# Towards a sorghum ideotype with optimized cell wall degradability: insights from histological, degradation kinetics, biochemical, and pre-treatment analyses

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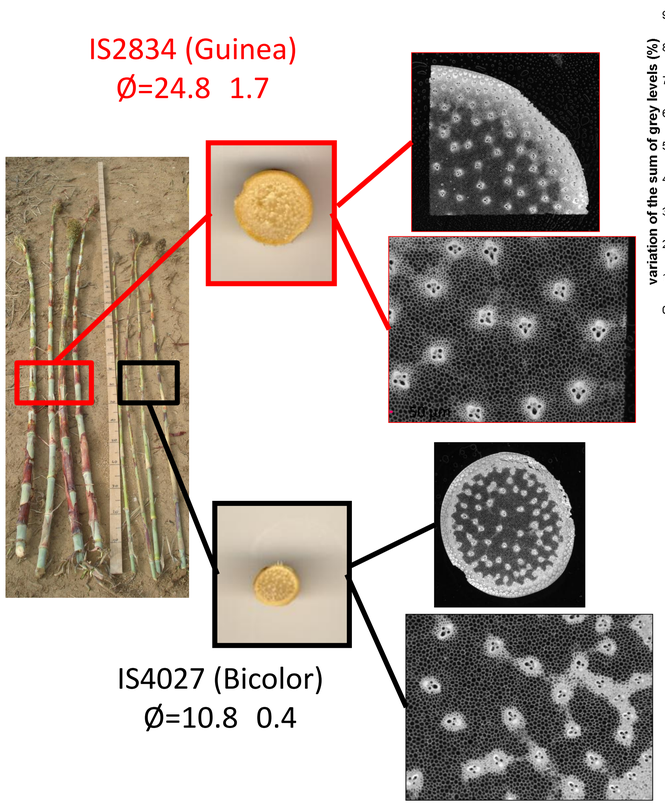
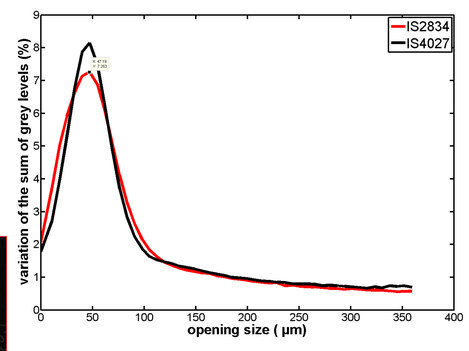
In addition to being the fifth cereal in terms of grain production, the interest of sorghum as a feed and energy crop is increasingly underlined. Selection of sorghum genotypes for fodder and energy production relies on an in-depth understanding of stem tissue organization and cell wall characteristics variability. In this context, a set of genotypes representative of the sorghum stem biomass variability was analyzed in order to define an ideotype with optimized cell wall degradability.

## Histological analysis revealed a limited variability of cell size but contrasting tissues proportions

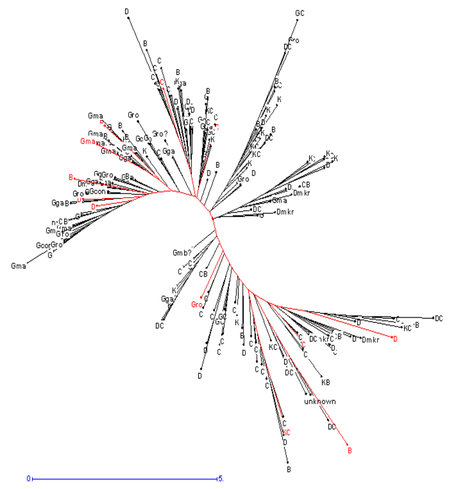



Two genotypes harboring different stem diameters were analyzed at the histological level. Although they did not differ in terms of cell size they presented contrasting tissue proportions, the one with the largest diameter presenting a larger parenchyma proportion.

## Cell Wall Characterization

In order to reach a better understanding of the differences detected in terms of cell wall degradability and identify the cell wall components driving these different behaviors an in-depth biochemical analysis of the cell wall composition of a set of 13 genotypes representative of the biochemical diversity (NIRS profile) of sorghum was performed (Bmr mutant were not included to this analysis). A significant variability for all the cell wall components analyzed was detected.



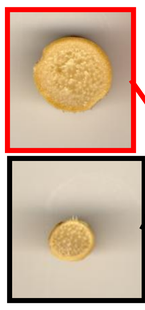
Selection of 13 genotypes representative of sorghum stem cell wall diversity

Variability of different cell wall characteristics in a panel represesentative of the worldwide sorghum diversity.

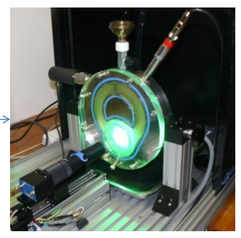
| Trait | mean | Cv % |
|---|---|---|
| Cell wall residue (% DM) | 70.32 | 14.83 |
| Lignin Klason content (% CWR) | 16.32 | 9.05 |
| G µmol/g LK | 407.00 | 10.57 |
| S µmol/g LK | 361.01 | 19.29 |
| Lignin S/G | 0.89 | 14.71 |
| Esterified p-coumaric acid (mg/g CWR) | 16.13 | 12.18 |
| Esterified ferulic acid (mg/g CWR) | 4.75 | 8.89 |
| Etherified ferulic acid (mg/g CWR) | 4.06 | 9.60 |
| Total Sugars (% CWR) | 75.56 | 4.35 |
| Arabinose (% CWR) | 4.58 | 21.21 |
| Galactose (% CWR) | 1.09 | 40.43 |
| Glucose (% CWR) | 41.18 | 14.81 |
| Xylose (% CWR) | 28.72 | 10.74 |

