JARQ 41 (3), 191 – 194 (2007) http://www.jircas.affrc.go.jp

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry for Rice (*Oryza sativa* L.) Seed Phytochemicals

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Abstract

A quick and simple method for the evaluation of rice varieties using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF-MS) was proposed and the results of the mass analysis using five kinds of matrixes were demonstrated here. Extracts from ground rice seeds could be analyzed within 30 min to detect mass fingerprints resulting from phytochemicals. Each variety showed a unique mass fingerprint pattern and the characters of rice seed varieties could be visualized by radar chart peaks to distinguish varieties.

Discipline: Biotechnology
Additional key words: matrix, variety

Introduction

Rice (*Oryza sativa* L.) has been a major agricultural product in Asia including Japan. The commercial value of specific rice varieties grown in good conditions can be very expensive, and sometimes the price of high-grade branded rice is ten times greater than ordinary varieties. Therefore, there have been several incidents of falsely labeling ordinary rice as high-grade branded rice. Quick and simple methods have been expected to distinguish between ordinary rice and high-grade branded rice on-site at a farm or storehouse, even if they are not complete methods for all rice varieties. These methods are also needed to evaluate whether the rice is of good quality or not. It is impossible for most people to distinguish varieties by color, smell, shape, and taste of raw rice, but some specialists of rice brands are believed to be able to do this. Although DNA analysis[3] has been the most reliable method to identify varieties, it needs much time, special techniques, chemicals, and equipment which has been sterilized. Protein analysis using electrophoreses[6] sometimes shows differences between varieties, but most varieties have similar electrophoresis patterns. Protein analysis also needs special techniques, and both DNA and protein techniques are impossible to do quickly on-site at a farm.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF-MS) instruments, which are smaller, cheaper and easier to use than before, have recently become common for biological studies. A handy type TOF-MS instrument has been developed for field research[1]. I proposed to use MALDI TOF-MS for the quick evaluation of rice brands by the analysis of phytochemicals in seed endosperm.

Materials and methods

All solvents used for sample preparation and mass analysis were HPLC grade. Matrixes used for mass spectrometry were 6-aza-2-thiothymine (AT) (Aldrich, USA), α-cyano-4-hydroxycinnamic acid (CA) (Fluka, Switzerland), 1,8-dihydroxy-9[10H]-anthracenone (DT) (Sigma, USA), gentisic acid (GA) (Nacalai Tesque, Japan), and sinapic acid (SA) (Fluka). Mature rice (*O. sativa* L., var. Nihonbare, Kotobuki mochi, Norin-22, a radiation mutant of Norin-22, Norin-8, and Nihon masari) seeds grown in a field of National Institute of Agrobiological Sciences were used.

Rice seeds were ground with a mortar and pestle after removing seed coats and embryos. Saturated solutions of matrixes (CA, DT, GA, and SA) in 66% acetonitrile containing 0.1% trifluoroacetic acid or 10 mg/mL AT in 30% ethanol were mixed with fine rice powder (1 mg/10 μL

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Received 9 August 2006; accepted 24 October 2006.

solution) and vortexed in an Eppendorf tube for 5 min at room temperature. Supernatants were obtained after 5 min centrifugation at maximum speed.

Extracts of 1 μL were dropped at several points on a steel target (Bruker Daltonics, Germany) and allowed to lyophilize. MALDI TOF-MS (Ultraflex, Bruker Daltonics) was used in a linear mode (*m/z* 50-1,000), changing the aim every 30 shots, and 300 shots were collected for one sample. Analyses were performed in both the positive and negative modes without any gating.

