On-Tissue Boronic Acid Derivatization for the Analysis of Vicinal Diol Metabolites in Maize with MALDI-MS Imaging

Trevor T. Forsman, Maria Emilia Dueñas[#], Young Jin Lee*

Department of Chemistry, Iowa State University, Ames, IA 50011, USA

[#] Current address: Newcastle University Biosciences Institute, Newcastle University, Newcastle upon Tyne, NE2 4HH, UK

* To whom correspondence should be addressed.

E-mail: yjlee@iastate.edu

Abstract

Derivatization reactions are commonly used in mass spectrometry to improve analyte signals, specifically by enhancing the ionization efficiency of those compounds. Vicinal diols are one group of biologically important compounds that have been commonly derivatized using boronic acid. In this study, a boronic acid with a tertiary amine was adapted for the derivatization of vicinal diol metabolites in B73 maize tissue cross-sections for mass spectrometry imaging analysis. Using this method, dozens of vicinal diol metabolites were derivatized, effectively improving the signal of those metabolites. Many of these metabolites were tentatively assigned using high resolution accurate mass measurements. In addition, reaction interference and cross-reactivity with various other functional groups were systematically studied to verify data interpretation.

Introduction

Mass spectrometry imaging (MSI) is an important analytical tool that provides spatial and chemical information of metabolites in tissue samples.[1,2] As MSI has become more prevalent, so has the desire to improve the spatial resolution, thus allowing for the localization of metabolites down to the cellular and sub-cellular level.[1,2] Matrix-assisted laser desorption/ionization (MALDI)-MSI is an attractive technique towards high-spatial resolution imaging due to its soft ionization and small sampling size.[3, 4, 5, 6] In MALDI-MSI achieving high spatial resolution leads to a decrease in the sampling size per pixel. Due to small sampling size, low abundance compounds may not be detected, especially those with low ionization efficiency. This limitation can be resolved by performing on-tissue chemical derivatization to selectively enhance targeted classes of compounds.

On-tissue chemical derivatization reactions can increase the ionization efficiency of analytes by either providing the analyte a permanent charge or high proton affinity.[7,8,9] Derivatization reactions using Girard's reagent T, 2-picolylamine, and coniferyl aldehyde have been successfully applied to modify carbonyl, carboxylic acid, and primary amine functional groups on tissue, respectively.[7,8,9]

Boronic acids have been used to derivatize sugars, brassinosteroids, and other vicinal diol metabolites to form boronic esters in solution.[10, 11, 12] Recently, this method has been adapted by Kaya *et al.* for MALDI-MSI using a custom synthesized boronic acid, 4-(N-methyl)pyridinium boronic acid. In this study, the synthesized boronic acid was used to visualize catecholamines in adrenal tissues.[13] Here, we adopt a commercially available boronic acid with a tertiary amine, 4-(dimethylamino)phenylboronic acid (DBA), for *in situ* chemical modification MSI of vicinal diols and applied this strategy to explore the metabolite coverage in maize stems, roots, and leaves, determining as many possible features that were the result of this reaction. As a result, a reactivity screening had to be conducted to determine what side-reactions occur, so false identification could be minimized.

Material and Methods

Materials

Catechol (>95%), HPLC grade acetonitrile (ACN), HPLC grade methanol (MeOH), and HPLC grade water were purchased from Fisher Chemical (Pittsburg, PA, United States). Myristic acid (99.7%) and vanillic acid (>97%) were purchased from Fluka (Munich, Germany). Adenine (99%), 2-methoxy-4-propylphenol (≥99%), 4-isoquinolinecarboxaldehyde (97%), 5-

methoxysalicylic acid (98%), citronellol (95%), glucose (99.5%), glucose-6-phosphate (≥98%), glycerol-3-phosphate (>93%), progesterone (>99%), sodium lactate (98%), trifluoroacetic acid (TFA) (99.5%), and the matrices, 2,5-dihydroxybenzoic acid (DHB) (98%), 1,5-diaminonaphthalene (DAN) (97%) and α-cyano-4-hydroxycinnamic acid (CHCA) (≥98%), were purchased from Sigma Aldrich (St. Louis, MO, United States). D-(+)-glyceraldehyde (85-90%) was purchased from Carbosynth (Compton, United Kingdom). Hexadecanal (≥98%) was purchased from Cayman Chemical (Ann Arbor, MI, United States). DBA (96%), and 3-hydroxy-4-methoxybenzoic acid (99%) were purchased from Acrōs Organics (Geel, Belgium). Gelatin from porcine skin (300 bloom) was purchased from Electron Microscopy Sciences (Hartfield, PA, United States). B73 maize seeds were obtained from Dr. Marna Yandeau-Nelson at Iowa State University.

