Received: 23 November 2018 | Revised: 21 January 2019 | Accepted: 23 January 2019

DOI: 10.1002/rcm.8397

RESEARCH ARTICLE

WILEY Rapid Communications in Mass Spectrometry

Spatial distribution mapping of molecules in the grains of different rice landraces, using desorption electrospray ionization mass spectrometry

Arunan Suganya[1] | Debal Deb[2] | Thalappil Pradeep[1]

[1] DST Unit of Nanoscience, and Thematic Unit of Excellence, Department of Chemistry, Indian Institute of Technology Madras, Chennai 600 036, India

[2] Centre for Interdisciplinary Studies, Barrackpore, Kolkata 700 123, India

Correspondence
Thalappil Pradeep, DST Unit of Nanoscience, and Thematic Unit of Excellence, Department of Chemistry, Indian Institute of Technology Madras, Chennai 600 036, India.
Email: pradeep@iitm.ac.in

Funding information
Department of Science and Technology

Rationale: Documentation of the metabolite profiles of rice landraces is essential as most of them have been lost due to the conventional practices of cultivation. Therefore, application of mass spectrometry imaging (MSI) will be an appropriate analytical platform for molecular profiling, as it can provide a detailed understanding of the site-specific localization patterns of biomolecules, and the cues concerning metabolic pathways in organisms.

Methods: Desorption electrospray ionization mass spectrometry (DESI-MS) is a relatively non-destructive analytical technique for surface sampling in natural conditions. Here, we report the spatial distribution of diverse molecules in the grains of different rice landraces of India using DESI-MSI. Molecules were identified by ESI-MS and tandem MS analysis of rice extracts. Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) were used for the elemental mapping on the rice grains.

Results: DESI-MSI showed a uniform distribution of choline (*m/z* 104.1), sucrose in the form of its sodium (*m/z* 365.1) and potassium (*m/z* 381.0) adducts, linoleic acid (*m/z* 279.2), 13-HODE-9-HODE (*m/z* 295.2), unidentified molecules with *m/z* 535.3, 559.5, and 561.5 and isoschaftoside (*m/z* 563.1) in the endosperm of rice grains. Gluconic acid (*m/z* 195.0) and signalling phospholipid intermediate molecules were localized in the embryo whereas oryzanol A (*m/z* 601.5) and oryzanol C (*m/z* 615.5) had a restricted localization in the bran region of the grain. SEM-EDS mapping showed the localization of potassium and phosphorus along the bran and embryo.

Conclusions: DESI-MSI revealed the distribution of lipids and sugar molecules in the specific regions of the rice grains. Thus, molecules unique to some rice varieties were identified with this analytical platform. Mass spectrometry imaging of rice along with the elemental mapping by SEM-EDS will be of use in understanding the localization pattern of certain molecules in the context of metals present in the grain.

 © 2019 John Wiley & Sons, Ltd.

1 | INTRODUCTION

Metabolomics is one of the rapidly evolving key "omics strategies" employed in plant research. Understanding the complex metabolome of a biological sample is feasible today with the availability of various forms of mass spectrometry. The capability to combine the spatial information along with the chemical fingerprints of the specimen makes imaging mass spectrometry a useful, and much preferred, analytical platform for plantomics.[1] Based on the nature of ionization, a number of imaging mass spectrometry methods such as matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), secondary ion mass spectrometry (SIMS), desorption electrospray ionization mass spectrometry (DESI-MS) and several other variants of these broad techniques are available for capturing the site-specific molecular mapping of biological samples.

Rice is the staple cereal of about half of the world's population.[2] Adverse environmental effects and the use of toxic agrochemicals have resulted in the accumulation of metals in rice which are issues of serious concern. Arsenic accumulation in rice has led to stringent quality restrictions on its imports in many parts of Europe. Besides such environmental effects, natural bioaccumulation of prevalent minerals such as silver has also been reported.[3] Although environmental effects have been significant and have been studied largely from the point of view of toxicity, mineral fortification and other dietary enhancements of rice are also important.[4] A rapid fingerprinting of molecular species expressed in rice and their spatial distribution in the rice grain would help build up an important dietary database for rice consumers. Vitamins and mineral fortification in specific regions of rice grain, along with socio-cultural practices involved in the processing of rice, will contribute to health benefits.[5] The natural accumulation of organic osmolytes such as glycine betaine, proline, trehalose, mannitol, and fructan will enable the plant to thrive under adverse environmental conditions.[6] Some rice landraces inherently possess heritable traits for tolerance of diseases and edapho-climatic stress such as drought and salinity.[7] Application of molecular imaging platforms to scout for such rice varieties and an understanding of the key molecules linked with their inherent stress tolerant properties will be useful in crop improvement.

Previous reports on rice grain imaging using MALDI-MS provided information about the spatial distribution of phospholipids and anthocyanins.[8-10] Although MALDI-MSI allows identification of macromolecules like proteins, it is not efficient to image the molecules in the low molecular weight range due to the interferents from the matrices applied on the sample. Therefore, any imaging tool of this kind employed on biological materials would be more beneficial if performed under natural conditions. This would enable such studies at various developmental stages of the tissue such as during germination. Mass spectrometry, especially using ambient ionization methods, can be advantageous in undertaking such studies. One of the most prominent methods of ambient ionization imaging mass spectrometry is desorption electrospray ionization mass spectrometry (DESI-MS) where ionization is achieved by the impingement of charged solvent droplets at surfaces.[11] DESI-MSI was widely used to study the molecules expressed on the surfaces of delicate plant or animal tissues.[12,13] Compared with imaging of lipids in mammalian samples, the lipid imaging in plant samples is not well addressed by DESI-MSI.[14] This is the first work to report the localization of lipids and other unknown molecular entities on rice grains by DESI-MSI. We present a systematic investigation of this imaging methodology performed on several rice landraces of India.

2 | EXPERIMENTAL

2.1 | Chemicals and materials

Commercially available methanol (MeOH) (Honeywell Riedel-de Haen LC/MS ultra chromasolv tested for UHPLC/MS, Sigma Aldrich) was used in our experiments without any further purification. HPLC grade *N*,*N*-dimethyl formamide (DMF), acetonitrile (ACN) and propan-2-ol (IP) were purchased from Rankem. Samples of ten rice (*Oryza sativa* ssp. *indica*) landraces G2 (Garib-sal), G28 (Gouri sundari), G38 (Gazep xali), H24 (Hugi bhatta), H34 (Hende baihar), K11 (Kataribhog), K86 (Kala nuniya), R09 (Radha tilak), T05 (Tiki), and T11 (Tike churi) were obtained from the seed bank of the Centre for Interdisciplinary Studies, Kolkata (http://www.cintdis.org/basudha). G28, H24, K11, K86 and R09 are aromatic. All these rice varieties are cultivated and conserved in Basudha farm in the southern Odisha district, Rayagada (19°42′32.0″N, 83°28′8.4″E), and grown in an organic environment without application of chemical fertilizers and pesticides.

