

ToF-SIMS Analysis of Plant Seed Interactions with Plant-Growth Promoting Bacteria

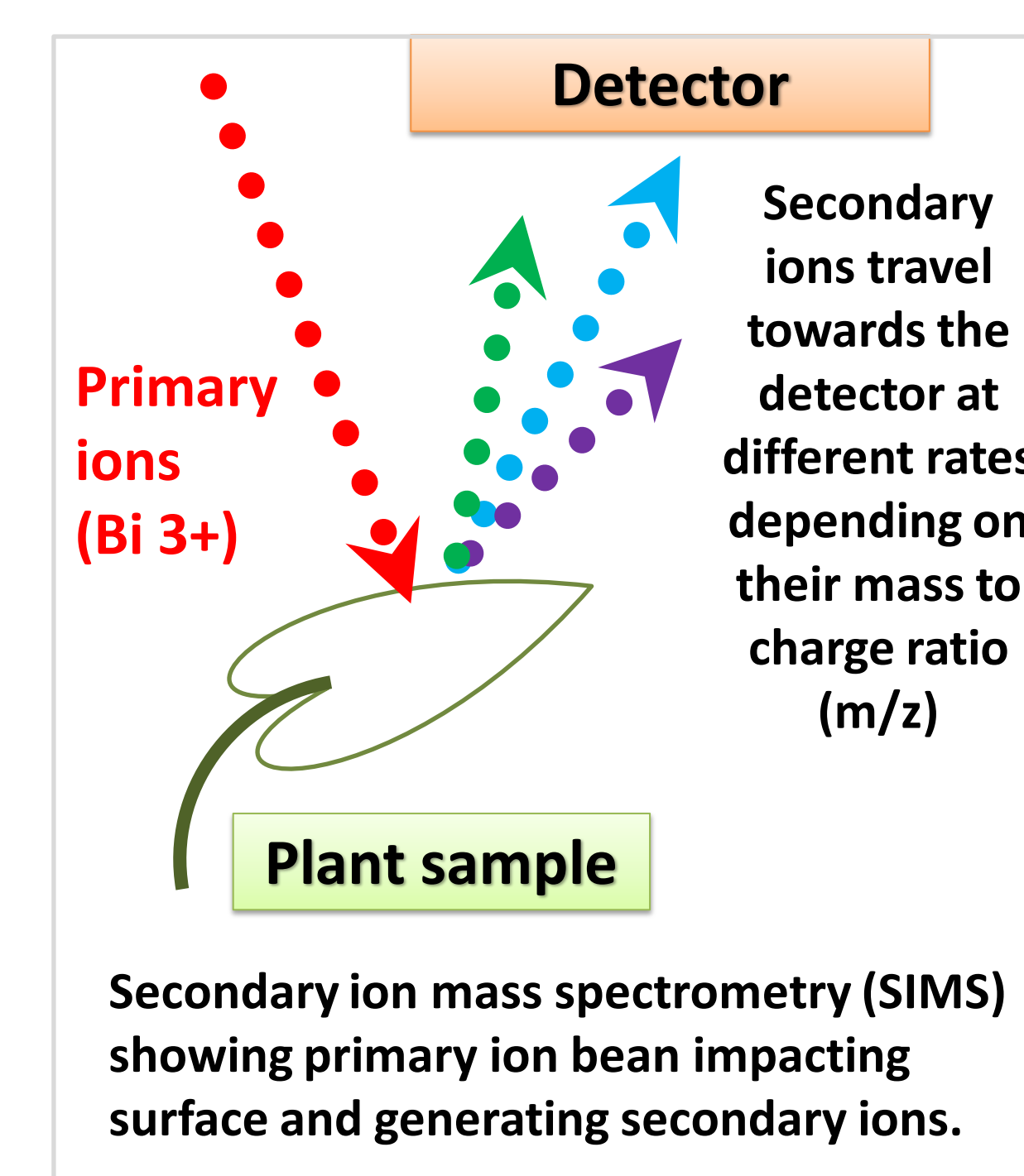
Tammy Pheuphong^{1,2}, Rachel Komorek², and Xiao-Ying Yu²

1. Science Department, Infinity Math, Science and Technology High School, Chicago, IL 60623, USA
2. Earth and Biological Sciences, Pacific Northwest National Laboratory, Richland, WA 99354, USA

Introduction

Mass spectrometry imaging (MSI) provides an in situ and/or in vivo spatial analysis of a plant sample [1]

