

# Statistical Analysis of Rice Samples for Compositions of Multiple Light Elements (H, C, N, and O) and Their Stable Isotopes

Takashi KORENAGA, Masaaki MUSASHI,<sup>†</sup> Rumiko NAKASHITA, and Yaeko SUZUKI

*Department of Chemistry, Tokyo Metropolitan University, 1-1 Minamiosawa, Hachioji, Tokyo 192-0397, Japan*

Stable isotopic compositions and elemental contents of the H, C, N, and O in 163 rice samples were analyzed. The samples were taken from three different farming countries; Japan ( $n = 103$ ), United States of America ( $n = 30$ ), and Australia ( $n = 21$ ), in addition of Asian rice samples from Thailand ( $n = 2$ ), Vietnam ( $n = 1$ ), and China ( $n = 6$ ) as comparison. They were mostly short grain samples known as “Koshihikari,” with several samples of middle and long grains included. All samples were grown in the presence of either natural manure or artificial fertilizer. The climate of the rice farming environment was diverse, from arid to humid. Excluding  $\delta D$  data showing large uncertainty, according to the statistical analysis of the principal components based on the stable isotopic compositions such as  $\delta^{13}C$ ,  $\delta^{15}N$ , and  $\delta^{18}O$  of rice samples, the Japanese rice samples were clearly distinctive from the Australian and the American rice samples. This fact may be explained by the regional differences in isotopic signatures of the climate, utilized nutrition, and/or quality of irrigation water among the farming countries. This statistical distinction could be one of the useful tools to extract the rice samples grown in Japan from those grown in the other countries.

(Received May 24, 2010; Accepted June 7, 2010; Published August 10, 2010)

## Introduction

The “Koshihikari” is the highest quality rice cultivated not only all over Japan but also in various countries such as the United States of America, Australia, and some Asian countries. In order for scientists to identify the rice cultivar and cultivation area, several techniques have been developed: *i.e.*, gene analysis using polymerase chain reaction (PCR);<sup>1</sup> strontium and boron isotope classification;<sup>2,3</sup> characterization by fatty acid compositions;<sup>4</sup> and a rapid screening by trace elemental analysis.<sup>5</sup> Alternatively, multi-stable isotopic analysis of biogenic light elements such as hydrogen, carbon, nitrogen, and oxygen in food materials has received their great attention as a rapid screening and a cost-friendly routine method to identify their origin, promptly and economically.<sup>6-11</sup> Practically, they have employed this method to solve food authenticity problems: *i.e.*, an evaluation of the adulteration of honey, juice, and wine,<sup>12-14</sup> or an identification of the geological origin of the meat,<sup>15</sup> dairy products,<sup>16,17</sup> wine,<sup>18</sup> cereal crops,<sup>9</sup> and long-grain rices.<sup>8</sup> Compared to the other techniques, the stable isotopic method especially for the plants could provide a wider range of information such as different kinds of the photosynthetic pathways and soil nutrition, and those of the quality and origin of irrigation water plants. This is because that those isotopic compositions ( $\delta^{13}C$ ,  $\delta^{15}N$ , and  $\delta^{18}O$ ) reflect various factors such as isotopic compositions of source materials (*e.g.*,  $CO_2$ ,  $H_2O$ ,  $NH_3$ , and  $NO_2$ ) and their assimilation processes as well as growth environments. It has known that the carbon isotopic composition of plant materials strongly depends on the carbon fixation process such as the C3 and C4 cycle.<sup>19</sup> The nitrogen

isotopic composition mainly depends on soil nutrition.<sup>20-22</sup> The oxygen isotopic composition of the plant materials mainly reflects that of local ground water: meteoric water and/or ice-melt water.<sup>23,24</sup> By optimizing such usefulness of the light elemental isotopic compositions, we have applied a multi-elemental stable isotope technique for rice samples in order to classify the rice cultivars and cultivar areas. In this study, we first focused on the stable isotopic compositions ( $\delta D$ ,  $\delta^{13}C$ ,  $\delta^{15}N$ , and  $\delta^{18}O$ ) of rice samples as climatic parameters related to temperature and humidity, and on the agrochemical parameters in terms of the soil nutrients and irrigation waters. Secondary, we classified the rice samples by performing the principal component analysis (PCA) for their  $\delta^{13}C$ ,  $\delta^{15}N$ , and  $\delta^{18}O$  values, to discuss the factors influenced on the classification.