2.2 | Sample preparation

Dehusked, unpolished rice grains were used for our study. To remove moisture, the rice grains were vacuum desiccated for 2 days at 10^{-2} mbar. For embedding, two parts of epoxy resin (Technovit EPOX resin and EPOX hardener regular (A) procured from Chennai Metco, India, Cat. No: TECH001 & TECH002) mixed with one part of hardener was poured over the desiccated rice samples aligned on a silicone mould. The embedded samples were air cured at room temperature for 24 h. A rotary cutter (Discoplan TS, Struers, Denmark) was used to cut the resin blocks to a thickness of 3 mm. The cut resin blocks were further polished to a flat surface for DESI-MS imaging using a belt sander machine. The resin block with embedded rice grains was fixed directly on to the DESI-MS stage using double-sided adhesive tape.

2.3 | Metabolite extraction

Fresh rice samples were frozen in liquid nitrogen and ground to form a powder. The powdered rice samples were stored at −20°C until further analysis. The ground rice samples were extracted with methanol, and centrifuged at 20,000 *g* for 20 min, and refrigerated at −20°C for 2 days to precipitate the large molecules. The samples

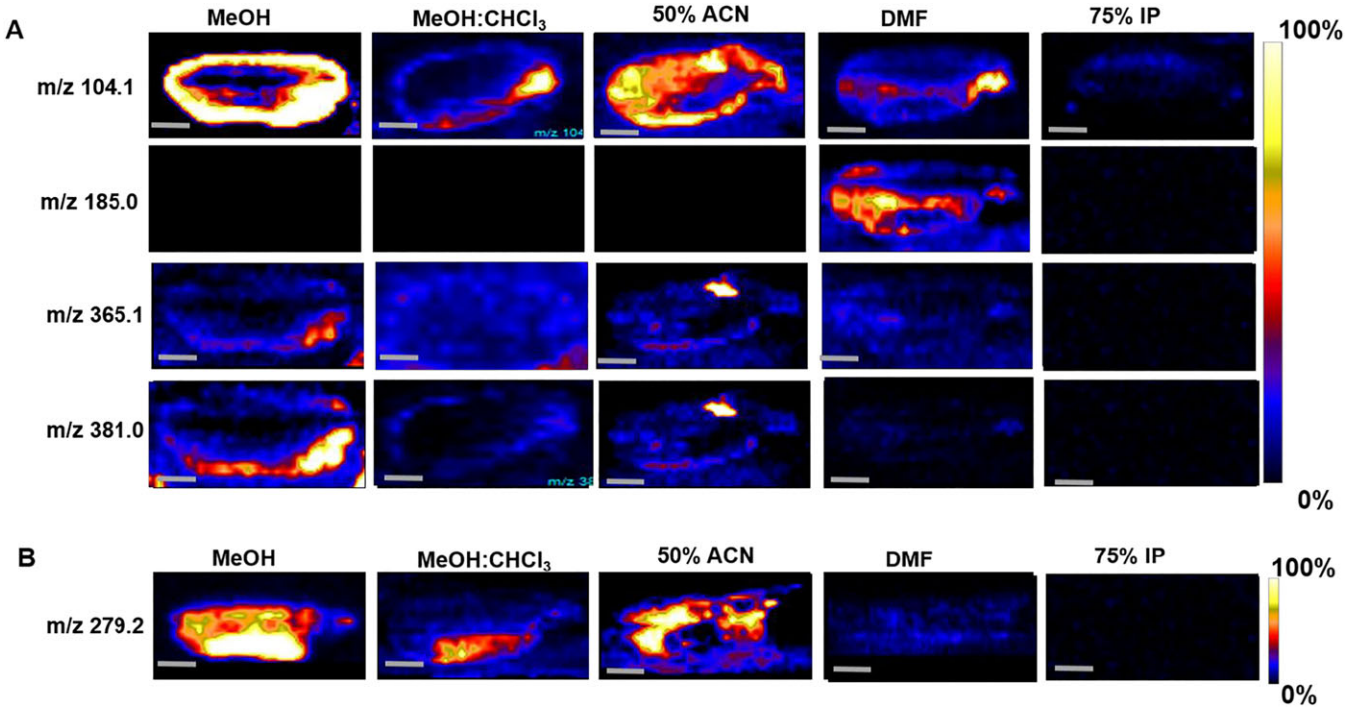

FIGURE 1 DESI-MS imaging of H24 rice using different solvents as spray solvent. Molecular images from A) positive and B) negative ion mode imaging. The ion intensities of the images are normalized across the rows. All the images have a uniform scale bar of 1 mm [Color figure can be viewed at wileyonlinelibrary.com]

were centrifuged again at 20,000 *g* for 20 min and metabolites were concentrated using a rotary evaporator. The concentrated samples were diluted 100 times prior to MS analysis.

3 | INSTRUMENTATION

3.1 | DESI-MS imaging of rice grains

A LTQ XL linear ion trap mass spectrometer (Thermo Scientific, San Jose, CA, USA) fitted with a DESI source (Prosolia, Indianapolis, IN, USA) was used for the imaging experiments. The flat rice sections were mounted directly onto the 2D moving stage of the mass spectrometer using double-sided tape. The samples were scanned over a mass range of *m/z* 50–1000. Methanol was the spray solvent for imaging in both positive and negative ionization mode. The operating conditions were as follows: spray tip to sample distance 2 mm, tip to mass spectrometer inlet 2 mm, solvent spray angle 60°, solvent flow rate 5 µL/min, spray voltage ±5 kV, capillary temperature 250°C, capillary voltage ±45 V, tube-lens voltage ±100 V and nebulizing gas pressure (N_2) 100 psi. The area of imaging was fixed depending on the rice grain size (~3 mm). The pixel size (spatial resolution) was set to 200 µm × 200 µm. Imaging was performed on the single rice grains of different varieties