- Applications include: responses to stress & defense mechanisms, ecophysiologically important processes, and symbiotic relationships
- This project aims to characterize the interaction between plant growth-promoting bacteria (PGPB) using a C3 plant as a model



Time-of-flight secondary ion mass spectrometry (ToF-SIMS) allows lateral resolutions <100 nm [1]

- High sensitivity and gives a full spectra of molecular ions.
- Produces distribution maps of ions by plotting the intensities of peaks against their x-y coordinate

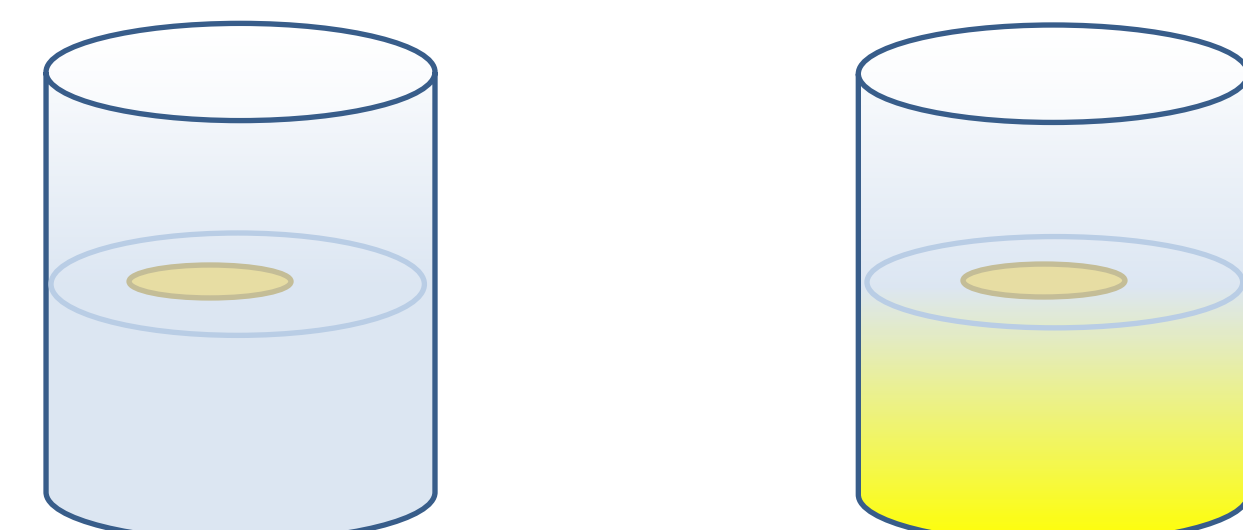
Brachypodium and *Pseudomonas* as a plant and PGPB model [2, 4]

- Brachypodium has a fully sequenced genome and a short life cycle
- An accepted model plant for studying grain crops like wheat and barley
- Previous studies show “increased biomass and adventitious root growth”

Methods

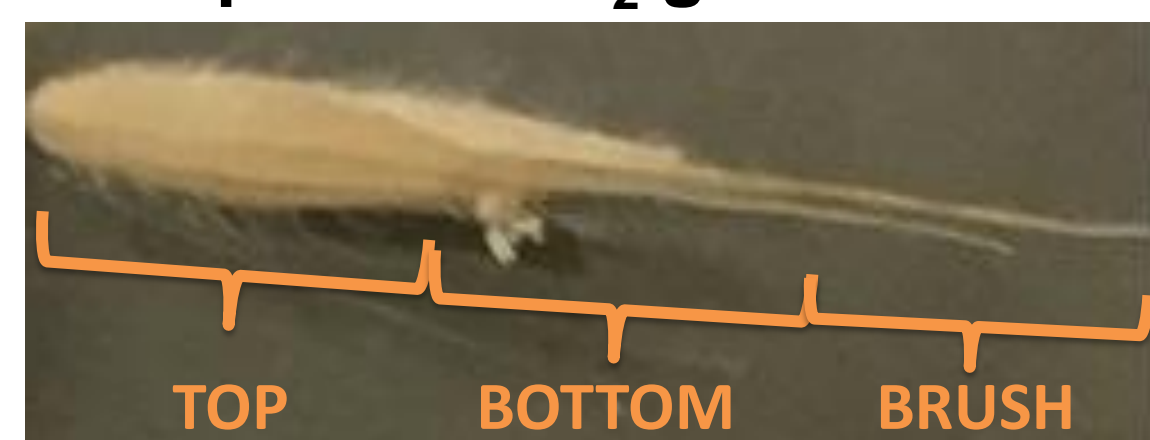
Control Seed
40 mL of DI water

Seed in *Pseudomonas*
inoculated DI water (400 μ L in 40 mL)