Preparation for Standard Analysis

For the analysis of standard samples, 1 mM of each standard (catechol, glucose, myristic acid, sodium lactate, glyceraldehyde, vanillic acid, 2-methoxy-4-propylphenol, 4-isoquinolinecarboxaldehyde, 5-methoxysalicylic acid, citronellol, progesterone, hexadecanal, and 3-hydroxy-4-methoxybenzoic acid) was prepared individually in MeOH and spotted (2 μL) onto a μFocus LDI plate (Hudson Surface Technologies, Old Tappan, NJ, United States). For adenine, glucose-6-phosphate, and glycerol-3-phosphate, standard samples were prepared to 1 mM in H_2O. The spots were completely dried prior to the application of the derivatization agent and the matrix. When calculating reaction efficiency, three sets of replicates were analyzed for both derivatized and underivatized samples in positive ion mode using silver as a matrix. Acidic compounds were also analyzed in negative ion mode using DAN as a matrix.

Maize Tissue Growth

B73 maize stems and roots were grown as described in Dueñas *et al.*[9] In brief, maize seeds were arranged along the top of a moist paper towel. The seeds were staggered with their embryos facing down and DI water was used to wet the paper towel. Then, the paper towel was rolled tight enough to prevent the maize seeds from moving. The roll was then secured with tape and placed in a 1L beaker half-filled with DI water. The beaker was placed in the dark and the seeds were allowed to grow. Once the roots had grown to 10-14 cm in length (ca. 10 days), the tissue was harvested and collected 1 cm above and below the seed, for the stem and root, respectively.

For maize leaf imaging, maize seeds were planted in soil and grown in a climate-controlled green house as described in Dueñas *et al.*[9] In the greenhouse, 30% humidity was maintained under a diurnal cycle of 16 hours of light at 27 ^{o}C and 8 hours of dark at 24 ^{o}C. The sections of leaves were harvested 11 days after planting and collected at the midpoint of the third true leaf.

Following harvest, the plant tissue samples were flash-frozen in 10% (w/v) porcine gelatin solution in a cryo-mold and flash-frozen using liquid nitrogen. Tissue samples were then cryo-sectioned at 10 μm thickness at -20 ^{o}C, and collected using Cryo-Jane tape (Leica Biosystems, Wetzlar, Germany) as described in Korte *et al.*[2] Prior to derivatization and matrix application, the samples were vacuum dried while gradually warming to ambient temperature.

Derivatization Reaction

A solution of the derivatization reagent, DBA, was prepared at 50 mM in methanol. This solution was applied to tissue sections or a μFocus LDI plate using a heated nebulizer (TM-Sprayer; HTX-Scientific; Chapel Hill, NC, United States). The TM-Sprayer conditions for the derivatization agent solution were the following: nebulizer temperature of 30 °C, flow rate of 30 μL/min, sprayer velocity of 1200 mm/min, track spacing of 3 mm, and a nitrogen curtain gas pressure of 10 psi. Eight passes were made under these conditions resulting in the surface density of DBA to be 3.3 nmol/mm^2.

Matrix Application

A sputter coater (Ted Pella, Redding, CA, United States) was used to apply a 1.7 nm thick layer of silver as a matrix (sputter time of 5 seconds at 40 mA).[15] A TM-Sprayer was used to apply the organic matrices. DHB solution was made to 40 mg/mL in 70:30 MeOH:H_2O and applied using the following conditions: nebulizer temperature of 75 °C, flow rate of 100 μL/min, sprayer velocity of 1200 mm/min, track spacing of 3 mm, and a nitrogen curtain gas pressure of 10 psi. Eight passes were made under these conditions resulting in the surface density of DHB to be 0.0576 μmol/mm^2. CHCA solution was made to 10 mg/mL in 70:30 ACN:H_2O with 0.1% TFA and applied using the following conditions: nebulizer temperature of 75 °C, flow rate of 120 μL/min, sprayer velocity of 1200 mm/min, track spacing of 3 mm, and a nitrogen curtain gas pressure of 10 psi. Four passes were made under these conditions resulting in the surface density of CHCA to be 7.05 nmol/mm^2. DAN solution was made to 10 mg/mL in 90:10 ACN:H_2O and applied using the following conditions: nebulizer temperature of 30 °C, flow rate of 100 μL/min, sprayer velocity of 1200 mm/min, track spacing of 2.5 mm, and a nitrogen curtain gas pressure

of 10 psi. Four passes were made under these conditions resulting in the surface density of DAN to be 8.43 nmol/mm^2.

Mass Spectrometric Data Acquisition and Analysis

A MALDI-linear ion trap-Orbitrap hybrid mass spectrometer (MALDI-LTQ-Orbitrap Discovery; Thermo Scientific, San Jose, CA, United States) equipped with an external laser of diode pumped Nd:YAG (355 nm; UVFQ, Elforlight Ltd.; Daventry, UK) was used for analysis. The laser spot size was 15 μm measured from a laser burn mark on a slide sublimation-coated with DHB. The laser energy was ~1 μJ/shot. Mass spectra of tissue samples were acquired in both positive and negative ion mode for scan range of *m/z* 100-1000 using an Orbitrap analyzer (mass resolution of 30,000 at *m/z* 400), with 10 laser shots per spectrum. MS imaging data were acquired at a raster step of 20 μm. For calculating reaction efficiency, mass spectra were acquired in the linear ion trap analyzer, using zoom mode, 5 laser shots, and a raster step of 50 μm. MS/MS was performed using the linear ion trap analyzer with normalized collision energy of 50. Data analysis was performed using Image Quest (Thermo Scientific) and MSiReader software.[14]

Results and Discussion

Mass spectrum of boronic acid reaction with catechol standard.