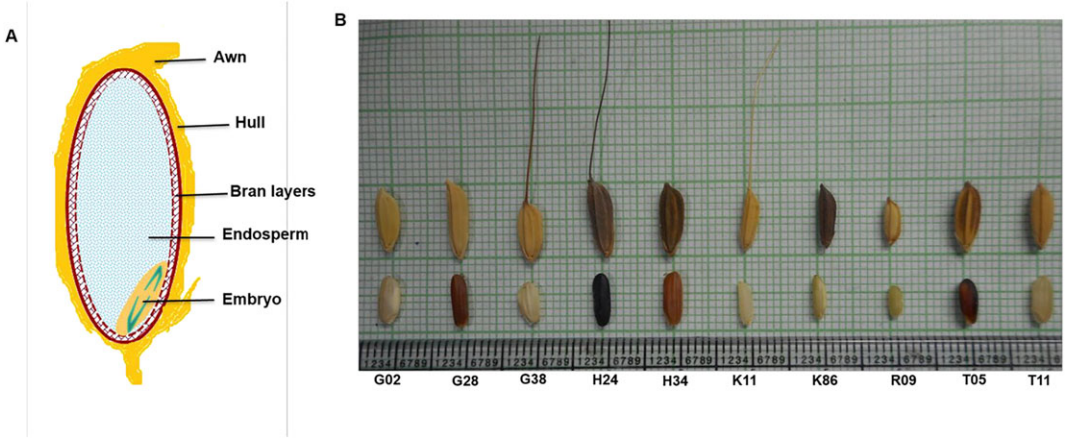

FIGURE 2 A, Schematic representation of the anatomy of the rice grain. B, Photograph of the spikelets (top row) and dehusked (bottom row) grains of ten rice landraces of India used in this study. The grains were placed on a graph paper with grid lines of 1 mm width [Color figure can be viewed at wileyonlinelibrary.com]

individually. For DESI-MSI experiments, the auto gain control (AGC) was off. Each line scan spectrum obtained as an Xcalibur raw file was processed using the Firefly software for creating an image file. The constructed 2D ion images were visualized in BioMap software (http://www.maldi-msi.org) in an interpolate display method.

3.2 | ESI-MS analysis

The electrospray ionization (ESI) source of the Thermo LTQ mass spectrometer was used for analysis. The data were acquired using Xcalibur Quant software. The diluted samples were sprayed by applying a voltage of 5 kV in both the ionization modes with a mass range of *m/z* 50–1000. The capillary temperature was set to 250°C, N_2 sheath gas flow rate was 10 arbitrary units, solvent flow rate was set at 5 μL/min, and tube lens voltage was ±100 V. Collision-induced dissociation (CID) fragmentation was carried out for the specific ion peaks from the MS analysis with an isolation width of 1.0 *m/z*. Utilizing the spectral details, the metabolites were annotated using several publicly available databases such as METLIN, ReSpect, LipidMaps, KEGG, MassBank, and also published literature.

3.3 | Electron microscopy imaging and elemental characterization.

The surface morphology imaging by scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) analysis for the elemental composition and mapping on the rice grain surface was performed using a FEI QUANTA 200 scanning electron microscope.

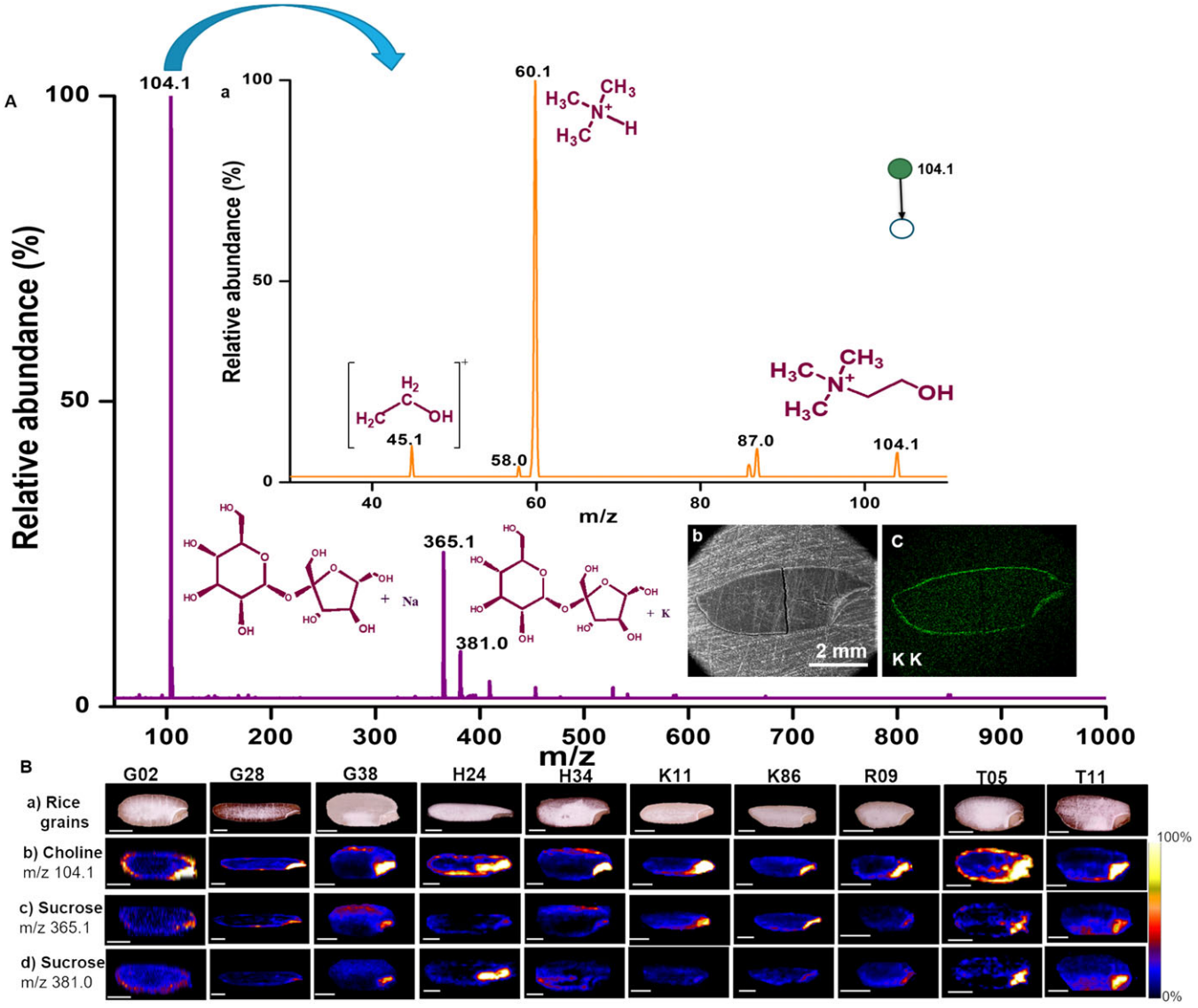

FIGURE 3 A, A representative line scan spectrum collected from the grain surface in positive ion mode of DESI-MS, the inset represents: a) tandem MS spectrum of choline at *m/z* 104.1, b) SEM image of G2 rice grain section, and c) EDAX image showing the distribution of potassium in the G2 grain. B, a) optical images of rice grain cut surface, molecular images of b) protonated choline, c) sodiated sucrose, and d) potassiated sucrose. The scale bars in the images correspond to 1 mm [Color figure can be viewed at wileyonlinelibrary.com]

