Contribution of Multispectral Autofluorescence Imaging to Histochemistry in Understanding Sorghum Internode Hydrolysis Pattern

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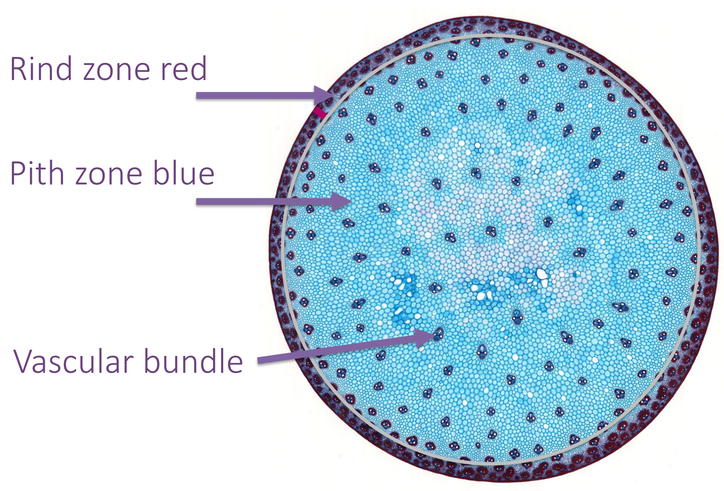
- Sorghum is a candidate species to support the development of new biomass based value chains
- Digestibility depends of overall lignification
- Fasga staining reveals regions of differentiated tissues lignification
- Multispectral autofluorescence reveals differences in phenolic compounds

The objective of this work was to evaluate the relevance of multispectral autofluorescence imaging to better understand contrasted hydrolysis patterns observed inside the sorghum stem internode



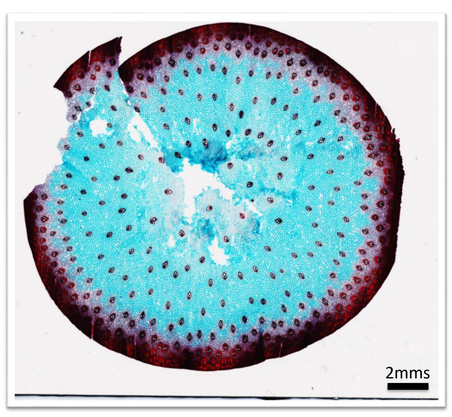
Sorghum internode cross-section after fasga staining

Hydrolysis

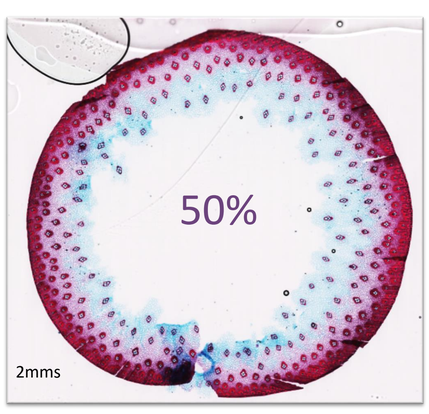
- 2 Genotypes : Biomass 140 (G01), IS28409 (G28) and 4 plants/genotype
- Bottom internodes/150µm cut serial sections
- Hydrolysed during 72h at 37°C with an enzyme cocktail of 4% Cellulase/Hemicellulase

Fasga staining

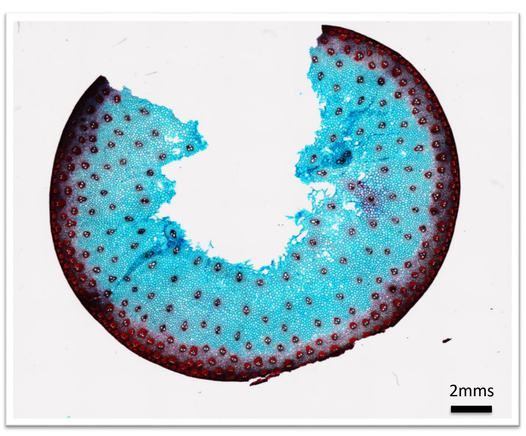
- This staining method highlights internode's regions possibly differing in lignin content : cell walls stained red (high lignin) vs blue (low lignin)
- Images of samples and hydrolysed sections were analysed with Image J