Scheme 1A shows the reaction of DBA with vicinal diols resulting in the formation of a boronic ester. When this reaction was performed by spraying the DBA solution to the sample,

the microdroplets formed on surface rapidly evaporated minimizing the reverse reaction (i.e. hydrolysis of boronic ester). This reaction was first performed on a catechol standard as shown in **Figure 1**. For this analysis, a thin layer of sputtered silver was used as a matrix.[15] Interestingly, as shown in the zoomed-in region (**Figure 1A**), the unreacted DBA signal is dominated by the peak at *m/z* 164.088 corresponding to $[DBA\text{-}H]^+$, followed by the radical ion at *m/z* 165.095, $DBA^{+\bullet}$. As it is unlikely for neutral DBA to lose a hydrogen anion, we propose the loss of a hydrogen molecule from protonated DBA as shown in **Scheme 1B**. This reaction is attributed to occur during the MALDI process, presumably induced by the laser, since only protonated DBA is observed when performing electrospray ionization (ESI) (**Figure S1**). The protonated molecule peak, $[DBA+H]^+$, is also present at *m/z* 166.104 in **Figure 1A**, although the ion signal for the hydrogen loss peak, $[DBA\text{-}H]^+$, is more than ten times greater for a wide pH range (pH= 4-9), suggesting that this reaction is highly efficient and pH independent. The loss of hydronium ion at *m/z* 146.077, $[DBA\text{-}H_2O\text{-}H]^+$, is assigned as the consecutive reaction of losing water after the loss of hydrogen molecule in **Scheme 1B**.

Similarly, the loss of hydrogen molecule is dominant at *m/z* 238.103 for the derivatized catechol as shown in the zoomed in spectrum, **Figure 1B**, which is also the base peak of the mass spectrum (**Figure 1D**). The molecular radical ion of derivatized catechol exists at *m/z* 239.110, but only at 25% of the hydrogen loss peak. The unique isotope pattern of ^{10}B verses ^{11}B is observed for all DBA related peaks with the mass difference of 0.996 Da and 1:4 abundance difference, such as *m/z* 237.107 and 238.103 corresponding to the hydrogen loss of ^{10}B- and ^{11}B-derivatized catechol, respectively. This unique pattern provides confidence in the identification of DBA derivatized compounds. Unreacted catechol is detected as a silver ion adduct at *m/z* 216.941 and 218.940, as ^{107}Ag and ^{109}Ag adduct, respectively, but in very low abundance (see

Figure 1D). When comparing the signal of catechol before and after the derivatization reaction, the conversion percentage was 98% at the optimized condition and the signal improved about six-fold. Moreover, there is also a cluster of peaks around *m/z* 440.249 corresponding to the boroxine of DBA (**Figure 1C, Scheme 1C**), three boronic acids forming a cyclic structure with the loss of three waters, which has been previously reported to be formed by heating boronic acid[16, 17]. Boroxine synthesis may be favored when boronic acid is applied to sample surface under nitrogen curtain gas, even at temperatures just above room temperature. The boroxine signal could be reduced by using a lower concentration of boronic acid; however, a slight excess of boronic acid is used to ensure the maximal derivatization of diols.

Investigation of side reactions of boronic acid.

Due to using excess boronic acid in the reaction, double derivatization (i.e. two boronic acids reacting with a single compound) is expected. Double derivatization can occur with any compound that has at least two sets of vicinal diols. To confirm the likelihood of double derivatization, boronic acid was sprayed on a glucose standard spot. As shown in **Figure 2**, the doubly derivatized ion signal was about four times the intensity of the singly derivatized ion. Both singly and doubly derivatized ions were of significant intensity, with three times more doubly derivatized ion signals than singly derivatized. No triple derivatization was observed for glucose; however, triple derivatization might still be possible for a metabolite with more than two sets of diols. Hence, caution is advised when analyzing a complex mixture or a tissue sample.

Even though DBA derivatization enhanced the analyte signal when performed on standards, a few problems were encountered. The first major issue was that sodium ions interfered with the reaction between boronic acid and the diol compounds. For example, when performing the boronic acid derivatization reaction for a mixture of standards that contained a sodium counter ion, such as sodium glucose-6-phosphate and sodium glycerol-3-phosphate, the signal for the derivatization product significantly suffered. Sodium ions form a complex with vicinal diols, interfering with the derivatization reaction. To confirm that sodium ions suppress the reaction, sodium acetate was sprayed on standard catechol to a final surface density of 0.13 nmol/mm^2 prior to derivatization. This resulted in a 3-fold signal decrease compared to sodium-free conditions. This was not a concern for the MS imaging of plant tissues used in this work due to low sodium ion content. Moreover, sodium interference does not seem to be a major problem for other samples, such as animal tissues, unless sodium ion content is too high. For instance, Kaya *et al.* successfully performed on-tissue derivatization of catecholamines on pig adrenal gland tissues using a pyridinium boronic acid.[13]