## Experimental

### Sample materials

This study employed 163 rice samples collected from six different countries. Japanese rice, including the 103 unpolished and 12 polished rice samples, were collected from eight different farming regions (Fukushima, Nagano, Tochigi, Niigata, Chiba, Mie, Ibaragi, and Shimane) all over Japan. They were all short grain Koshihikari species grown using artificial fertilizer, and were harvested in the year of 2006.

American rice, including the 30 polished and 3 unpolished rice samples, were collected from California, United States of America in the years of 2005 and 2006. They were three different grain sizes: short ( $n = 12$ ), middle ( $n = 10$ ), long grains ( $n = 3$ ), and 5 unidentified samples. Their species were Koshihikari or Akitakomachi with some unidentified samples. They were grown mostly in the presence of artificial fertilizer and some of them were grown using natural manure.

<sup>†</sup> To whom correspondence should be addressed.  
E-mail: mmusashi@tmu.ac.jp

Australian polished rice: 21 samples were collected from New South Wales, Australia, in 2006. The samples contained two sizes of grain: the short ( $n = 17$ ) and the middle ( $n = 4$ ). The rice samples were all Koshihikari, excluding 5 unidentified samples. Their soil nutrition was not identified.

Additionally, rice samples from three other Asian countries were included in this study. Two samples of polished rice were collected from Thailand in 2006. The samples were short grain, and their species were Koshihikari and Sasanishiki; their soil nutrition was not identified. One polished rice sample collected from Vietnam in 2006 was a short grain Koshihikari without the soil nutrition being known. Six polished rice samples collected from China in 2006 and 2007 were the short grain Akitakomachi and unidentified species without the soil nutrition being identified.

#### Sample preparation, elemental and isotope analyses

Dried rice, 10 – 15 g, was ground to a fine powder before analysis. For carbon and nitrogen, 1.1 to 1.2 mg of the powdered rice was weighed into a tin capsule,  $5 \times 9$  mm. Then, each sample was analyzed by elemental analyzer-isotope ratio mass spectrometry (EA-IRMS); we used a Finnigan Delta plus XP interfaced with Flash EA 1112 manufactured by Thermo Electron Corporation, to determine the C and N isotope ratios. For oxygen, after 0.2 to 0.3 mg of the powdered rice was weighed into a silver capsule,  $5 \times 9$  mm, oxygen isotope analysis was carried out by a thermo-combustion EA-IRMS (TCEA-IRMS) using the Finnigan Delta plus XP interfaced with Flash EA 1112 manufactured by Thermo Electron Corporation. Hydrogen isotope analysis of a few number of the samples were carried out at the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) using a high temperature conversion elemental analyzer (TCEA) coupled to an isotope ratio mass spectrometer (IRMS; Thermo Finnigan Delta plus XP) via a ConFlo III interface.

The  $\delta$  notation is used to describe the isotopic difference between the sample and an international standard, which was defined as a following equation:  $\delta (\text{‰}) = (R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}} \times 1000$ , where  $R_{\text{sample}}$  is the isotope ratio (*i.e.*, D/H,  $^{13}\text{C}/^{12}\text{C}$ ,  $^{15}\text{N}/^{14}\text{N}$ , and  $^{18}\text{O}/^{16}\text{O}$ ) of the sample, and  $R_{\text{standard}}$  is the isotope ratio of the international standard: for carbon, Peedee Belemnite (PDB); for nitrogen, Air; and for oxygen, Standard Mean Ocean water (SMOW). Each isotope value is given as permil, ‰. C, N, and O isotopic compositions can normally be measured with an analytical uncertainty of  $\pm 0.2$  to  $0.3\text{‰}$ . The  $\delta\text{D}$  value was given in permil (‰) relative to SMOW, which was calibrated using Greenland Ice Sheet Precipitation (GISP,  $\delta\text{D} = -190\text{‰}$ ) and Standard Light Antarctic Precipitation (SLAP,  $\delta\text{D} = -428\text{‰}$ ). In the replicate analyses, the standard deviation of the  $\delta\text{D}$  values for purified sample was always better than  $3\text{‰}$  as average.<sup>25</sup>

The elemental contents of the C, N, and H of the samples were determined by using CNH-coder, Yanaco MT-5, of the Analytical Laboratory, Tokyo Metropolitan University. The O content was calculated as a residual amount of a sample subtracted the C, N, and H contents.