- Soak at room temperature, sealed with Parafilm, no direct sunlight for 24 hrs

Dry all samples with N_2 gas then cut into three segments:



- ToF-SIMS imaging was conducted using **delayed extraction mode**

- **Bi^{3+} ion source; 25 keV**
Top: 200 μ m X 200 μ m
Bottom: 200 μ m X 200 μ m
Brush: 50 μ m X 50 μ m



Results

Spectral Comparison

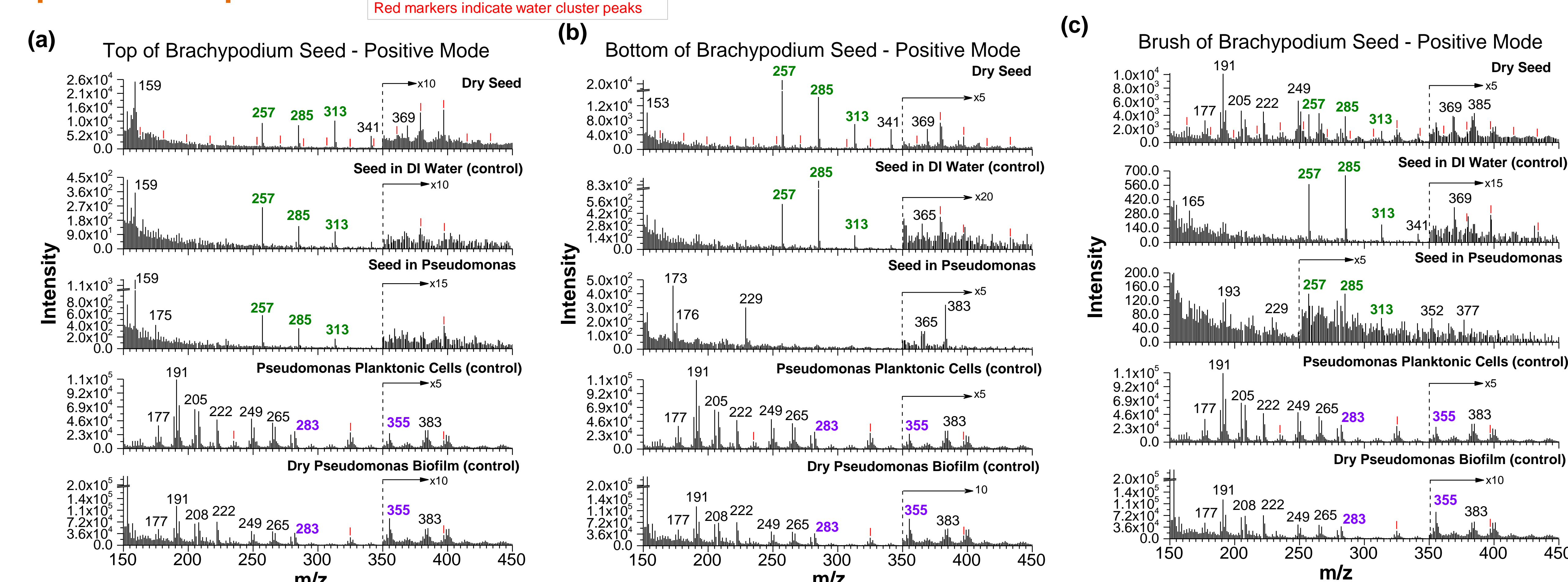


Figure 1. Comparison of SIMS mass spectra between m/z 150-450 in the positive mode for each sample: dry seed, seed in DI water, seed in *Pseudomonas*, *Pseudomonas* planktonic cells, and a dry *Pseudomonas* biofilm: (a) top of seed, (b) bottom of seed, and (c) brush of seed.