Side reactions were also investigated between DBA and the organic matrices, DHB and CHCA. **Figure 3** shows the MALDI mass spectra of DBA when DHB and CHCA were used as matrices. Both matrices reacted with DBA, even though neither has a vicinal diol, suggesting that this reaction is not completely selective to vicinal diols. DHB reacts with DBA at high efficiency as its reaction product is dominant in MALDI-MS spectrum (**Figure 3A)** with almost no boronic acid or boroxine present. This efficiency is attributed to the vicinal position between the carboxylic and phenol group of DHB. Interestingly, a hydrogen loss at *m/z* 282.096, ^{11}B-[DHB + Δm_{der} - H]$^+$, is present less than the radical ion at *m/z* 283.103, ^{11}B-[DHB + Δm_{der}]$^{+\bullet}$, unlike other derivatized compounds of vicinal diols such as catechol in **Figure 1B**. The reaction

of CHCA with DBA differed from the reaction displayed in **Scheme 1A**. This reaction resulted in a single water loss, presumably due to an esterification between a carboxyl group in CHCA and one of the hydroxyl groups in boronic acid. This side reaction has low reaction efficiency with the derivatized signal being <10% of the combined CHCA peak intensities at *m/z* 212.032, $[CHCA+Na]^+$, and *m/z* 234.014, $[CHCA+2Na-H]^+$, as shown in **Figure 3B**. This type of side reactions with carboxylic acids is common as confirmed using a myristic acid standard, but the signal is very low as shown in **Figure 4**. To avoid side reactions with organic matrices, LDI-MS can be performed without matrix as suggested by Kaya *et al.*[13] or non-organic matrices can be adopted such as silver sputtering which was used in this study. The ion signal increased by 20-fold when using silver as a matrix for the DBA derivatized catechol compared to when no matrix was used.

To better understand the side reactions of DBA, a systematic study was performed to determine the reaction efficiency of selected standard compounds reacting with DBA (**Table 1**). Various chemical functional groups are included such as aldehyde, hydroxyl, phenol, primary amine, secondary amine, and ketone. Compounds containing both a phenol and carboxylic acid group, similar to DHB, were also tested in the ortho-, meta-, and para-position. DHB was not included in this study because of large experimental error coming from background DHB contamination in the MALDI source. The ionization efficiency is significantly different between reactant and product, especially due to the addition of a t-amine group; therefore, the reaction efficiency cannot be simply calculated from the intensity ratio of product and reactant. For the generalized reaction of DBA including **Scheme 1A, 1B** and any side reaction, $M + DBA \rightarrow P$, where M is analyte of interest and P is any reaction product(s), the reaction efficiency can be calculated as in equation 1.

$$Reaction\ efficiency\ (\%) = \frac{reacted\ amount}{initial\ amount} = (1 - \frac{M_f}{M_i}) \times 100\% \qquad (1)$$

Here M_i and M_f indicate the initial and final amounts of analytes, respectively. The ratio of M_f over M_i can be accurately measured in mass spectrometry, as they are the same molecule with the same ionization efficiencies.

Acidic analytes are measured in negative ion mode while others are measured in positive ion mode. Other than vicinal diol (catechol, 95%), only carboxylic acids are reactive with DBA. Aliphatic carboxylic acid (myristic acid) has low conversion (3%), but aromatic carboxylic acid (benzoic acid) has high conversion when hydroxyl group is present at o- (5-methoxysalicylic acid) and m- position (3-hydroxy-4-methoxybenzoic acid), 76% and 49%, respectively. When hydroxy group is in p-position (vanillic acid), the conversion percentage (4%) is similar to myristic acid. In addition, the reaction product has only a single water loss, just like the fatty acid reaction, suggesting that it only reacted with the carboxylic acid functional group. It is consistent with the fact phenol itself does not react (0%). The high reaction efficiency of benzoic acid with o-hydroxyl group, followed by m-, suggests the importance of geometric constraint for a compound to effectively react with boronic acid. The reactions of DBA with o- and p- positioned phenol and carboxylic acid groups were both significant and were considered when performing derivatization using DBA.

On-tissue derivation of boronic acid for MS imaging.

With better understanding of DBA reactivity with various chemical functional groups, this reaction was applied to maize tissues for MALDI-MS imaging. Reaction conditions including derivatization reagent concentration, TM-sprayer flow rate, number of TM-Sprayer

passes, and matrix selection were optimized based on the number of new unique features observed only on the derivatized maize tissue (leaves, roots, and stems). These same conditions were used when determining the reaction efficiency of selected standards in the previous section. In this analysis, metabolite features in derivatized tissues were compared to those of underivatized tissues. Unique features due to derivatization were determined using a python script (Supplementary Info) to find all the peaks that appeared only in the derivatized tissues and have a $^{10}B/^{11}B$ isotope pattern. Then, peaks that differed by the mass of one or two hydrogens were removed to avoid redundant counting due to various ion forms of hydrogen-loss, radical form, and H^+ adduct. Additionally, peaks that were not localized to the tissue were removed; this was determined by visually inspecting MSiReader generated images. Following the removal of non-derivatized and duplicate features, the number of uniquely derivatized metabolite features were 27, 40, and 43 in the leaf, root, and stem cross-sections, respectively, a total of 84 unique features after the removal of features that appear in multiple tissues (**Table S1**).