#### Principal component analysis

In order to extract any factors affecting the relations among the  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{18}\text{O}$  values of the rice samples in this study (Figs. 1 and 2), we performed one of the multi-variant analyses, the principal component analysis (PCA). The PCA was employed for three variable quantities,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{18}\text{O}$  values with using the PASW® Statistics Ver. 17.0. For the analysis, data were standardized as mean values of one and variations of one. By the statistical analysis in this study, the

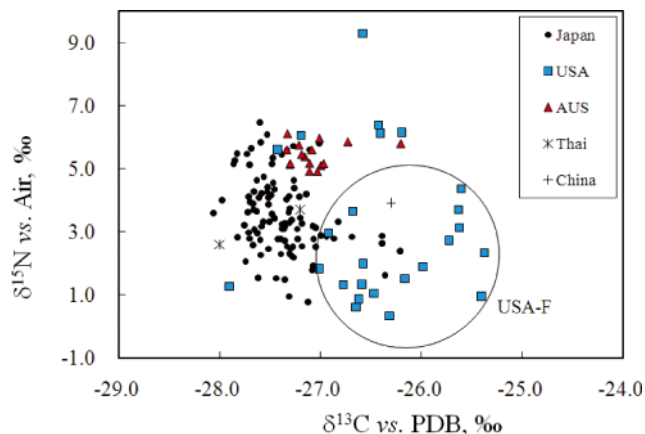


Fig. 1 Two-dimensional distribution of the carbon-nitrogen stable isotopic values of rice samples obtained in this study. The plots encircled approximately and named as USA-F are the American rice samples using artificial fertilizer with some outliers.

three variables were summarized into two equations, so-called “principal component equations”, here named as  $Z_1$  and  $Z_2$ , which were the axes in rectangular coordinates. All of the data on the variables could be eventually integrated into the two components or two meanings.

## Results

Analytical results of the rice samples from Japan (JPN), the United States of American (USA), Australia (AUS), Thailand, Vietnam, and China, together with published data of those from India, Pakistan, France, Italy, Spain, and three States (Arkansas, Missouri, and Texas) of the USA,<sup>8</sup> are summarized in Table 1 and Figs. 1 and 2, and the details are in Supporting Information.

#### Stable isotopic compositions

Japanese rice samples ( $n = 103$ ) collected from eight different cultivation regions had average values of  $3.5 \pm 1.0\text{‰}$  in  $\delta^{15}\text{N}$ ,  $-27.4 \pm 0.2\text{‰}$  in  $\delta^{13}\text{C}$ , and  $21.3 \pm 0.8\text{‰}$  in  $\delta^{18}\text{O}$ . When we compared the results using the average value of each region, the heavier  $\delta^{15}\text{N}$  values were found in Fukushima,  $4.7\text{‰}$ ; Tochigi,  $4.6\text{‰}$ ; and Ibaragi,  $4.6\text{‰}$ ; whereas the lighter values were found in Nagano,  $2.8\text{‰}$  and Shimane,  $1.7\text{‰}$ . For the  $\delta^{13}\text{C}$  values, the heaviest was in Nagano,  $-26.9\text{‰}$ , whereas the lightest was in Fukushima,  $-27.8\text{‰}$ . For the  $\delta^{18}\text{O}$  values, the heaviest was in Mie,  $22.7\text{‰}$ , whereas the lightest was in Nagano,  $21.0\text{‰}$ . Notably, Nagano rice was enriched in  $^{14}\text{N}$ ,  $^{13}\text{C}$ , and  $^{16}\text{O}$ , whereas Fukushima rice was enriched in  $^{15}\text{N}$  and  $^{12}\text{C}$ . In the USA rice harvested in 2005, the short grain samples grown with artificial fertilizer (named US-2005SF in Table 1),  $\delta^{15}\text{N} = 4.0\text{‰}$ , had lower  $\delta^{15}\text{N}$  values than those grown using natural manure (named US-2005SM in Table 1),  $\delta^{15}\text{N} = 6.2\text{‰}$ . An average  $\delta^{15}\text{N}$  value of the middle grain samples (named US-2005MF in Table 1),  $1.3\text{‰}$ , was the lowest of all samples, whereas that of the long grain samples (named US-2005LF in Table 1) was  $3.3\text{‰}$ . The average  $\delta^{15}\text{N}$  values of the short grain and middle grain grown using natural manure were generally higher ( $6.0$  to  $7.0\text{‰}$ ) than those grown with artificial fertilizer. The  $\delta^{13}\text{C}$  values of the USA rice were independent of their species, grain size, and soil nutrition. The  $\delta^{18}\text{O}$  values were also independent of their species, grain size, and soil nutrition, although the average  $\delta^{18}\text{O}$  value of the samples harvested in 2005 was  $3\text{‰}$