4 | RESULTS AND DISCUSSION

DESI-MSI aids in the direct detection of metabolites expressed on a tissue/organ surface under ambient atmospheric conditions without tedious sample preparation protocols. However, in the present case, embedding and sectioning of rice grain was necessary to obtain a flat cross section of the specimen and rice otherwise is too hard to cut. The charged solvent spray based ionization of DESI-MSI is nearly non-destructive to the sample as it does not use high-energy lasers as in MALDI-MSI. The literature suggests that mounting of the sample with epoxy resin does not generate any interfering mass signals.[14] Figure S1 (supporting information), showing the DESI-MS spectrum collected from the embedded grain surface, proves that the resin does not interfere with the molecular imaging of rice. The imaging resolution in DESI-MS is greatly influenced by its operating parameters like the shape of the emitter, gas flow rate, the distance between the spray tip and mass spectrometer inlet, the nature of the spray solvent, and so on. A number of modifications were made to the shape and size of the emitter tip in order to achieve a resolution below 100 µm in DESI-MS.[15-17] In our work, we tried to image rice grain features using a typical DESI-MS spray emitter without any modifications. The composition of solvent and the influence of solvent on surface wettability are other crucial factors which influence the signal ion intensity and the image quality.[18] Although most of the studies employ organic solvent mixtures as spray solvent, 100% methanol gave good signal ion intensity for rice metabolites compared with 50% and 95% methanol (Figure S2, supporting information).We tested an array of solvents, e.g. 100% MeOH, 50% ACN, DMF, 75% IP and MeOH:$CHCl_3$(1:1 v/v). (Figure 1) 50% ACN, DMF, MeOH: $CHCl_3$ and MeOH gave almost the same chemical information in positive ion mode. DMF is considered to be a morphologically friendly spray solvent as the

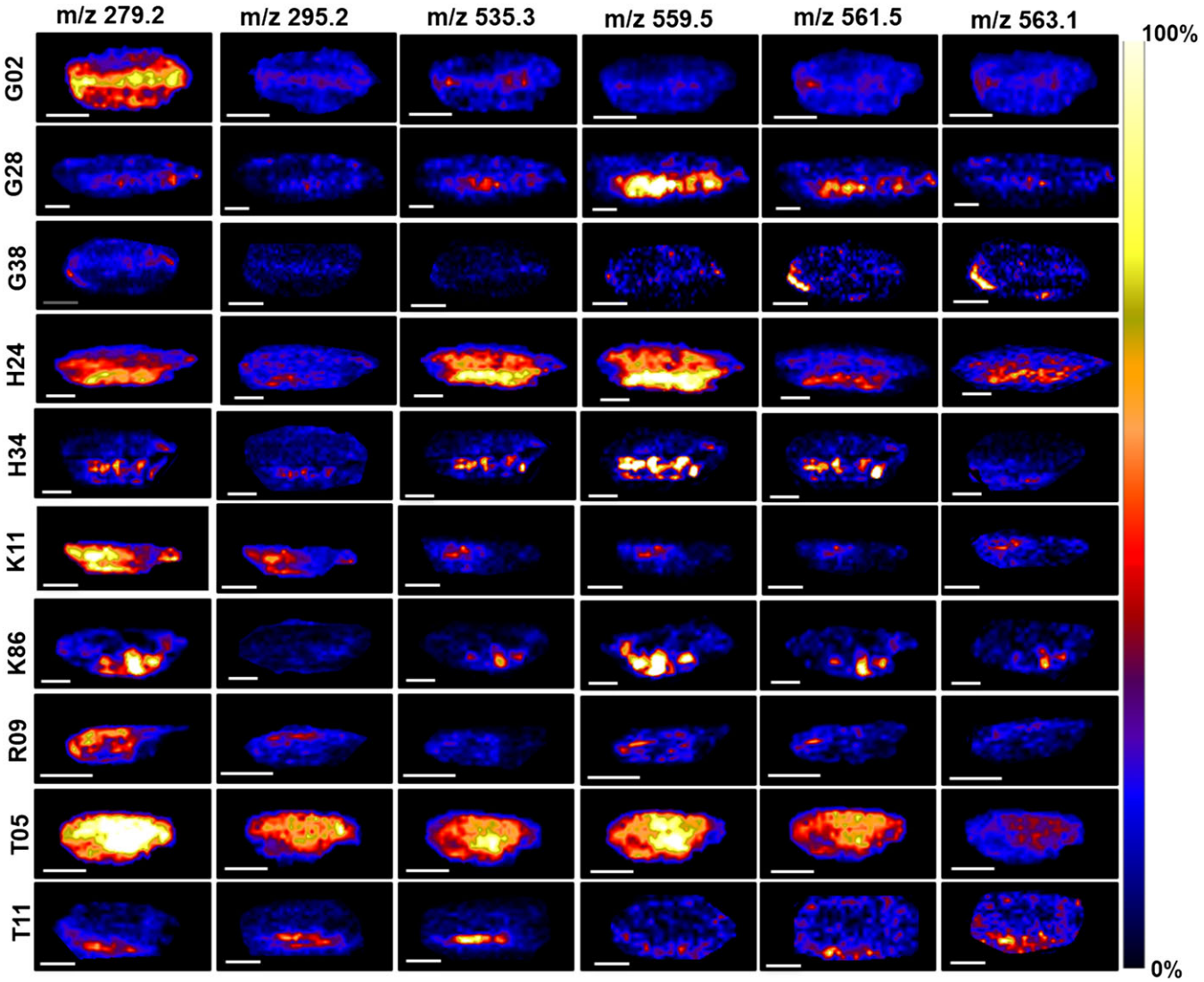

FIGURE 4 Negative ion mode DESI-MS images of rice grains showing the distribution of linoleic acid (*m/z* 279.2), 13-HODE-9-HODE (*m/z* 295.2), and unidentified molecules at *m/z* 535.3, 559.5, 561.5 and isoschaftoside (*m/z* 563.1) along the endosperm regions. The ion intensity is normalized along the columns and all the images have a uniform scale bar of 1 mm [Color figure can be viewed at wileyonlinelibrary.com]

samples analyzed with DMF can be further used for histopathology studies.[18] However, in our study, a slight disturbance in the specimen was noticed when DMF was electrosprayed over the rice grain surface. The molecular images obtained in negative ion mode were relatively better with MeOH in comparison with other solvents tested. Table S1 (supporting information) presents the characteristic features of the rice grains based on which they were selected for our study. The grain morphologies of the rice varieties used in this investigation are presented in Figure 2.