Principal Component Analysis

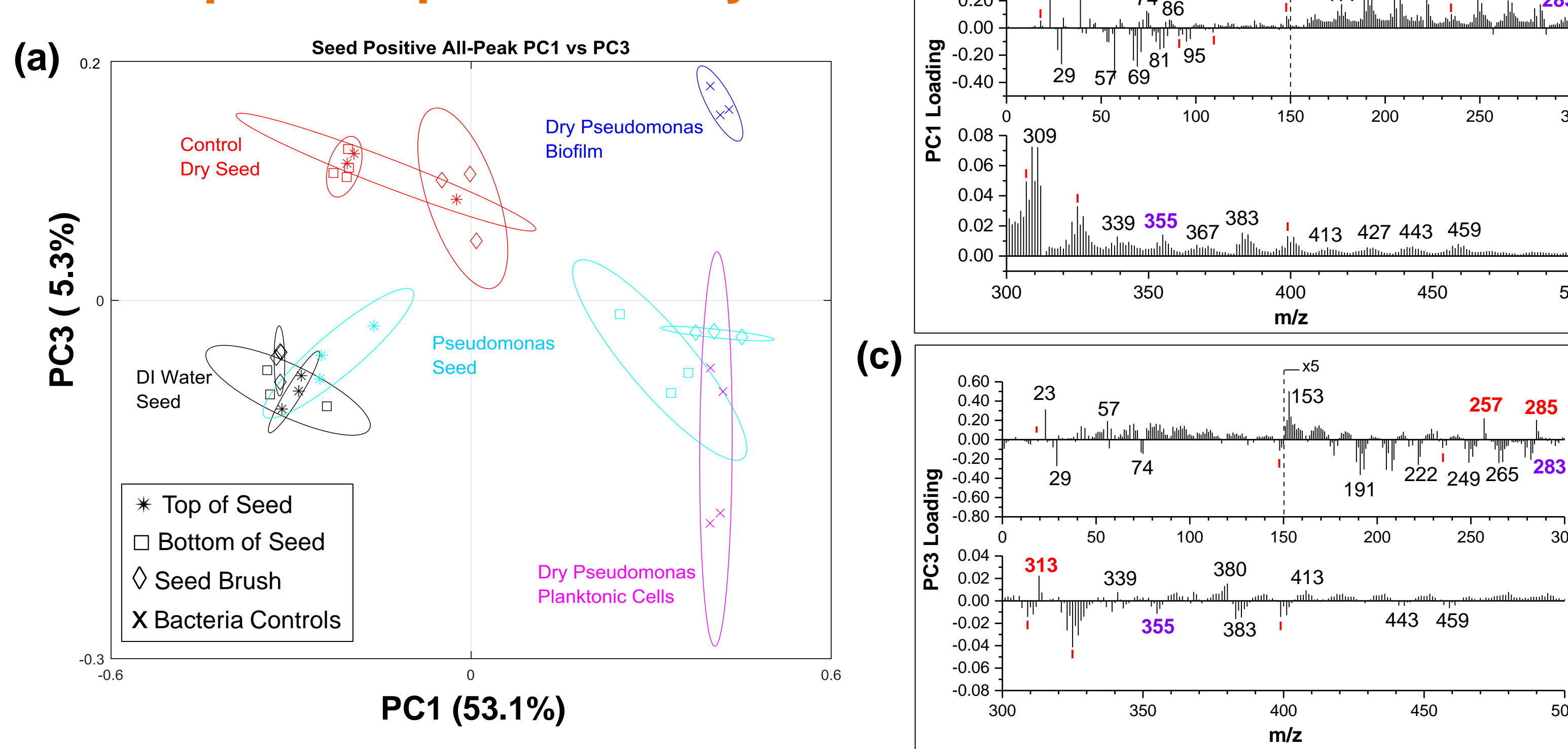


Figure 2. Spectral principal component analysis results: (a) PC1 vs. PC3 score plot, (b) PC1 loading plot, and (c) PC3 loading plot using all peaks in the positive mode.

Table 1. Tentative assignments for selected peaks in Figure 1

Observed m/z	Theoretical m/z	Formula	Assignment	Reference
257.13	257.25	$[C_{16}H_{32}O_2 - H]^+$	Protonated form of Palmitic acid	
285.14	285.28	$[C_{17}H_{34}COOH - H]^+$	Protonated form of Stearic acid	Keune, et al., 2005
313.17	313.30	$[C_{20}H_{32}O_2 - H]^+$	Protonated form of Arachidic acid	
283.10	283	----	Fragment of cardiolipin	Thompson, et al., 2004
355.16	355	----	Fragment of cardiolipin	