Tentative assignments were made using the Maize Genetics and Genomics Database and the METLIN library using a mass tolerance of 10 ppm.[18,19] Features were assigned accounting for the potential of hydrogen loss (**Scheme 1A**), molecular radical ion, and protonated ion, as well as possible adduct formation with a sodium ion, potassium ion, or silver ion. Using this screening method 10 features were tentatively assigned by comparing the exact masses of known maize metabolites in the Maize Genetics and Genomics Database. An additional 23 peaks were tentatively assigned by exact mass matching using the METLIN library and having a known presence in plant tissues, although not necessarily in maize. These 33 tentative identifications are listed in **Table 2** and their MS images are shown in **Figure 5**, **6**, and **7** for leaf, root, and stem cross-sections, respectively.

The side reaction between DBA and compounds containing a carboxylic acid and hydroxyl group in close proximity are not able to be distinguished from the reaction between DBA and diols. Both reactions resulted in a mass change corresponding to two water losses and the addition of DBA. Due to the inability to distinguish these reactions, tentative assignments were made among the above 84 potential unique features which can be also assigned as metabolites containing both phenol and carboxylic functional groups. Of the tentative assignments made, 6 were known maize metabolites from the Maize Genetics and Genomics Database and 3 were from the METLIN library. These 9 metabolites are listed in **Table S2** and shown in **Figure S2B**, **Figure S3B**, and **Figure S4B** for leaf, root, and stem cross-sections, respectively.

The potential side reaction with carboxylic acids was also explored among the 84 unique features in the derivatized tissue sections by subtracting the corresponding mass change and comparing with Maize Genetics and Genomics Database and METLIN library. There were 34 features that could be assigned as a carboxylic acid metabolite based on exact mass and the compound having a known presence in plant tissue. Since the reaction efficiency with fatty acids is low (e.g., 3% for myristic acid), many of 34 assignment might not be real. Considering the fact that the ion signal of unreacted fatty acids is much higher in negative mode than that of the derivatized in positive mode (e.g., 50 times for myristic acid), maize tissues were analyzed in the negative mode to test how many of them are consistent among the 34 potential assignments as carboxylic acids. Among those, only 5 (**Table S3**) appeared underivatized in the negative ion mode and none of them have higher signals than that of derivatized in positive mode. Hence, we conclude that the side reaction is minimal between DBA and carboxylic acid maize metabolites.

Nevertheless, the five potential fatty acid metabolites derivatized with DBA are shown in **Figure S2C**, **Figure S3C**, and **Figure S4C** for leaf, root, and stem cross-sections, respectively.

Most of the tentatively assigned features that contain vicinal diols were not observed in the underivatized sample, with the exception of monosaccharides or their derivatives. In **Figure 5**, **6**, and **7**, underivatized ions are mostly shown as a silver adduct as they are typically most abundant. Monosaccharides were observed in both the derivatized and underivatized conditions at similar or slightly higher intensity, including $C_5H_{10}O_5$, and $C_6H_{12}O_6$. Aside from monosaccharides, many compounds were observed only in the derivatized sample and have unique localization as shown in **Figure 5**, **6**, and **7**. These selected compounds consist of multiple groups of biologically significant compounds including a lipopolysaccharide (LPS) precursor, keto-deoxy-manno-octulosonic acid (KDO), glycosides such as arbutin, vanilloloside, dihydroxyphenyl-galloyl-glucopyranoside, and hydroxy-inol[glucosyl-glucoside], as well as other small metabolites. KDO is a metabolite produced by both bacteria and higher-level plants.[20] In plants, KDO is observed predominantly in young leaves, and is present in the epidermis as shown in **Figure 5**. This metabolite is a precursor to lipid A, a part of LPS, which has a role as an inducer of systematic acquired resistance. In a previous study, LPS was determined to be localized to the epidermis of Arabidopsis leaves, matching the localization that we observed with KDO in maize.[21]

Many glycosides were also tentatively assigned including arbutin, vanilloloside dihydroxyphenyl-galloyl-glucopyranoside, and hydroxy-inol[glucosyl-glucoside]. The peak at *m/z* 401.164 was assigned as arbutin, which was present in the epidermis of the leaf as shown in **Figure 5**. Arbutin is a glycosylated hydroquinone, which has been reported to be present in leaves as a defense against drought.[22] The localization of arbutin to the epidermis supports this

finding. The cuticle wax of leaves protects plants from losing water during drought and other instances of stress. Since arbutin serves a protective role against drought, its localization to the epidermis is expected. The derivatized peak at *m/z* 218.098 is most likely glyceraldehyde (diol) or lactate (hydroxyl and carboxylic acid in vicinal position) and observed in both root and stem tissues (**Figure 6** and **7**). In both these tissues, this metabolite is distributed throughout the pith, pericycle, endodermis, cortex, and epidermis, but is absent from the xylem (tissue anatomy in **Figure 6**). Unfortunately, glyceraldehyde and lactate cannot be distinguished with accurate mass alone as they all have the same molecular formulae, $C_3H_6O_3$. In addition, performing MS/MS on these derivatized compounds mostly results in fragments that correspond to the derivatization agent, DBA; thus, we were not able to confidently confirm compound identifications. Nevertheless, it is very likely both are present in plant tissues.

