INTRODUCTION

With the expansion of populations across the globe, challenges faced in the scientific community are evolving continuously. Just as concerns of agricultural sustainability in the form of weeds are rising, similar situations are arising in the agriculture world. Farmers are often treated to herbicides to control the growth of weeds which cause for nutrients and ultimately reduce crop yields. However, just as microbes develop resistance to antibiotics, weeds are also developing resistance to herbicides.

DESI-MS imaging has been increasingly employed given its simplicity, with virtually no sample preparation required, its atmospheric conditions and weight of molecular information. The objective of this work was to determine the unique patterns of molecular biomarkers that could be identified using DESI-IMS-MSI and their applicability to phenotyping important agronomic traits.

METHODS

Sample preparation

Batches of laboratory cultivated grass weed and crop species were removed from the overall growth and adhered to a standard glass microscope data using double-sided tape.

Instrumentation and setup

All analyses were performed as a hybrid TOF-OF-MS-IMS system, with the integrated TOF ion trap used to isolate the ions of interest used to separate ions by ion mobility in the gas phase. The instrument was operated in mass isotopomer mode, in both positive and negative modes of analysis.

Data management and analysis

DESI-IMS-MS datasets were examined, with Masslynx, Driftscope, then processed and visualized by HDI, version 1.4. Multivariate analysis tool (EzInfo). Each square area represents a single ROI and covers a 100 pixel area. The 2D stage DESI (Fisons) stage was mounted directly using wave ion guide optics used in HDI software, subsequent to scanning of the sample preparation required, its atmospheric conditions and weight of molecular information. The objective of this work was to determine the unique patterns of molecular biomarkers that could be identified using DESI-IMS-MSI and their applicability to phenotyping important agronomic traits.

RESULTS

For all initial experiments two species of weed grasses were analyzed: Black-grass (Alopecurus myosuroides) ryegrass (Lolium rigidum) and a cereal crop, wheat (Triticum aestivum). Within the wheat two different cultivars (i.e. Coronet and Revelation) were selected to give early indication of the possibility for intra-species differentiation.

Typically, DESI imaging of both and other cereal cultivars is performed by a solvent composition of merely methanol, with a smaller portion of water. However, when analyzing the blades of weed grass, the addition of chloroform (up to 4% of the total volume) to the spray solvent greatly enhanced the number of molecular details detected. For example, the rice plants were coated with a mixture containing all samples for comparison and relative variability. Figure 3 shows a comparison of the mass spectra generated, plotted to the m/z range of 400-600. Although there is a lot of similarity between the spectra, some potentially characteristic peaks are observed, m/z 621 is highlighted in this case.

Figure 3. Example of acquisition area setup as shown and implemented in NIX software, subsequent to scanning of the sample preparation required, its atmospheric conditions and weight of molecular information.

Figure 4. Unsupervised PCA score plot (A) and Loadings plot (B) resulting from the ROI that were exported as shown in Figure 4. These ion images demonstrated the majority of selected rice was separated from the rice samples combination of ryegrass and black-grass. Fewer m/z were observed as being located within the wheat shows however, whereas one example is shown in figure 4.

Figure 5. Images of NE tiles generated from grass species analyzed. From left to right: Blackgrass, ryegrass and wheat. (Coronet then Revelation). All images are displayed on individual greyscale intensity gradients (as depicted on image). All data for images was normalised against the TIC in HDI 1.4 software.

Figure 6. Mass spectra across the m/z range of 400-600 for all four species (as described). A potential species specific ion is observed in the mass spectrum acquired from the rice plant in HDI software. The ion was identified at a relative error of 1.5 ppm with a signal intensity four times higher than the next highest signal. All data for images was normalised against the TIC in HDI 1.4 software.

Data management and analysis

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Conclusions

In addition to the initial work, further investigation began into the mass spectrometric differentiation of the same grass species (cv. Coronet and Revelation). Two populations of the same black grass species were chosen. Further investigations are required to identify any possible differentiators for wild species that were common across different biological replicates within the two populations are shown in Figure 8 and were used to confirm reproducibility across biological replicates within the two populations.

Figure 7. The drift plot as exported from HDI 1.4 shows the presence of m/z 202 with near identical retention times and showed different localization in the black grass and wheat samples. These ions could truly belong to two different structural species (isomers). Or alternatively, they might possess the same structure but with one ion having been generated by fragmentation after the IMS cell and therefore sharing the same drift time. To establish the identity of the ions it would be necessary to perform a transfer IMS-MS/MS experiment directly from the samples containing the potential precursors.

CONCLUSION

• Endogenous species with the potential to differentiate between black-grass, ryegrass and wheat were detected with the aid of unprocessed PCA and the corresponding ion images shown in HDI.

• It was possible to detect a number of small molecule species that were common across different biological replicates of the same and differing populations of different species.

• Further investigations are required to identify any possible differentiators for wild type and multi-resistant populations of weed-grasses.

REFERENCES


