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

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CONTRIBUTION OF MULTISPECTRAL AUTOFLUORESCENCE IMAGING TO HISTOCHEMISTRY IN UNDERSTANDING SORGHUM INTERNODE HYDROLYSIS PATTERN

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Sorghum is the world's fifth largest cereal crop and exhibits high biomass yield potentials. This cereal is suitable to support the development of biomass value chains in temperate and semi-arid environments. Digestibility of plant feedstock is one of the key properties and is largely dependent on the overall lignification of the plant which is monitored at the tissue scale by the relative amounts and composition of plant organ. To obtain details on organ anatomy and spatial distribution of main components, medium-throughput histochemical methods have been developed on maize and sorghum internodes [1,2]. FASGA staining procedure is based onthe competition between 2 dyes and reveals regions with different staining colour (essentially red and blue color) which may be interpreted in terms of tissue ligninification [3]. In addition to staining procedures, the specific spectral properties of phenolic compounds could also be exploited to map their distributions using spectral imaging technics. Multispectral autofluorescence imaging was successfully applied to maize stem internode to reveal differences in cell wall phenolic compounds distribution (lignin and hydroxycinnamic acids) [4]. 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.

Two sorghum genotypes differing in their hydrolysis yield were selected. Internodes (at the bottom of the stem) from four healthy plants harvested at the grain filling stage were characterized for histochemistry in relation to spatial-susceptibility to enzymatic hydrolysis. Cell wall digestion was realized with a combined cellulase/hemicellulase 4% mix during 72h. The hydrolyzed sections were stained with FASGA and the hydrolysis yields were estimated by comparing the area of the section before and after hydrolysis (Figure 1A,B).. Images were analyzed with the open-source ImageJ freeware4 and a dedicated script to quantify anatomical traits. Multispectral autofluorescence images (Figure 1C) were acquired using a multi-zoom macroscope AZ100M (Nikon, Japan) equipped with 4 filter blocks to acquire images under UV, blue and green excitation lights. Emission light was recovered through long pass filters and, taking advantage of the RGB channels of the color camera, each image was split in three channels and stack alltogether to obtain a 12 channels multispectral image. In such multispectral images, each pixel is characterized by an intensity profile that can be considered as a spectral signature (Figure 1D).

Observed after FASGA staining, both genotypes showed typical sorghum internode organization with two main anatomical zones: (1) a mainly red-stained peripheral zone Z1 (rind), which is rich in vascular bundles and sclerenchyma cells and is surrounded by an epidermis on the most external part, and (2) an inner zone Z2 (pith), composed of vascular bundles spread into blue-stained parenchyma. As already observed, no hydrolysis of the red-stained zone (Z1) was evidenced for these two genotypes. Different hydrolysis pattern in the low-lignified zone (Z2) was revealed. One genotype showed a limited losse of material (below 10% for two plants and up to 25 % for the other two plants). For the second genotype hydrolysis

yields were about 40-50%, corresponding to an almost complete degradataion of the Z2 region. For both genotypes, the hydrolysis was more pronounced in the middle of the internode cross section.

Comparison of the brightfield images with the color representation of the average multispectral images shows that all the cell wall showed autofluorescence under UV or visible excitation. Differences in color in this multispectral image highlight the main tissues with highly lignified tissue appearing as pink/orange. No visual difference between parenchyma was evidenced and more precise analysis of spectral signature was carried out. Spectral signature of blue-stained parenchyma cell walls were extracted in both hydrolysed and not hydrolysed regions. Whole spectral signatures were compared through principal component analysis. Slight differences were observed, in particular under UV-excitation light and must be faced to overall spectral variability (within a cross section and between plants).

The relevance of combined classical histochemical and multispectral imaging was emphasized in this study allowing to highlight the different behaviours of constratsing genotypes and different regions of the internodes.

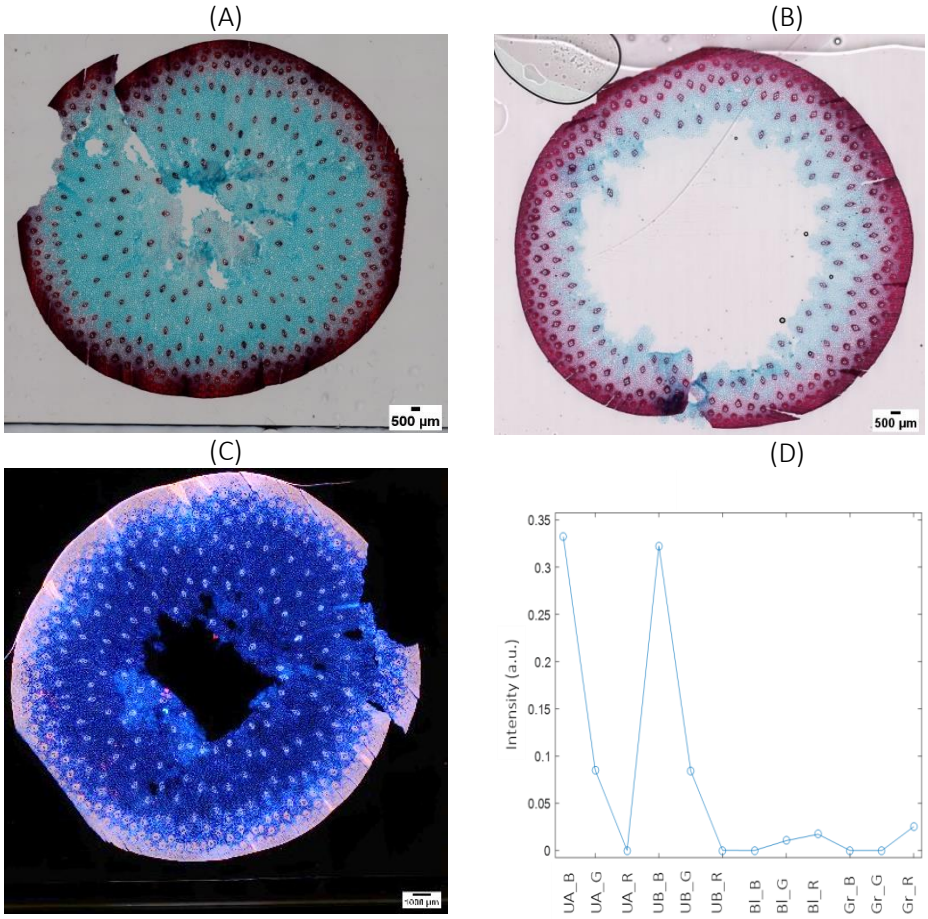

Figure 1 : Cross section of sorghum maize stem internode. A,B. Fasga staining before (A) and after (B) digestion. C. Color representation of the average multispectral autofluorescence image. D. Autofluorescence pseudo-spectrum from parenchyma cell walls.

Acknowledgments

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