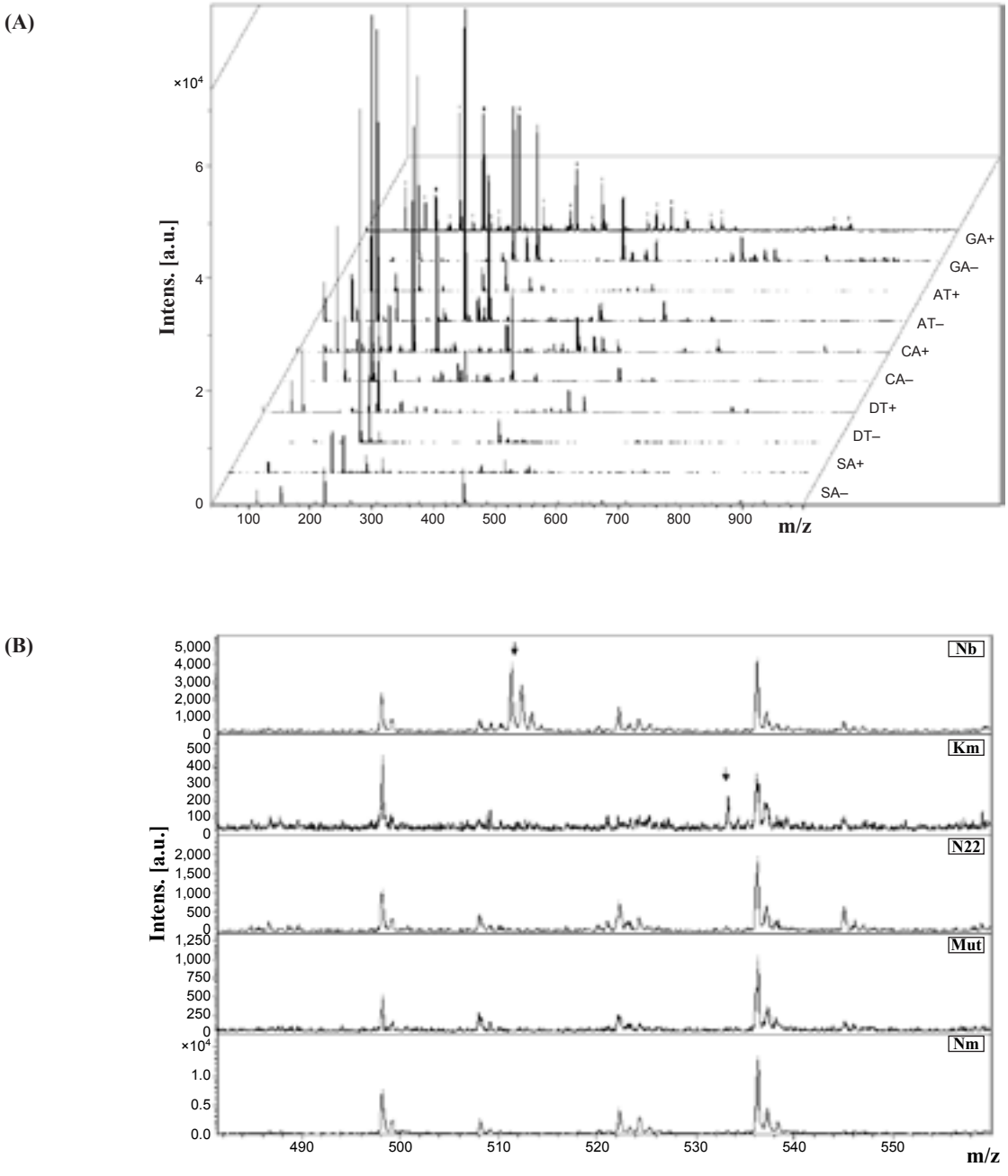

Fig. 1. Mass spectrometry patterns of phytochemicals extracted from rice seeds

Five kinds of matrixes, AT, CA, GA, DT, and SA, were used and rice seed extracts were analyzed by mass spectrometry from *m/z* 50–1,000 in both the positive and negative modes. (A): Spectra from Nihonbare using five matrixes in both the positive (+) and negative (–) modes. (B): Detailed peak pattern differences among rice varieties. Nb: Nihonbare, Km: Kotobuki mochi, N22: Norin-22, Mut: radiation mutant of Norin-22, N8: Norin-8, Nm: Nihon masari. Intensity was represented as artificial units.

Results and discussion

It was difficult to use a mortar and pestle for grinding rice seeds, and ground rice seeds can be obtained more easily using a device such as a coffee mill. The phytochemicals can be extracted in 5 min or less with a vortex and centrifugation. Supernatants obtained using a handy centrifuge at low speed (about 5,000 rpm) were enough for the mass analysis (Data not shown). Globes used in the case of proteomic analysis[2] were not needed during the sample preparation in this phytochemical analysis. Only 1 μL of extract was needed for each matrix, and therefore it was possible to analyze a single rice seed.

Mass spectra are shown in Fig. 1. Variety Nihonbare showed several peaks in each matrix and some of them showed the same mass in the charts (Fig. 1A, e.g. *m/z* 103.7, *m/z* 382.3, and *m/z* 560.3 in all of the matrixes). However, their intensities were different because the matrixes used in this experiment had the peculiarity that the ionization depended on the phytochemical molecules. For example, AT was good for organometallic complexes. Many peaks were observed in a positive mode but were not observed in a negative mode, and vice versa (e.g. *m/z* 154.9 and *m/z* 231.3 in GA). Although we could not identify which peaks were which phytochemicals in this experiment, they were one of the phytochemicals that were identified before[5].

Varietal differences were very clear between Nihonbare and Kotobuki mochi, waxy rice. They can be distinguished by the difference in the color of the endosperm because the basic differences between them were the components of starch. Starches were not visualized in this mass fingerprint experiment, but significant differences were observed in the low molecular weight substances, the phytochemicals (Fig. 1). MALDI TOF-MS analysis showed that the differences between normal rice and waxy rice were not restricted to the characters of starches.