Conclusion

In this study, *in situ* derivatization of vicinal diol compounds with a tert-amine containing boronic acid was investigated to improve the ion signals of vicinal diols for MALDI-MSI. Potential issues of this reaction were investigated including reaction suppression by sodium ions and side reactions with organic matrices. A systematic study was performed for the reaction with various chemical functional groups. There was minimum side reaction for compounds with a carboxylic acid (conversion percentage, 3-4%), but significant side reaction was observed for aromatic compounds with carboxylic and hydroxyl group in o- or m- position (50-80%). By identify these side-reactions, more confident tentative assignments could be made. In addition, a significant increase in ion signal was demonstrated for vicinal diols and the efficient detection of new metabolite features was made possible from the $^{10}B/^{11}B$ isotope pattern. The optimized

reaction condition was successfully applied to maize tissues of leaf, root, and stem, detecting dozens of metabolite features and their localizations, which was not possible without derivatization.

Acknowledgements

This work was partially supported by National Science Foundation (NSF) (Award# 1905335) and United States Department of Agriculture-National Institute of Food and Agriculture (USDA-NIFA) (2017-67007-25949).

References

1. G. Thiery-Lavenant, A.I. Zavalin, R.M. Caprioli. Targeted multiplex imaging mass spectrometry in transmission geometry for subcellular spatial resolution. *J. Am. Soc. Mass Spectrom*. **2013**, *24*, 609

2. A.R. Korte, M.D. Yandeau-nelson, B.J. Nikolau, Y.J. Lee. Subcellular-level resolution MALDI-MS imaging of maize leaf metabolites by MALDI-linear ion trap-Orbitrap mass spectrometer. *Anal. Bioanal Chem*. **2015**, *407*, 2301

3. A.D. Feenstra, M.E. Dueñas, Y.J. Lee. Five micron high resolution MALDI mass spectrometry imaging with simple, interchangeable, multi-resolution optical system. *J. Am. Soc. Mass Spectrom*. **2017**, *28*, 434

4. A. Römpp, B. Spengler. Mass spectrometry imaging with high resolution in mass and space. *Histochem Cell Bio*. **2013**, *139*, 759

5. B. Spengler, M. Hubert. Scanning microprobe matrix-assisted laser desorption ionization (SMALDI) mass spectrometry: Instrumentation for sub-micrometer resolved LDI and MALDI surface analysis. *Am. Soc. Mass Spectrom*. **2002**, *13*, 735

6. B. Heijs, A. Potthoff, J. Soltwisch, K. Dreisewerd. MALDI-2 for the Enhanced Analysis of N-Linked Glycans by Mass Spectrometry Imaging. *Anal. Chem.* **2020**, *92*, 13904

7. C. Esteve, E.A. Tolner, R. Shyti, A.M.J.M. van den Maagdenberg, L.A. McDonnell. Mass spectrometry imaging of amino neurotransmitters: a comparison of derivatization methods and application in mouse brain tissue. *Metabolomics*. **2016**, *12*, 30

8. Q. Wu, T.J. Comi, B. Li, S.S. Rubakhin, J.V. Sweedler. On-Tissue Derivatization via Electrospray Deposition for Matrix-Assisted Laser Desorption/Ionization Mass

Spectrometry Imaging of Endogenous Fattey Acids in Rat Brain Tissue. *Anal. Chem*. **2016**, *88*, 5988

9. M.E. Dueñas, E.A. Larson, Y.J. Lee. Toward Mass Spectrometry Imaging in the Metabolomics Scale: Increasing Metabolic Coverage Through Multiple On-Tissue Chemical Modifications. *Front. Plant Sci.* **2019**, *10*, 860

10. P. Xin, J. Yan, J. Fan, J. Chu, C. Yan. An improved simplified high-sensitivity quantification method for determining brassinosteroids in different tissues of rice and arabidopsis. *Plant Physiol.* **2013**, *162***,** 2056

11. T. Deng, D. Wu, C. Duan, Y. Guan. Ultrasensitive quantification of endogenous brassinosteroids in milligram fresh plant with a quaternary ammonium derivatization reagent by pipette-tip solid-phase extraction coupled with ultra-high-performance liquid chromatography tandem mass spectrometr. *J. Chromatogr. A* . **2016**, *1456*, 105

12. M. Faraco, D. Fico, A. Pennetta, G.E. De Benedetto. New evidences on efficacy of boronic acid-based derivatization method to identify sugars in plant material by gas chromatography-mass spectrometry. *Talanta*. **2016**, *159***,** 40

13. I. Kaya, S.M. Brülls, J. Dunevall, S. Lange, J. Mårtensson, A.G. Ewing, P. Malmberg, J.S. Fletcher. On-Tissue Chemical Derivatization of Catecholamines Using 4 - (N - Methyl)pyridinium Boronic Acid for ToF-SIMS and LDI-ToF Mass Spectrometry Imaging. *Anal. Chem.* **2018**, *90*, 13580