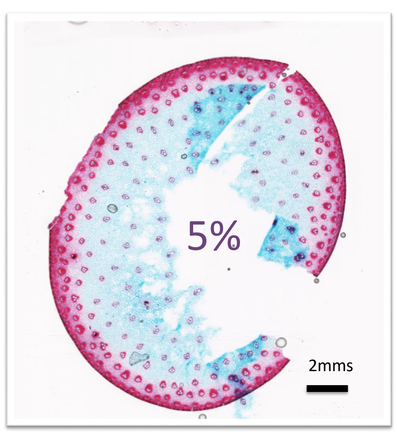
G01 before



G01 after



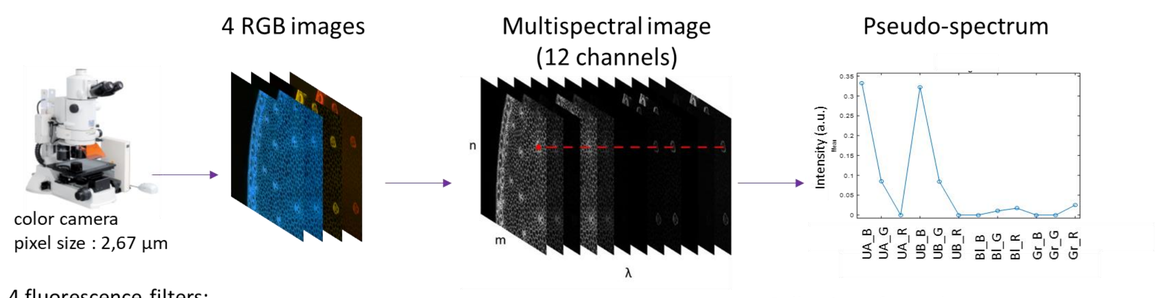
G28 before



G28 after

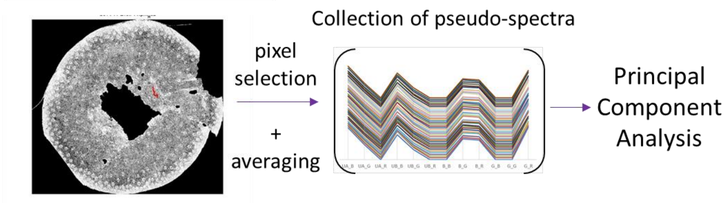
Fasga staining before and after hydrolysis

Multispectral Images

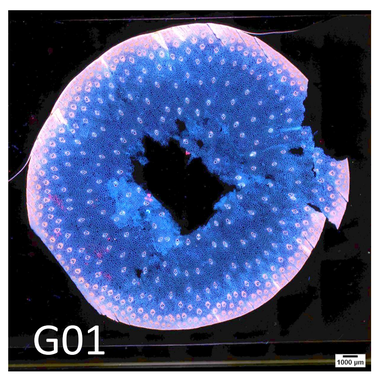


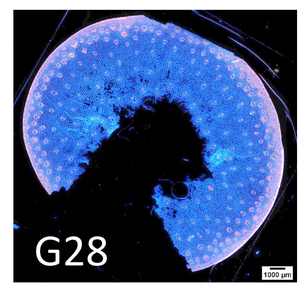
4 fluorescence filters:

	Excitation	Emission
UA	360-370	>400
UB	325-375	>420
Blue	450-490	>515
Green	510-560	>590

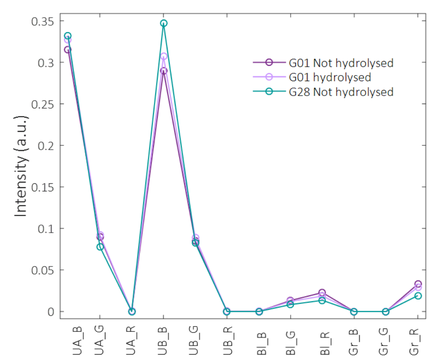


Multispectral autofluorescence image acquisitions and data analysis



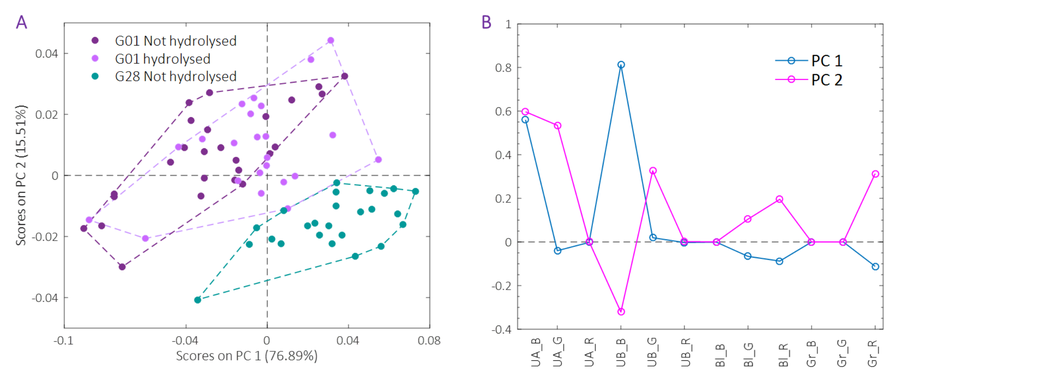


Color representation of the average multispectral autofluorescence images of G01 and G28



Mean pseudo-spectra from parenchyma cell walls in the pith (G28, G01)

- All cell walls are autofluorescence under UV or visible lights
- Parenchyma cell walls in fasga blue zone harbour intense fluorescence under UV excitation and low under visible



Principal component analysis on averaged spectra extracted from blue stained parenchyma in G28 and G01 (2 zones according to hydrolysis results at 72h). A. similarity map, B. loadings

- No hydrolysis of the fasga red rind zone
- Different hydrolysis patterns between the fasga blue pith zone and in the middle of internode
- G28 showed a lower hydrolysis yield (4 to 25%) than G01 (40%-50%)

- Differences in spectral signature in G28 compared to G01: higher intensity under short UV excitation in shorter emission range (blue), lower intensity under visible excitation

  Hypothesis : higher ferulic acid amount and lower p-coumaric acid/lignin amount in G28 *(Berger et al. 2021)*

Autofluorescence imaging reveal the possible role of hydroxycinnamic acids in explaining the hydrolysis pattern of sorghum cell walls in the internode pith

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