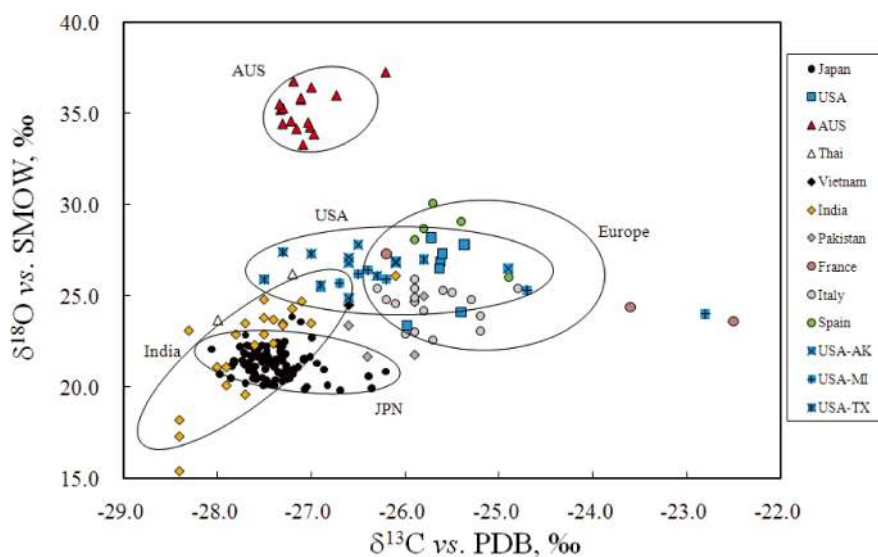


Fig. 2 Two-dimensional distribution of the carbon-oxygen stable isotopic values of rice samples obtained in this study, together with some Asian and European data in the literature.<sup>8</sup> The samples with some outliers are approximately encircled by the regions: Japan (JPN), USA, Australia (AUS), India, and Europe.

higher than that of those in 2006. In the Australian rice reaped in 2006, the short grain samples had the average values of  $\delta^{15}\text{N} = 5.4\text{‰}$ ,  $\delta^{13}\text{C} = -27.1\text{‰}$ ,  $\delta^{18}\text{O} = 33.6\text{‰}$ . The Thai, Vietnamese, and Chinese rice were in a range of  $\pm 0.5\text{‰}$  in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , and in a range of  $\pm 2\text{‰}$  in  $\delta^{18}\text{O}$ . The variation, however, may be significant, but the total numbers of the data were too small to discuss in detail. There was no significant correlation of the isotopic compositions with their species, condition, and grain size. The  $\delta\text{D}$  values of America:  $-85.7\text{‰}$ , and Australia:  $-72.6\text{‰}$ , seemed to show slightly heavier than those values of three Japanese regions (Nagano,  $-88\text{‰}$ ; Niigata,  $-92\text{‰}$ ; and Shimane,  $-94\text{‰}$ ), although the uncertainty of each analysis was too large to pursue the  $\delta\text{D}$  value further in the following Discussion section.