14. G. Robichaud, K.P. Garrard, J.A. Barry, D.C. Muddiman. MSiReader: An Open-Source Interface to View and Analyze High Resolving Power MS Imaging Files on Matlab Platform. *J. Am. Soc. Mass Spectrom*. **2013,** *24*, 718

15. R.L. Hansen, M.E. Dueñas, Y.J. Lee. Sputter-Coated Metal Screening for Small Molecule Analysis and High-Spatial Resolution Imaging in Laser Desorption Ionization Mass Spectrometry. *J. Am. Soc. Mass Specrom*. **2018**, *30*, 299

16. H.R. Snyder, J.A. Kuck, J.R. Johnson. Organoboron compounds, and the study of reaction mechanisms. primary aliphatic boronic acids. *J. Am. Chem. Soc*. **1938**, *60*, 105

17. J.B. Crumpton, W. Zhang, W.L. Santos. Facile Analysis and Sequencing of Linear and Branched Peptide Boronic Acids by MALDI Mass Spectrometry. *Anal. Chem*. **2011**, *83*, 3548

18. C.M. Andorf, E.K. Cannon, J.L. Portwood, J.M. Gardiner, L.C. Harper, M.L. Schaeffer, B.L. Braun, D.A. Campbell, A.G. Vinnakota, V.V. Sribalusu, M. Huerta, K.T. Cho, K. Wimalanathan, J.D. Richter, E.D. Mauch, B.S. Rao, S.M. Birkett, T.Z. Sen, C.J. Lawrence-Dill. MaizeGDB update: new tools, data and interface for the maize model organism database. *Nucleic acids research*, **2015**, *44*, D1195

19. C.A. Smith, E.J. Want, C. Qin, S.A. Trauger, T.R. Br, D.E. Custodio, R. Abagyan, G. Siuzdak. METLIN: A metabolite mass spectral database. *Ther. Drug Monit*. **2005**, *27*, 747

20. M. Séveno, E. Séveno-Carpentier, A. Voxeur, L. Menu-Bouauiche, C. Rihouey, F. Delmas, C. Chevalier, A. Driouich, P. Lerouge. Characterization of a putative 3-deoxy-D-*manno*-2-octulosonic acid (Kdo) transferase gene from *Arabidopsis thaliana*. *Glycobiology*, **2010**, *20*, 617

21. D. Zeidler, I.A. Dubery, P. Schmitt-Kopplin, U.V. Rad, J. Durner. Lipopolysaccharide mobility in leaf tissue of *Arabidopsis thaliana*. *Mol. Plant Pathol*, **2010**, *11*, 747

22. C. Pop, L. Vlase, M. Tamas. Natural Resources Containing Arutin. Determination of Arbutin in the Leaves of *Bergenia crassifolia* (L.) *Fritsch.* acclimated in Romania. *Not. Bot. Hort. Agrobot. Cluj*, **2009**, *37*, 129

Table 1. Reaction efficiencies of standard compounds with DBA.

Compound	Functional Group(s)	Reaction Efficiency*
catechol	vicinal diol	95 (± 2) %
5-methoxysalicylic acid	ortho phenol & carboxylic acid	76 (± 1) %
3-hydroxy-4-methoxybenzoic acid	meta phenol & carboxylic acid	49 (± 6) %
vanillic acid	para phenol & carboxylic acid	4 (± 5) %
myristic acid	carboxylic acid	3 (± 5) %
4-isoquinoline-carboxaldehyde	aryl aldehyde	0 %
2-methoxy-4-propylphenol	phenol	0 %
citronellol	hydroxyl	0 %
progesterone	ketone	0 %
adenine	1^o and 2^o amine	0 %
hexadecanal	alkyl aldehyde	0 %

* Reaction efficiency is calculated using Equation (1). Numbers in parenthesis are standard deviation from three replicates.

Table 2. Tentative assignments of potential diol compounds in maize tissue based on accurate mass and presence in the Maize Genetics and Genomics Database or the METLIN Database.

