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High resolution imaging of maize stem with time-of-flight secondary ion mass spectrometry

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In the study of converting cellulosic plants into biofuels, much attention has been paid to maize stalk for its vast biomass production and great potential in bioethanol generation [1-3]. However, high lignification of cell walls in maize stem has significantly prevented the accessibility of enzymes, microorganisms or chemicals to cell wall polysaccharides, thus leading to a low efficiency in the conversion [4]. Our objective is to analyze lignin distribution and evaluate the lignification in maize stems by TOF-SIMS imaging [5,6], which will facilitate the selection of potential phenotypes for biofuel production as well as to provide clues in building new genotypes to obtain desired lignin distribution pattern or to downregulate general lignin production.

Preliminary imaging experiments were performed with a commercial TOF-SIMS IV mass spectrometer (ION-TOF GmbH, Münster, Germany) with bismuth cluster ions (Bi_3^+ , 25 keV) as the primary ion beam. The so-called burst alignment ion beam focusing mode was utilized to obtain a high spatial resolution, followed by applying a delayed extraction of secondary ions to retain the high mass resolution routinely generated in high current bunched ion beam focusing mode [7]. To neutralize the charges accumulated on the insulating surface, a low energy pulsed electron flood gun was applied during all the acquisitions. Small areas of $400\text{ }\mu\text{m} \times 400\text{ }\mu\text{m}$ corresponding to vascular bundle and ground tissue of maize stem were mapped, respectively.

With above instrumental settings, the distribution of different lignin types on maize stem section was mapped with high mass and spatial resolution. G/S ratios of cell walls in different cell types were calculated to predict the degradability of cell wall lignin. Moreover, lignin deposition pattern in the side wall of metaxylem vessel was directly visualized thanks to the high spatial resolution. In addition, the localization of inorganic calcium might prove its association with cell wall pectine, which could further strengthen the cell walls in maize stem.

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