4.1 | Distribution of choline and sugar molecules

The positive ion mode DESI-MS spectrum from the rice grain surface in Figure 3A and molecular images in Figure 3B show the expression of choline and sucrose with their sodium and potassium adducts in the whole grain of ten different rice landraces. Choline (*m/z* 104.1) is a water-soluble *N*-methyl-substituted molecule which plays a role in both glycine betaine and phosphatidyl choline biosynthetic pathways in plants.[19] As rice is a non-natural accumulator of glycine betaine, the role of choline is restricted to phospholipid biosynthesis. Endosperm, the storage tissue of the mature cereal grain, is chiefly composed of starch and a minor amount of soluble sugars such as glucose, fructose, sucrose, etc.[20,21] The soluble sugar content in rice is known to vary between the cultivars.[22] Carbohydrates are mainly transported in the form of sucrose in the plant system through the phloem.[23] In Figure 3B, the accumulation of sucrose is seen to be uniform in the endosperm of G02, G38, K11, K86, R09 and T11 rice varieties. Sucrose is known to form adducts with cations. The literature suggests that potassiated sucrose does not fragment easily as compared with sodiated and lithiated sucrose under the same applied collision energy, and thus the potassiated sucrose imparts structural integrity to sucrose during molecular transport unlike its other cationic adducts.[24] The DESI-MS images of potassiated sucrose shows enhanced signal intensity in the embryo of the G38, H24, T05, and T11 rice varieties. SEM-EDAX mapping of G2 rice in the inset of Figure 3A showed a strong localization of potassium in the embryo. Interestingly there may be a correlation between molecular localization and metal ion distribution, especially in rice grown in areas where surface water is contaminated with heavy metals such as arsenic, mercury, etc. Figure S3 (supporting information) shows the results from a quick experiment performed

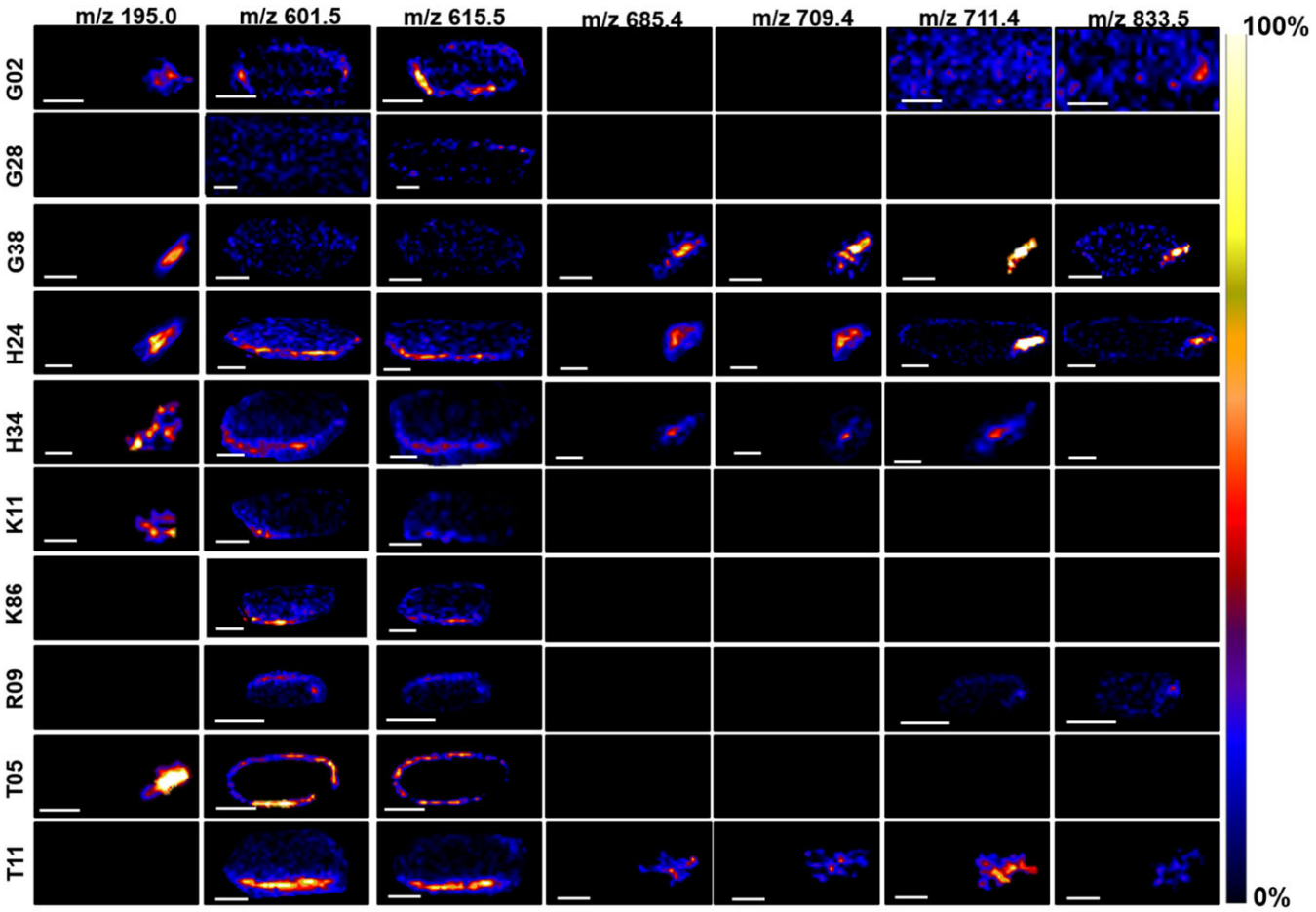

FIGURE 5 Negative ion mode DESI-MS images of rice grains showing the distribution of gluconic acid (*m/z* 195.0), oryzanol A (*m/z* 601.5), oryzanol C (*m/z* 615.5), methyl phosphoesters of PA 34:2 (*m/z* 685.4), PA 36:4 (*m/z* 709.4), PA 36:3 (*m/z* 711:4) and PI 34:2 (*m/z* 833.5) along the bran and embryo regions. All the images have a uniform scale bar of 1 mm [Color figure can be viewed at wileyonlinelibrary.com]