2D Imaging

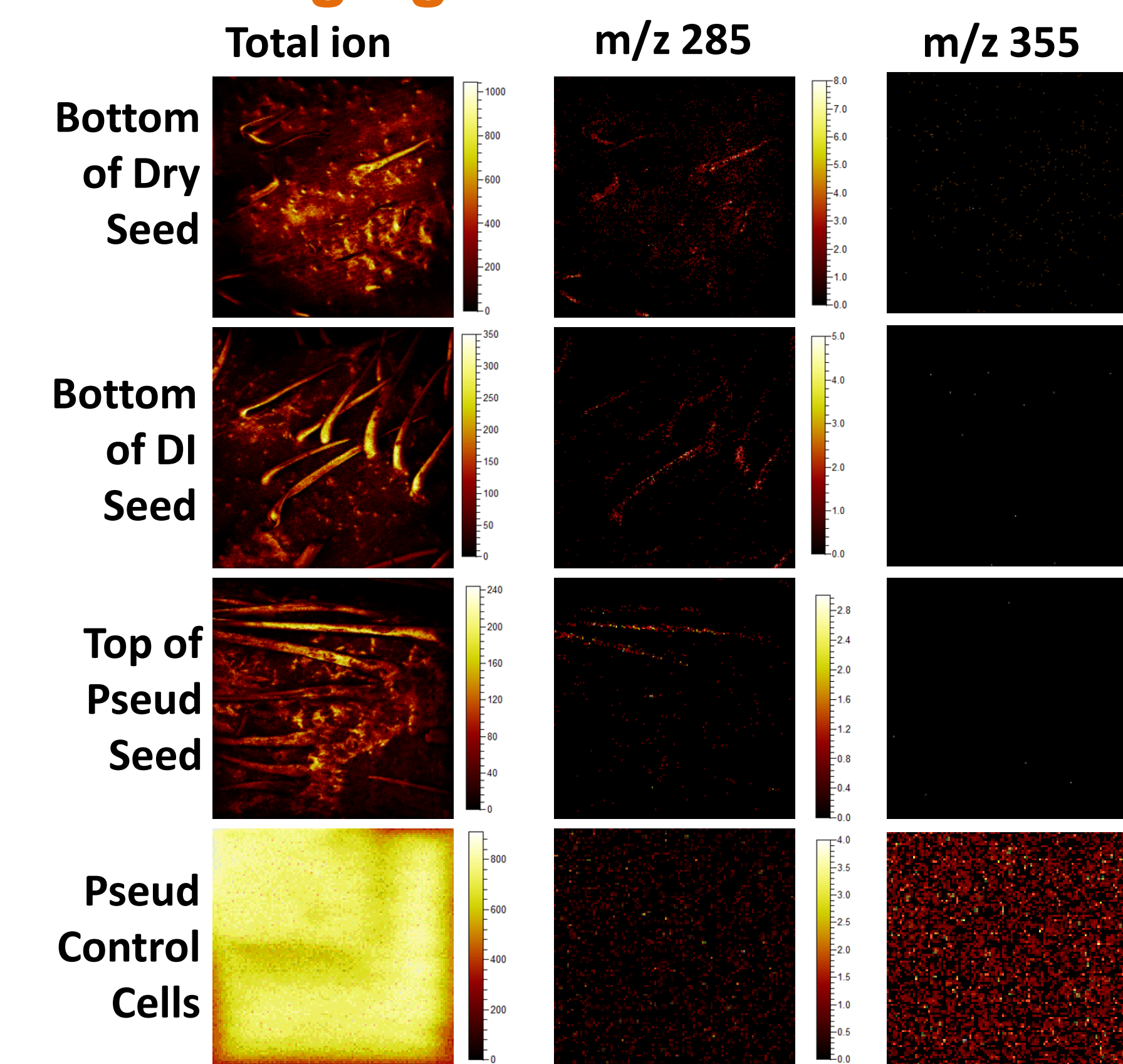


Figure 3. Distribution of selected peaks over the surface of each sample. m/z^+ 285 appears on the hairs of the seeds. Note: each image has a unique scale

Summary and Future Work

- Successfully used delayed image extraction to study *Brachypodium* seed and PGPB for the first time (**Figure 3**)
- Comparison of mass spectra, along with PCA and 2D imaging provides initial characterization of *Brachypodium* seed surface (**Table 1**)
- It is challenging to identify peaks since the number of spectra using MSI for plant and seeds (other than trees) is relatively low [1]
- Future work could use tandem mass spectrometry (MS/MS) in order to distinguish overlapping peaks, isobars, and/or isomers [1]
- Consider comparing methods of sample preparation in future work. Sample handling is the most crucial step in MSI. [2]

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References

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