performed to investigate the expression of *Arabidopsis KNOX* genes and their downstream target genes supported by phenotypic observation of secondary growth development in hypocotyl. The secondary growth development was investigated by Phloroglucinol staining and Toluidine blue staining of hypocotyls of 12 week old plants.

| No. | Locus | Gene | Relative Expression Ratio | p Value (*) |
|---|---|---|---|---|
| 1. | AT3G59010 | *PME61* | - 44 x | 0.0212 |
| 2. | AT5g59310 | *LTP4* | + 47 x | 0.0319 |
| 3. | AT5G3170 | *FLA11* | - 39 x | 0.0243 |
| 4. | AT4G18780 | *CesA8* (*IRX1*) | - 30 x | 0.0009 |
| 5. | AT5G17420 | *CesA7* (*IRX3*) | - 186 x | 0.0317 |
| 6. | AT5G44030 | *CesA4*(*IRX5*) | - 76 x | 0.0296 |
| 7. | AT5G15630 | *COBL4*(*IRX6*) | - 42 x | 0.0248 |
| 8. | AT4G32880 | *ATHB-8* | - 3 x | 0.0150 |
| 9. | AT1G32770 | *SND1* | N.D. | N.A. |
| 10. | AT4G28500 | *SND2* | - 107 x | 0.0111 |
| 11. | AT2G46770 | *NST1* | - 278 x | 0.0164 |
| 12. | AT5G60450 | *ARF4* | - 3 x | 0.0507 |
| 13. | AT4G29080 | *IAA27* | - 57 x | 0.0005 |
| 14. | AT5G54690 | *Galacturonosyltransferase (IRX8)* | - 723 x | 0.0009 |
| 15. | AT2G38080 | *Laccase4* (*IRX12*) | - 404 x | 0.0116 |
| 16. | AT3G16920 | *CTL2 (chitinase like)* | - 100 x | 0.0033 |
| 17. | AT3G42950 | *GH28(polygalacturonase)* | - 2 x | 0.0899 |
| 18. | AT3G10340 | *PAL4* | - 3 x | 0.0167 |
| 19. | AT5G02030 | *BELL* | + 1 x | 0.1011 |

**Table 1.** The expression of coexpressed-downstream target gene candidates in the double mutant *stm-GK;knat1*$^{bp-9}$. Data were analyzed from 4 biological and 2 technical replica and normalized to the expression of *ACTIN2*. Negative ratios correspond to a decrease of expression compared to wild-type (Col-0), positive ratio to an increase. (*) Calculated based on t-test. (N.D) Not detectable, (N.A) not applicable.

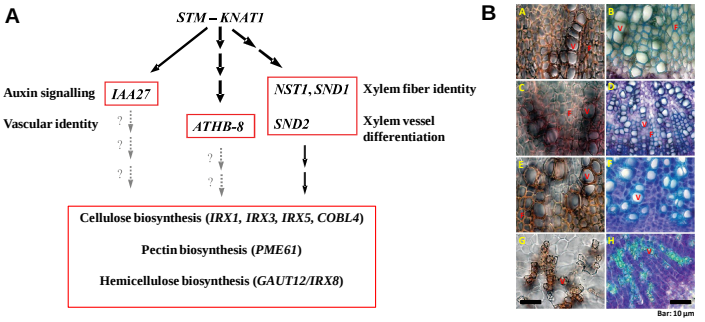


**Figure 3A**. A hypothetical model of *STM* and *KNAT1* functions in secondary xylem development. (One arrow) Direct interaction, (several arrows) indirect interaction, (black arrows) interaction shown in this work, (grey arrows) hypothetic interaction. **3B.** Vessel element structures of *Arabidopsis* hypocotyl in Col-0 (A, B), *stm-GK* (C, D), *knat1*$^{bp9}$ (E, F) and *stm-GK;knat1*$^{bp-9}$ (G, H). (A, C, E, G) Stained using phloroglucinol-HCl, (B, D, F, H) stained using toluidine blue. Bar: 10µm.