by soaking G2 rice grains in 10 mg g^{-1} of Cr^{3+} metal ion solutions for 5 h and checking for bioaccumulation of chromium in the rice grains. SEM-EDS mapping showed the distribution of chromium along the bran and embryo of the rice grain. The metal picking efficiency of the cereal grains depends on many factors such as form of the metal ions (organic or inorganic), rice variety, nature of the soil and water where the rice grows, temperature, susceptibility to stress, and so on. Therefore, an elaborate experiment has to be performed by screening a large number of rice samples. Certain molecules expressed on the bran or embryo surface did not have enough signal intensity when subjected to fragmentation in DESI-MS. Therefore, ESI-MS/MS analysis of rice extracts was performed to identify the molecule. Figure S4 (supporting information) shows the tandem MS data obtained from ESI-MS and DESI-MS for a representative molecular ion in positive and negative ion mode. The metabolite profile obtained from the ESI-MS analysis of rice extracts in positive ionization mode is shown in Figure S5 (supporting information). Data from ten rice varieties are presented.

4.2 | Distribution of endosperm-specific molecules

Figures 4 and 5 represent the distribution of molecules in the rice varieties from the negative ion mode of imaging. The composition and distribution of lipids in rice are not uniform as they vary with their genotypes and environmental conditions. Palmitic acid, linoleic acid, and oleic acid are known to be the major free fatty acids in rice.[25] Linoleic acid (*m/z* 279.2), also referred to as omega-6 fatty acid, is an essential fatty acid required for the normal growth and development of humans. Hydroxyoctadecadieonic acids (HODEs) are the stable oxidative derivatives of omega-6 fatty acid. In Figure 4, the free fatty acids linoleic acid (*m/z* 279.2), 13-HODE-9-HODE (*m/z* 295.2), three other unidentified molecules with *m/z* 535.3, 559.5, and 561.5, and isoschaftoside, a C-glycosylflavonoid (*m/z* 563.1), present a strong localization in the starchy endosperm of the rice varieties.

4.3 | Distribution of embryo-specific molecules

In Figure 5, gluconic acid, a glucose oxidation product (*m/z* 195.0), is seen to be localized in the embryo of G02, G38, H24, H34, K11 and T05 rice varieties. The multiple components of γ-oryzanol are made up of the esters of triterpene alcohol ferulates and sterol ferulates.[26,27] Two of the major γ-oryzanol components of the rice bran oil such as cycloartenyl ferulate (oryzanol A, *m/z* 601.5) and 24-methyl cycloartenyl ferulate (oryzanol C, *m/z* 615.5) were mapped along the bran region of the rice varieties. Oryzanol distribution is more pronounced in the T05 rice variety than the other rice landraces. A review suggests that the dietary intake of γ-oryzanol from rice bran oil helps in the treatment of hypercholesterolemia by lowering the plasma cholesterol levels, and, also, the ferulate part of oryzanol offers antioxidant properties.[28] Phosphatidyl inositol is a regulatory membrane lipid from the glycerophospholipid class with an inositol head group. The literature suggests the distribution and expression of specific molecular species of PIs will vary with external conditions such as stress states or the molecular function which has to be regulated.[29] PI 34:2 (*m/z* 833.5), a molecular species of PI, was localized along the bran and embryo.

4.4 | Methylation of phosphatidic acids

Phosphatidic acids (PAs), a minor class of total phospholipids, are known to be the key signalling molecules involved in plant development and metabolic pathways. The ions at *m/z* 685.4, 709.4, and 711.4 exhibit a site-specific localization in the embryo of G38, H24, H34 and T11. The DESI-MS spectrum shown in Figure 6 collected from the rice grain cross sections further confirmed that those molecules are from the grain surface. A study on maize embryo imaging by MALDI-MS reported that the ions at *m/z* 685.4 and 709.4 are methyl phosphoesters of PA 34:2 (*m/z* 671.4) and PA 36:4 (*m/z* 695.4), respectively. It was also mentioned that the phosphoesterification of phosphatidic acid was detected only when 5% methanol was used as the solvent for matrix recrystallization.[30]

To further confirm the influence of spray solvent on the methylation of phosphatidic acids, imaging was performed with an array of non-methanolic solvents. Figure 7 shows the absence of methylated PA species when non-methanolic solvents were used as spray solvents. Therefore, the methylation of PA molecules in our study is from methanol. The product ion mass spectra of unidentified molecules by ESI MS are shown in Figure S6 (supporting information).

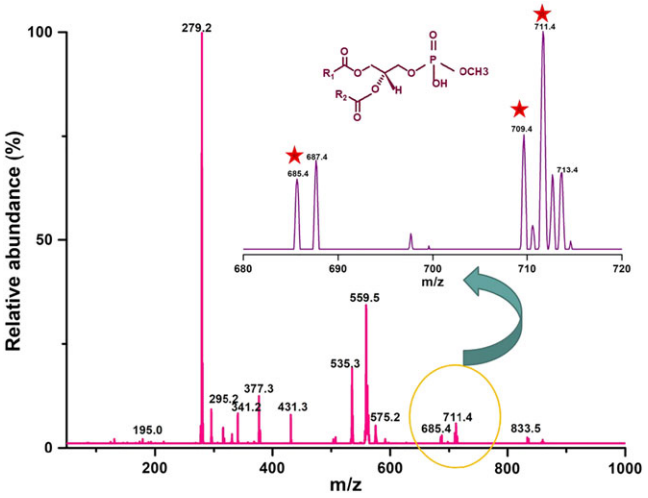

FIGURE 6 Negative ion mode DESI-MS spectrum collected from H24 rice section. The inset shows the molecular distribution pattern of methyl phosphoester of phosphatidic acids in the zoomed region of *m/z* 680–720 [Color figure can be viewed at wileyonlinelibrary.com]

