

SECONDARY CELL WALL MOLECULAR DETERMINISM IN SORGHUM IS NOT A SIMPLE COPY PASTE OF ARABIDOPSIS STORY



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Secondary cell wall composition is a key trait for sorghum breeding

Sorghum bicolor L. Moench is the world fifth crop for grain production. It is used for human and animal nutrition (grain and vegetative parts) and it is also increasingly mobilized for energy and natural fiber-reinforced polymers production. Sorghum biomass is mainly accumulated in stems and especially in secondary cell walls (SCW) and MYB and NAC transcription factors are expected to be key drivers of SCW composition (Zhong *et al.*, 2008). Understanding how is regulated SCW deposition in sorghum would allow fine tuning of SCW deposition and stem quality to improve selection of sorghum varieties that match farmers and value chains needs.

In order to identify candidate genes we used comparative genomics and co-expression gene network analyses. We were able to identify regulatory networks involved in SCW deposition and highlight the roles of NAC and MYB members that are the more likely to control SCW deposition in sorghum.

Yellow

Pink

1 - TWO CLUSTERS OF GENES INVOLVED IN SCW HAVE BEEN IDENTIFIED

- 45 modules of co-expressed genes were identified, each of them is named with a color randomly picked by WGCNA R package.
- 6 modules are enriched in Gene Ontologies (GO) related to SCW and CW and in CW enzymes (Fig. 2).

MATERIAL & METHODS

Identification of gene networks :

- 3 trials (2013, 2014, 2015) on a commercial biomass hybrid.
- Between 2 and 4 internodes (IN) sampled at 2 to 5 stages in 2 water availabilities (Fig. 1).
- Biochemial characterization of the IN composition.
- Yellow and pink modules genes are likely to contain SCW genes because their expression pattern is synchronous with lignin accumulation (Fig. 3A and 3B).

Lightsteelblue

Blue





Figure 2: Modules enrichement in CW enzymes and GO terms related to cell walls.

The number of genes considered per module is indicated by the bar heights (principal Y-axis) and the number of CW related GO significantly enriched is indicated by the line plot (secondary Yaxis). Modules significantly enriched in CW enzymes are indicated in blue. Grey modules are not significantly enriched in CW enzymes. **Figure 3**: **A-** Expression patterns of the 6 modules with CW related GO and CW enzymes enrichment detected (centered and normalized average expression of genes is represented) and **B-** Accumulation pattern of cell wall components (xylan, cellulose and lignin content expressed as percentage of dry matter (DM)) for IN 12 in the well-water treatment for 2013 trial (stages D1 to D5).

Turquoise

Steelblue

- 357 RNAseq libraries sequenced.
- Gene co-expression network built through WGCNA signed analysis (Langfelder & Horvath, 2008).
- High co-expression correlation between genes considered (>0.82).

Enrichment analysis:

- TopGO R package with PLAZA 4.0 GO database.
- Fisher t-test using a list of cell wall (CW) involved enzymes (Rai et al. 2016, McKinley et al. 2016, Petti et al., 2015).

Comparative genomic analyses of the MYB and NAC families:

- Use of 9 different dicotyledon and monocotyledon species.
- 1.170 NAC and 1.328 MYB protein used.
- Use of HMM signature, trimal, ML method to build the phylogenetic analysis and RAP-Green to root the tree.

Figure 1: 2013 trial sampling scheme (2 IN x 5 stages x 2 water treatments x 4 biological replicates).



3 – NEW MYB AND NAC POTENTIALLY INVOLVED IN SCW HAVE BEEN IDENTIFIED

• We identified SbMYB and SbNAC genes whose orthologs are not yet identified as key SCW regulators

2 - MOST OF At MASTER REGULATORS ARE ALSO KEY GENES IN SORGHUM

Fibers Vessels



- NAC and MYB sorghum orthologs of At functionally characterized genes have been identified thanks to published work (Fig. 4) and a phylogenic study of MYB and NAC families (Fig. 5).
- 122 SbNAC and 135 SbMYB in total were identified.
- SbNAC and SbMYB top co-expressed genes lists were extracted.
- Co-expressed genes lists of sorghum orthologs of most At master regulators are enriched in CW related GO (e.g. SbMYB46 in Fig. 6A, SbNST1 in Fig. 6B).
- 5 out of 8 VND family sorghum homolgs are not expressed in our sampling conditions and only one sorghum VND7 ortholog co-expressed gene list is enriched in CW related GO.



- but their co-expressed genes lists are enriched in CW related GO (e.g. SbMYB13a in Fig.5, 6C and 7 and SbNAC25 in Fig. 6D and 7).
- There are strong connections between co-expressed genes with SbMYB and SbNAC in the SCW yellow module.
- In this subnetwork, there are highly connected genes not annotated with CW related GO.



Figure 7: SbMYB and SbNAC (white rounded) within yellow module and their top coexpressed genes (purple gradient for correlation strength) that are enriched in CW related GO genes (blue). SbMYB and SbNAC with no ortholog SCW or CW annotated are red colored.

CONCLUSION AND DISCUSSION

- Genes in the 6 modules (Fig. 3A) identified are likely to be good candidate for a role in CW biosynthesis and especially SCW biosynthesis for pink and yellow module genes.
- Most orthologs of arabidopsis master regulators of SCW are likely to be involved in SCW regulation in sorghum. However, there are weaker evidences for involvement of some orthologs of At key SCW regulation genes such as VND7. As VND family is responsible for SCW regulation in vessels, this could be due to vessels and fibers pattern differences in the





Figure 5: MYB family tree including 9 species with a focus on SbMYB13a located in the pink clade along (but not closely related to) other MYB SCW regulators. Colors represent clades, classification inspired by Li *et al.*, 2016.

Aggregated GO per color : SCW Lignin Xylan Cellulose CW Pectin Vessels dvp GO not related to walls



Figure 6: Number of significantly enriched CW related GO in co-expressed genes with A-SbMYB46 B-SbNST1 C-SbMYB13a D-SbNAC25.

stem between monocotyledons and dicotyledons.

- New MYB and NAC candidates for SCW regulation in sorghum are identified.
- SbMYB and SbNAC SCW putative regulators top co-expressed genes are also top candidate genes for a role in SCW biosynthesis. It could be interesting to see how they overlap with other candidate genes lists such as differencially expressed genes between stem composition varying sorghum varieties or genes detected in stem composition association studies.

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