## Results and Conclusion

Hypocotyls of *stm* and *knat1* mutants displayed a strong reduction in diameter compared to the wild type (Figure 1A). The reduction was observed for almost all of *stm* and *knat1* alleles. Hypocotyl morphology of *knat2, knat2-5, knat3, knat4, knat5*, *knat6* and *knat7* was not different compared to the wild-type. As the observations on the intact hypocotyls already indicated, secondary growth of *stm* and *knat1* mutants was reduced (**Figure 1B**). These data suggest that *STM* and *KNAT1* are required for secondary growth of *Arabidopsis* hypocotyls. Formation of phase II xylem was significant reduced for *stm-GK and knat1*$^{bp-9}$ from wild-type (Figure 1B, *c, d,* and *e, f*). In the double mutant phase II xylem was completely absent underlying a synergistic genetic interaction between *STM* and *KNAT1* (**Figure 1B, *g, h***). *STM* was significantly down-regulated in *stm-GK*, but not reduced in *knat1*$^{bp-9}$ (**Figure 2**). Similary, *KNAT1* was strongly reduced in the *knat1*$^{bp-9}$, but not significantly different in *stm-GK*. The expression of *STM* and *KNAT1* was dramatically reduced in the double mutants of *stm-GK;knat1*$^{bp-9}$. These results indicate no evidence for an epistatic interaction. This is in line with the interpretation of the anatomical phenotype in the hypocotyl, which supports a synergistic interaction between *STM* and *KNAT1*.

Almost all genes selected from co-expressed genes by *STM* and *KNAT1* were significantly downregulated in the double mutant compared to the wild-type (**Table 1**), except for *Lipid transferase protein4* (*LTP4*) and *BELL* (*BELLINGER*). Other genes which have been previously reported to be involved in xylem fiber identity (*SND1* and *NST1*, Zhong et al. 2006; Mitsuda et al. 2007; Zhong et al. 2007) and xylem vessel identity (*SND2*) were also downstream targets of *STM/KNAT1* since their expression was significantly reduced in the double mutant. Besides genes associated with cellulose biosynthesis (*IRX1, IRX3, IRX5, IRX6*) and pectin formation (*PME61*), also hemicelluloses biosynthesis seemed to be a target of combined *STM/KNAT1* action, as seen in the down-regulation of the galacturonosyltransferase *IRX8* (**Table 1**). Collapsed or irregularly shaped vessels were attributed to changes in the secondary cell wall formation. Therefore, the vessel phenotype was examined more closely in *stm-GK*, *knat1*$^{bp-9}$ and the double mutant (**Figure 3B**). In wild-type, *stm-GK* and *knat1*$^{bp-9}$ the vessel elements appeared to be almost round (**Figure 3B, *a, b, c, d, e, f***), while in the double mutant vessel diameter was markedly reduced and the cells had a more angular shape, reminiscent to various *irx* mutants (**Figure 3B, *g, h***), . Additionally, the staining of the secondary cell walls in vessel elements appeared to be fainter. These results suggest that *STM* and *KNAT1* are a limiting factor for cell wall biosynthesis in vessels, which is in line with the function of their down-stream targets. A working model is presented in **Figure 3A**. A series of experiments has been outlined in this discussion, which will allow to test this model.

## Literature

Mitsuda N, Iwase A, Yamamoto H, Yoshida M, Seki M, Shinozaki K and Ohme-Takagi M (2007). NAC transcription factors, NST1 and NST3, are key regulators of the formation of secondary walls in woody tissues of *Arabidopsis*. The Plant Cell, 19:270-280.

Zhong R and Demura T, Ye Z-H (2006). SND1, a NAC domain transcription factor, is a key regulator of secondary cell wall synthesis in fibers of *Arabidopsis*. The Plant Cell, 18:3158-3170.

Zhong R, Richardson EA and Ye Z-H (2007). Two NAC domain transcription factors, SND1 and NST1, function redundantly in regulation of secondary wall synthesis in fibers of *Arabidopsis*. Planta, 225:1603-1611.

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**INDONESIAN-GERMAN NETWORK**
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KASSEL UNIVERSITY, INSTITUT PERTANIAN BOGOR, UNIVERSITAS BRAWIJAYA, UNIVERSITAS ANDALAS, UNIVERSITAS SAM RATULANGI, UNIVERSITAS JEMBER, UNIVERSITAS AIRLANGGA, UNIVERSITAS MULAWARMAN.

Bogor, 03 July 2012

To:. Dr. Widi Sunaryo
Mulawarman University.
Samarinda

Thank you for your interest to attend the first IGN-TTRC International Conference on Biotechnology for Human Life, that will be held in Bogor on July 17 and 18th, 2012.
We are happy to announce that your **poster abstract titled: "Molecular studies of genes controlling cambial cell differentiation and secondary cell wall formation" is accepted** to be presented in the conference. Your participation by attending the conference would be worthwhile. Meeting and talking with other scientists from foreign countries as well as from throughout Indonesia are always interesting.

We wish to meet you in the Conference.

Sincerely,

Dr Diah Ratnadewi
(Chairwoman of the Committee)