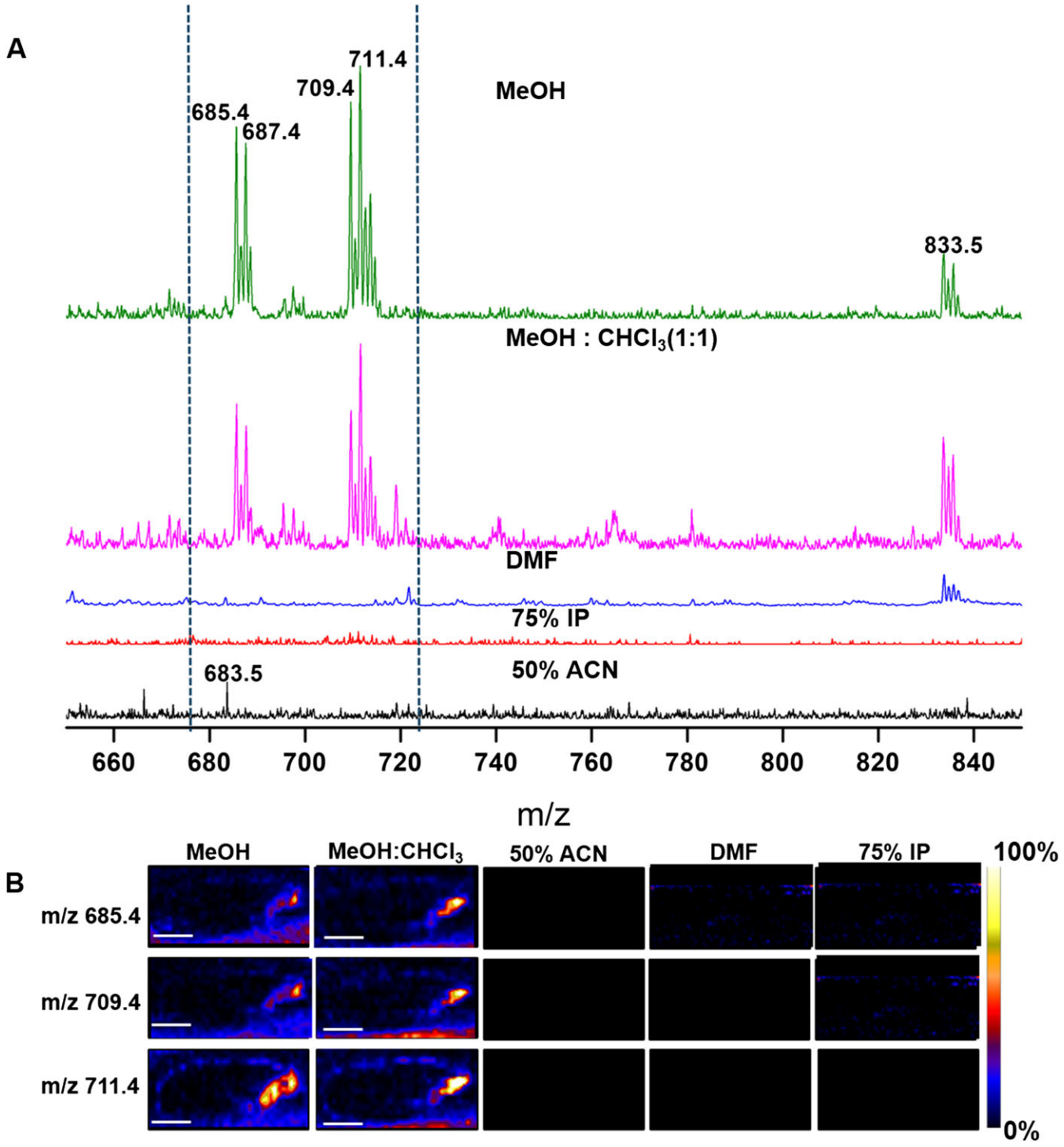

FIGURE 7 A, Negative ion mode DESI-MS spectrum collected from H24 rice section using an array of solvent systems. B, Molecular ion distribution pattern of methyl phosphoester of phosphatidic acids in H24 rice using different spray solvents. All the images have a uniform scale bar of 2 mm [Color figure can be viewed at wileyonlinelibrary.com]

5 | CONCLUSIONS

In this study, we reported the spatial mapping of different molecules of physiological importance in rice grains under ambient conditions. We introduced a systematic investigation into some of the rarest, traditional, and contemporary rice varieties. The DESI-MS based chemical imaging technique is non-invasive, and, therefore, the samples can be reused for imaging with further polishing to understand the details of molecular distribution at different depths of the sample. This approach can be advanced to studying tissues at the

developmental stages. The identification of molecules was made possible with tandem ESI MS analysis. An undesirable effect of spray solvent on the embryo-specific phosphatidic acid molecules was observed. Further optimization of spray solvents is necessary for obtaining lipid signals without interferents. The identified metabolites are not only important for human nutrition, but they also provide significant information regarding their role in plant metabolism. Thus, screening of rice samples by DESI-MS aided in understanding the distribution patterns of dietary metabolites and signalling molecules, which would be helpful in crop improvement for nutritional qualities. A compendium of metabolite distribution data of this kind along with the metal profile of rice grains may help people across the world to have the most appropriate rice varieties for consumption. A study correlating the metabolite expression under stress by different metal contaminants will be addressed in our upcoming work.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support by the Department of Science and Technology, Government of India, for nanomaterials research. A.S. thanks the Indian Institute of Technology Madras for a research fellowship. They would also like to thank Mr. Priyabrata Roy for his timely support in sample collection.

ORCID

Arunan Suganya https://orcid.org/0000-0002-2990-1916
Debal Deb https://orcid.org/0000-0002-7230-659X
Thalappil Pradeep https://orcid.org/0000-0003-3174-534X