Among the varieties, sometimes similar mass spectra were obtained using the same matrix. However, it was possible to find differences among varieties by mass spectra when different matrixes were used for mass analyses. Although it might be possible to apply hierarchical cluster analysis to these mass spectra for detailed classification using the mass spectra peak pattern[7] in future, finding specific differences between expensive brands and ordinary rice varieties are more important for practical field analysis. An example of the differences between varieties is shown in Fig. 1B. The peak at *m/z* 511.3 in the positive mode using CA was unique among the five varieties.

Radar charts were made for quickly understanding varietal differences (Fig. 2). The shapes of the radar charts were different from each other and therefore were helpful for visualization of differences. For example, the differences in the radar charts between Nihonbare and Kotobuki mochi using AT as the matrix in positive mode were very clear. Peaks 5 and 6 were prominent in the mutant but less conspicuous in Nihonbare and others.

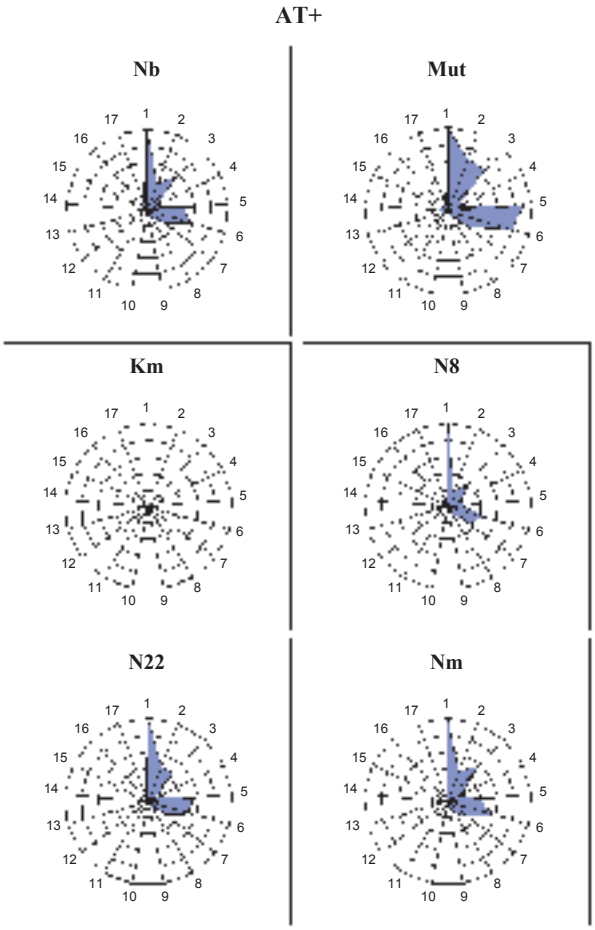

Fig. 2. Radar charts obtained from mass spectra peaks using AT in positive mode

Relative peak intensity of peak 1 was calculated as 1.000. Peaks were peak 1 (*m/z* 103.6), peak 2 (*m/z* 143.8), peak 3 (*m/z* 182.0), peak 4 (*m/z* 220.2), peak 5 (*m/z* 285.6), peak 6 (*m/z* 323.8), peak 7 (*m/z* 361.9), peak 8 (*m/z* 382.1), peak 9 (*m/z* 400.1), peak 10 (*m/z* 402.1), peak 11 (*m/z* 469.7), peak 12 (*m/z* 492.7), peak 13 (*m/z* 497.9), peak 14 (*m/z* 507.9), peak 15 (*m/z* 522.0), peak 16 (*m/z* 536.0), and peak 17 (*m/z* 560.1). Nb: Nihonbare, Km: Kotobuki mochi, N22: Norin-22, Mut: radiation mutant of Norin-22, N8: Norin-8, Nm: Nihon masari.

The purpose of this rapid analysis using MALDI TOF-MS is the evaluation of rice varieties in the field, and not the accurate classification of varieties by differences in phytochemicals. As it would be expected, phytochemicals in plant tissue including rice seeds would change with the environmental conditions. The mass fingerprints of phytochemicals in seeds might show differences between branded rice and the same variety which was grown in a poor condition. In the analysis of bacteria for the classification using mass spectrometry, cells must be grown in a strictly controlled condition[4]. In the case of crops, strictly controlled environments are impractical, and therefore the accuracy of this approach for the evaluation of varieties would be also limited. However, misevaluations of the same varieties as different varieties are preferable to evaluating them as the same brand, in spite of low quality because that would not be good quality from the viewpoint of phytochemicals. Using the handy type TOF-MS instrument[3] might help in the evaluation of quality rice brands in the field.

Acknowledgments

We acknowledge Ms. Jianping Yang and Ms. Kaori Nakane for their contributions.

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