Tissue	*m/z**	Molecular Formula (Tentative Assignments)	Δ ppm
root/stem	218.098	$C_3H_6O_3$ (glyceraldehyde)	3
root	220.113	$C_3H_8O_3$ (glycerol)	1
root	278.119	$C_5H_{10}O_5$ (ribulose)	0
leaf/root/stem	290.119	$C_6H_{10}O_5$ (dehydro-deoxy-rhamnonate)	0
stem	306.114	$C_6H_{10}O_6$ (glucono-lactone)	3
leaf/root/stem	308.129	$C_6H_{12}O_6$ (glucose/fructose)	1
leaf	331.142[!]	$C_6H_{13}NO_5$ (glucosamine/galactosamine /fructosamine)	6
leaf	355.144[#]	$C_7H_{14}O_8$ (glucoheptonic acid)	2
leaf	359.174[!]	$C_8H_{17}NO_5$ (deoxy-dimethylamino-glucose)	3
leaf	401.164[#]	$C_{12}H_{16}O_7$ (arbutin)	0
stem	418.167	$C_{12}H_{18}O_8$ (osmundalin/ furaneol glucoside)	2
root/stem	421.174	$C_{11}H_{19}NO_8$ (acetyl-muramate)	9
stem	435.188[$]	$C_{14}H_{21}NO_4$ (codonopsine)	5
root	440.227	$C_{16}H_{24}O_6$ (thymol-glucoside)	7
leaf/root/stem	443.154[$]	$C_{15}H_{17}NO_4$ (dubinidin)	0
root	445.194[#]	$C_{14}H_{20}O_8$ glycoside (vanilloloside/ hydroxytyrosol glucoside)	7
root	449.188	$C_{16}H_{19}NO_6$ (niazirinin)	1
root	454.288[$]	$C_{17}H_{34}O_3$ (hydroxy-heptadecanoic acid)	3
leaf/root/stem	461.166[#]	$C_{17}H_{16}O_7$ flavonoid (dihydrotricetin dimethyl ether/ deoxydryopteric acid)	5
root/stem	463.163[!]	$C_{14}H_{17}NO_7$ (dhurrin)	4
root	468.305[$]	$C_{18}H_{36}O_3$ (hydroxy-octadecanoic acid)	0
stem	496.179	$C_{17}H_{20}O_9$ (feruloylquinic acid/caffeoyl-methylquinic acid)	0
leaf	497.231	$C_8H_{13}O_8$ (keto-deoxy-manno-octulosonic acid)	5
stem	539.281[#]	$C_{25}H_{30}O_5$ (heliocide H)	5
root	569.170[#]	$C_{19}H_{20}O_{12}$ (dihydroxyphenyl-galloyl-glucopyranoside)	0
stem	584.288	$C_{19}H_{34}O_{12}$ (butyl hydroxybutyrate[arabinosyl-glucoside]/ (arabinopyranosyl-glucopyranosyl) butyl hydroxybutanoate)	2
stem	594.218	$C_{22}H_{26}O_{11}$ (symplocoside/ agnuside)	6
stem	649.324	$C_{24}H_{40}O_{12}$ glucoside (dihydrovomifoliol [apiosyl-glucoside]/ dihydroxy-ionol [apiosyl-(glucoside])	4
stem	663.339[#]	$C_{25}H_{42}O_{12}$ glucoside (hydroxy-ionol [glucosyl-glucoside]/ dihydrovomifoliol [rhamnosyl-(glucoside])	3
root/stem	694.206	$C_{29}H_{26}O_{12}$ (gladiatoside C1/ gladiatoside C2)	4
leaf	732.285[%]	$C_{27}H_{44}O_8$ (polypodine b)	3
leaf	792.436[$]	$C_{31}H_{61}O_{10}P$ (PG(12:0/13:0))	4
leaf	942.569	$C_{43}H_{75}O_{12}P$ (PI (34:4))	3

* All experimental *m/z* values correspond to $[M+DBA-2H_2O-H]^+$, unless denoted with an #, %, !, or $ which corresponds to $[M+DBA-2H_2O]^{\bullet+}$, a silver ion adduct, a sodium ion adduct, or a potassium ion adduct, respectively.

Figure Captions

Scheme 1. (A) Derivatization reaction of 4-(dimethylamino)phenylboronic acid (DBA) with vicinal diols. **(B)** Proposed mechanism for the loss of hydrogen molecule from DBA. **(C)** Formation of boroxine during the deposition of boronic acids on the MALDI plate.

Figure 1. MALDI-MS spectrum of catechol derivatized by DBA in positive mode, zoomed-in for the *m/z* range of **(A)** 144 to 168, **(B)** 236.5 to 240, and **(C)** 437 to 443, and **(D)** full *m/z* range of 100-460. DMA and Bor indicate dimethylaniline and boroxine, corresponding to products of DBA decomposition and self-dehydration, respectively. The standard catechol is indicated as 'Cat'.

Figure 2. MALDI mass spectrum of glucose derivatized with DBA. Δm_{der} is 129.075 Da which is the mass of DBA – $2H_2O$.

Figure 3. MALDI mass spectra of DBA using A) DHB and B) CHCA as a matrix. Dimethylaniline (DMA) is a product of DBA decomposition. Δm_{der} and Δm_{der}* are 129.075 Da and 147.086 Da, corresponding to the mass of DBA – $2H_2O$ and DBA – H_2O, respectively.

Figure 4. MALDI mass spectrum of DBA derivatized myristic acid acquired in the positive ion mode. Δm_{der}* is 147.086 Da corresponding to the mass of DBA – H_2O.

Figure 5. Optical images and MS images for maize leaf vicinal diol metabolites. The same intensity scale is used to produce false-color images between the derivatized and underivatized images for each *m/z* ion.

Figure 6. Optical images and MS images for maize root vicinal diol metabolites. The same intensity scale is used to produce false-color images between the derivatized and underivatized images for each *m/z* ion. Underivatized MS images correspond to the silver ion adduct, unless denoted with an # or * which are sodiated and protonated, respectively.

Figure 7. Optical images and MS images for maize stem diol metabolites. The same intensity scale is used to produce false-color images between the derivatized and underivatized images for each *m/z* ion. Underivatized MS images correspond to the silver ion adduct, unless denoted with an #, *, or % which are sodiated, protonated, and potassiated, respectively.