REFERENCES

1. Gemperline E, Keller C, Li L. Mass spectrometry in plant-omics. *Anal Chem*. 2016;88(7):3422-3434. https://doi.org/10.1021/acs.analchem.5b02938
2. Khush GS. Origin, dispersal, cultivation and variation of rice. *Plant Mol Biol*. 1997;35(1):25-34. https://doi.org/10.1023/A:1005810616885
3. Sen Gupta S, Baksi A, Roy P, Deb D, Pradeep T. Unusual accumulation of silver in the Aleurone layer of an Indian rice (*Oryza sativa*) landrace and sustainable extraction of the metal. *ACS Sustain Chem Eng*. 2017;5(9):8310-8315. https://doi.org/10.1021/acssuschemeng.7b02058
4. Bouis HE, Saltzman A. Improving nutrition through biofortification: A review of evidence from HarvestPlus, 2003 through 2016. *Glob Food Sec*. 2017;12:49-58. https://doi.org/10.1016/j.gfs.2017.01.009
5. De Moura FF, Moursi M, Donahue Angel M, et al. Biofortified β-carotene rice improves vitamin A intake and reduces the prevalence of inadequacy among women and young children in a simulated analysis in Bangladesh, Indonesia, and the Philippines. *Am J Clin Nutr*. 2016;104(3):769-775. https://doi.org/10.3945/ajcn.115.129270
6. Ks H, Nihal A, Faizan A, Raj K. Naturally occurring organic osmolytes: From cell physiology to disease prevention. *IUBMB Life*. 2010;62(12):891-895. https://doi.org/10.1002/iub.406
7. Deb D. Folk rice varieties, traditional knowledge and nutritional security in South Asia. In: Poyyamoli G, ed. *Agroecology, Ecosystems, and Sustainability in the Tropics*. Delhi: Studera Press, 2017; 2017:117-134. http://www.studerapress.com/wp-content/uploads/2017/06/6-Folk-Rice-Varieties-Traditional-Knowledge-and-Nutritional-Security-in-South-Asia.pdf.
8. Zaima N, Goto-Inoue N, Hayasaka T, Setou M. Application of imaging mass spectrometry for the analysis of *Oryza sativa* rice. *Rapid Commun Mass Spectrom*. 2010;24(18):2723-2729. https://doi.org/10.1002/rcm.4693
9. Yoshimura Y, Zaima N, Moriyama T, Kawamura Y. Different localization patterns of anthocyanin species in the pericarp of black rice revealed by imaging mass spectrometry. *PLoS One*. 2012;7(2):e31285. https://doi.org/10.1371/journal.pone.0031285
10. Zaima N, Yoshimura Y, Kawamura Y, Moriyama T. Distribution of lysophosphatidylcholine in the endosperm of *Oryza sativa* rice. *Rapid Commun Mass Spectrom*. 2014;28(13):1515-1520. https://doi.org/10.1002/rcm.6927
11. Wiseman JM, Ifa DR, Venter A, Cooks RG. Ambient molecular imaging by desorption electrospray ionization mass spectrometry. *Nat Protoc*. 2008;3(3):517-524.
12. Claude E, Jones EA, Pringle SD. DESI Mass Spectrometry Imaging (MSI) In: Cole LM, ed. *Imaging Mass Spectrometry: Methods and Protocols*. New York, NY: Humana Press; 2017:65-75. https://doi.org/10.1007/978-1-4939-7051-3_7
13. Boughton BA, Thinagaran D, Sarabia D, Bacic A, Roessner U. Mass spectrometry imaging for plant biology: A review. *Phytochem Rev*. 2016;15(3):445-488. https://doi.org/10.1007/s11101-015-9440-2
14. Dong Y, Li B, Malitsky S, et al. Sample preparation for mass spectrometry imaging of plant tissues: A review. *Front Plant Sci*. 2016;7:60. https://doi.org/10.3389/fpls.2016.00060
15. Kertesz V, Van Berkel GJ. Improved imaging resolution in desorption electrospray ionization mass spectrometry. *Rapid Commun Mass Spectrom*. 2008;22(17):2639-2644. https://doi.org/10.1002/rcm.3662
16. Campbell DI, Ferreira CR, Eberlin LS, Cooks RG. Improved spatial resolution in the imaging of biological tissue using desorption electrospray ionization. *Anal Bioanal Chem*. 2012;404(2):389-398. https://doi.org/10.1007/s00216-012-6173-6
17. Tillner J, Wu V, Jones EA, et al. Faster, more reproducible DESI-MS for biological tissue imaging. *J Am Soc Mass Spectrom*. 2017;28(10):2090-2098. https://doi.org/10.1007/s13361-017-1714-z
18. Green FM, Salter TL, Gilmore IS, Stokes P, O'Connor G. The effect of electrospray solvent composition on desorption electrospray ionisation (DESI) efficiency and spatial resolution. *Analyst*. 2010;135(4):731-737. https://doi.org/10.1039/B924208B
19. Rhodes D, Hanson AD. Quaternary ammonium and tertiary sulfonium compounds in higher plants. *Annu Rev Plant Physiol Plant Mol Biol*. 1993;44(1):357-384. https://doi.org/10.1146/annurev.pp.44.060193.002041
20. Smyth DA, Prescott HE. Sugar content and activity of sucrose metabolism enzymes in milled rice grain. *Plant Physiol*. 1989;89(3):893-896.
21. Singh R, Juliano BO. Free sugars in relation to starch accumulation in developing rice grain. *Plant Physiol*. 1977;59(3):417-421.
22. Smyth DA, Repetto BM, Seidel NE. Cultivar differences in soluble sugar content of mature rice grain. *Physiol Plant*. 1986;68(3):367-374. https://doi.org/10.1111/j.1399-3054.1986.tb03367.x
23. Truernit E. Plant physiology: The importance of sucrose transporters. *Curr Biol*. 2001;11(5):R169-R171. https://doi.org/10.1016/S0960-9822(01)00085-9
24. Yuan H, Wu Y, Liu W, et al. Mass spectrometry-based method to investigate the natural selectivity of sucrose as the sugar transport form for plants. *Carbohydr Res*. 2015;407:5-9. https://doi.org/10.1016/j.carres.2015.01.011
25. Fujino Y. Rice lipids. *Cereal Chem*. 1978;55(5):559-571. https://www.aaccnet.org/publications/cc/backissues/1978/Documents/chem55_559.pdf
26. Aladedunye F, Przybylski R, Rudzinska M, Klensporf-Pawlik D. γ-Oryzanols of north American wild rice (*Zizania palustris*). *J Am Oil Chem Soc*. 2013;90(8):1101-1109. https://doi.org/10.1007/s11746-013-2252-x

27. Xu Z, Godber JS. Purification and identification of components of γ-oryzanol in rice bran oil. *J Agric Food Chem*. 1999;47(7):2724-2728. https://doi.org/10.1021/jf981175j

28. Patel M, Naik SN. gamma-Oryzanol from rice bran oil – A review 2004; 63:569–578. https://pdfs.semanticscholar.org/a78c/6655599f92e7732a066c03ad07b33b0b32f6.pdf

29. Heilmann I. Phosphoinositide signaling in plant development. *Development*. 2016;143(12):2044 LP-2055. https://doi.org/10.1242/dev.136432

30. Dueñas ME, Carlucci L, Lee YJ. Matrix recrystallization for MALDI-MS imaging of maize lipids at high-spatial resolution. *J Am Soc Mass Spectrom*. 2016;27(9):1575-1578. https://doi.org/10.1007/s13361-016-1422-0

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Suganya A, Deb D, Pradeep T. Spatial distribution mapping of molecules in the grains of different rice landraces, using desorption electrospray ionization mass spectrometry. *Rapid Commun Mass Spectrom*. 2019;33: 727–736. https://doi.org/10.1002/rcm.8397