## Degradation kinetic analysis underlined different degradation behaviors

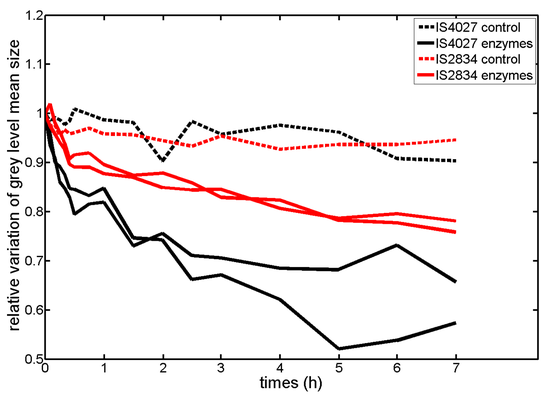
The two samples characterized at the histological level were then submitted to enzymatic degradation using a cocktail of cellulases, glucosidase and xylanase and their degradation dynamics were evaluated in terms of particle size and percentage of sugar released.

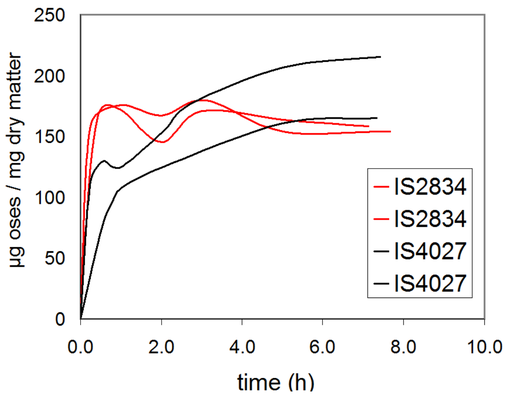


Isolation and freeze-drying of water-insoluble material



A bioreactor « Imatore » allowing a follow up of tissue degradation and recovery of samples for biochemical analysis was build (INRA Nantes).

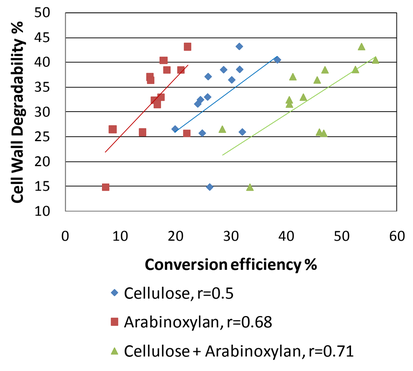


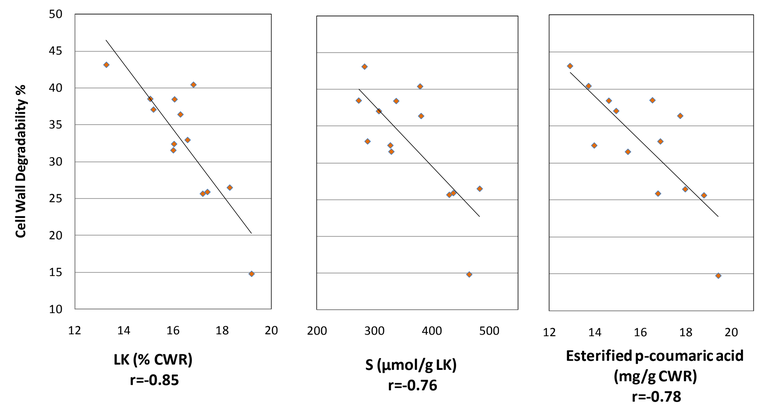




The genotype with the largest stem diameter presented a faster degradation and a smaller particle size at the end of the degradation process. Nevertheless, the smaller diameter genotype allowed a slightly higher percentage of sugar released.

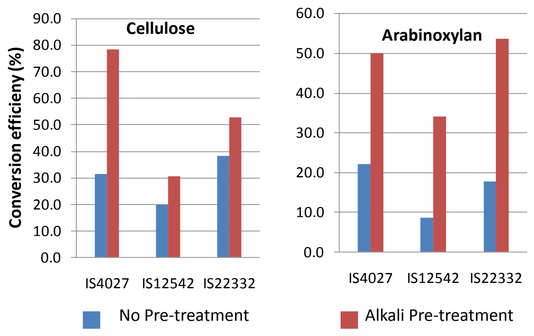
## Saccharification efficiency

In parallel with the characterization of the cell wall composition of 13 accessions, enzymatic saccharification was performed using a cocktail of cellulases, glucosidase and xylanase. Cell wall degradability varied amongst sorghum accessions and showed an important contribution of the hemicellulose degradation (Arabinose + Xylose). This analysis also underlined the well known negative impact of lignin content (LK) on cell wall degradability but mostly highlighted the importance of lignin structure and composition together with the esterified p-coumaric acid content on cell wall degradability.




## Alkali Pre-treatment effects




Alkali pre-treatment induced significant improvement in the conversion yields of cellulose and arabinoxylan but contrasting responses of the genotypes in terms of degree of improvement.

**Conclusion:** A large variability exists among the sorghum accessions in terms of cell wall degradability. According to our results, these differences depend on complex interactions between tissue proportions and the biochemical content of the cell walls with a central role for the phenolic compounds. In addition, if alkali pre-treatments enhance significantly the cell wall degradability they did not (in our conditions) eliminated the genetic variability underlying the importance to understand the genetic architecture of cell wall related traits.

Acknowledgments

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