

Imaging Plant Using Time-of-Flight Secondary Ion Mass Spectrometry

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This work presents initial imaging and spectra results of plant seed using time-of-flight secondary ion mass spectrometry (ToF-SIMS), showing the application of delayed image extraction to study root biology. Compared to MALDI (Matrix Assisted Laser Desorption Ionization), an imaging mass spectrometry technique widely used in plant studies,[1] SIMS is less destructive and provides submicrometer spatial mapping of molecular species of importance in metabolic processes. *Brachypodium distachyon* (Brachypodium), a genomics model for bioenergy and native grasses, is used due to its small diploid genome, close phylogenetic links to other grass species, relative ease of genetic transformation, short life cycle, small stature, and simple growth requirements.[2] Plant growth-promoting bacteria (PGPB) were introduced to Brachypodium seeds prior to germination, and their potential effect on seeding was studied using ToF-SIMS imaging. Specifically, delayed image extraction was used in data acquisition. This approach is chosen to obtain high mass and high spatial resolutions.[3]

The Brachypodium seed was defined as top, bottom, and brush shown in Figs. 1a-c. The seed samples were cut into sections and held flat in the sample holder prior to ToF-SIMS analysis. An IONTOF GmbH TOF-SIMS V spectrometer was used in this work. The primary ion beam was a 25 keV Bi₃⁺ beam, which was focused to about 400 nm diameter with a beam current of ~0.32 pA. The delay extraction mode was used to collect secondary ions, and the mass resolution was in a range of 2500-4000. The effective mass range was about m/z 25-1000.

Figs. 1d & e show submicron SIMS chemical mapping of different sections of a Brachypodium seed top vs. brush (Figs. 1b & c). Morphological information of the seed can be obtained using SIMS imaging. Besides excellent imaging of the seed sections exhibiting morphological differences, detailed chemical spatial mapping can be reconstructed from the SIMS spectra. Figs. 2a & b show the normalized SIMS positive spectra collected in the seed bottom and brush. Distinctive characteristic peaks are observed, indicating chemical composition is relevant to seed germination potential. Our initial results demonstrate that ToF-SIMS can be used as a viable tool to study root biology and root-microbe interactions.

References:

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- [2] T Girin *et al.*, Brachypodium: a promising hub between model species and cereals, *J. Experimental Botany* (2014), **65** (19), 5683-96.
- [3] QP Vanbellinggen *et al.*, Time-of-flight secondary ion mass spectrometry imaging of biological samples with delayed extraction for high mass and high spatial resolutions, *Rapid Comm. Mass Spectrom.* (2015), **29** (13), 1187-95.

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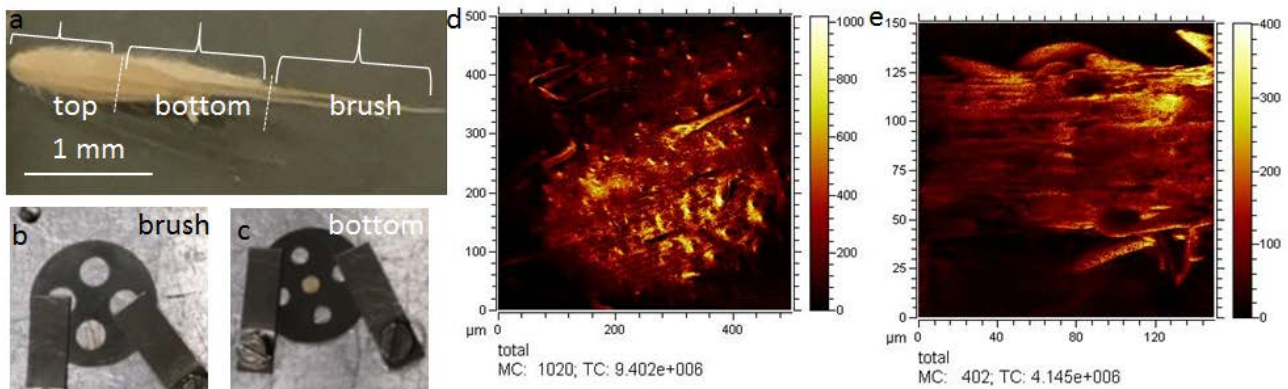


Figure 1. Brachypodium seed (a) brush (b) and bottom (c) prepared for ToF-SIMS imaging. Corresponding total ion images of seed brush (d) and bottom (e) are shown respectively.

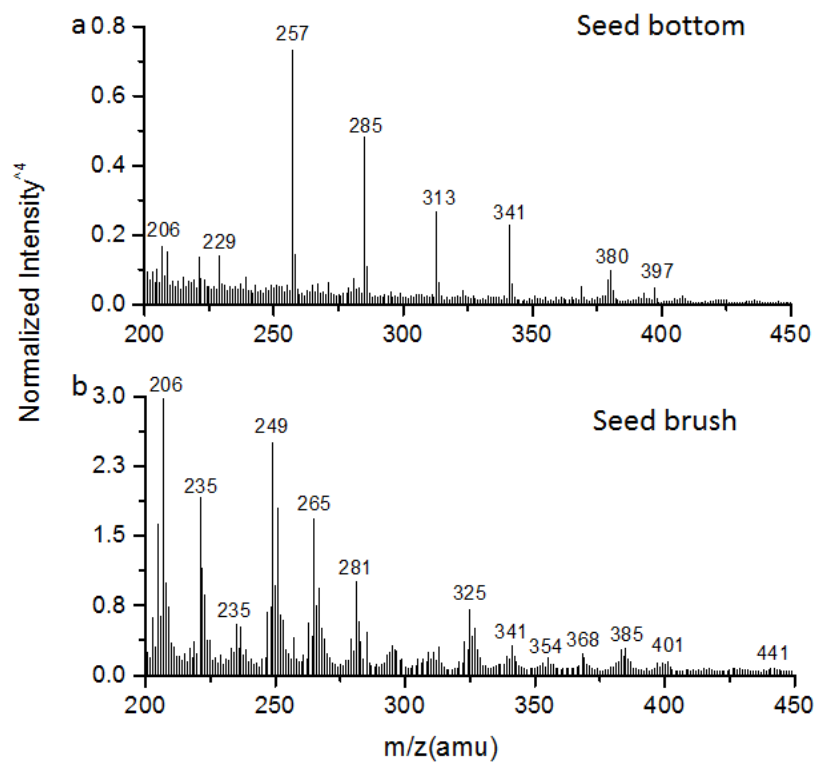


Figure 2. Normalized ToF-SIMS positive m/z spectra of seed bottom (a) and seed brush (b).