As far as the USA rice samples were concerned, the  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values were invariant within a few permil, indicating the values were independent of the rice species or grain sizes. The  $\delta^{15}\text{N}$  values were likely different between the fertilized and the manure cultivated rice samples, although the species and grain size were the same.

#### Elemental compositions

The nitrogen content of most samples centered at 1.0%, with some exceptions. For instance, USA's long grain rice (2005LF) and Chinese rice were as high as 1.3%. Carbon content of the rice samples were centered at 39.3 or 40.2%: the former belongs to USA and Chinese rice, while the latter to AUS, Thai, and Vietnamese. Hydrogen content was invariant and independent of the rice farming locality, grain size, and different kinds of nutrients. The oxygen content was slightly varied at around 52%. However, since the content was the residue subtracted the C, N, and H contents, some minor elements coexisting in the rice might influence on the O content. The C/N ratio showed a large variation from 30 to 44. The variance was likely controlled by the N content: the smaller the N content, the larger the C/N ratio.

## Discussion

#### Soil nutrition and climate

A correlation between  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of all rice samples employed in this study is drawn in Fig. 1. Japanese rice samples grown using artificial fertilizer gather tightly, although they were taken from seven different farming locations all over Japan. Similarly, Australian rice samples closely group together and locate within a distribution of the Japanese rice samples, with some outliers. Most of the American rice samples grown in the presence of fertilizer are encircled (named USA-F) in Fig. 1. They are statistically distinct from a distribution of the Japanese rice samples. Their  $\delta^{15}\text{N}$  values are slightly lower than those of Japanese and Australian. The reason is still unknown. However, the difference could be indicating that the  $\delta^{15}\text{N}$  of fertilizer is itself different among the farming countries. Some of the American samples situating outside of the USA-F group encircled in Fig. 1 are mostly the rice grown using manure, which has a  $\delta^{15}\text{N}$  value larger than that of fertilizer. As for the  $\delta^{13}\text{C}$  values, Japanese and Australian rice samples are hardly distinguishable. American rice samples in the USA-F group are apparently heavier in  $\delta^{13}\text{C}$  value than Japanese and Australian samples. The  $^{13}\text{C}$  enrichment in rice is generally attributed to a degree of dryness at the farming environment: when a plant grows in the more arid condition, the  $\delta^{13}\text{C}$  of the plant becomes heavier. Indeed, American rice is grown in dry fields in California, whereas Japanese rice is in high humidity. New South Wales, where the Australian rice is grown, is in between humid and arid climate. As a summary, this study confirmed that the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  diagram could show a difference in climate and source of fertilizer.

#### Irrigation water

Irrigation water using for plant growth is surface water, which originates somehow from meteoric water or snowmelt water. The  $\delta^{18}\text{O}$  value of the irrigation water reflects that of meteoric water precipitating at the cultivating area. A correlation between  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  of all rice samples employed in this study with

Table 1 Summary of analytical results on the multi-elemental compositions and their isotopic compositions of rice samples together with published data of India, Pakistan, France, Spain, and three states of the USA (Arkansas, Missouri, and Texas)<sup>8</sup>

Locality of rice farming and sample name	Sample size, <i>n</i>	Stable isotopic composition, ‰								Elemental composition, %				C/N
		$\delta^{15}\text{N}$ vs. Air	s.d.	$\delta^{13}\text{C}$ vs. PDS	s.d.	$(\delta^{18}\text{O}$ vs. SMOW)	s.d.	$(\delta\text{D}$ vs. SMOW)	s.d.	N	C	H	O	
Japan														
Fukushima	4	4.7	0.7	-27.8	0.1	21.6	0.6							
Nagano	10	2.8	0.7	-26.9	0.4	21.0	0.6	(-88)	5	0.92	39.47	6.87	52.74	42.90
Tochigi	28	4.6	1.0	-27.5	0.2	21.4	0.6			1.18	40.48	6.92	51.42	34.31
Niigata	44	3.2	0.6	-27.4	0.2	21.2	0.5	(-92)	57	0.90	40.06	6.84	52.20	44.51
Chiba	7	2.9	0.4	-27.3	0.1	21.6	0.5			1.04	40.02	6.84	52.10	38.48
Mie	3	3.5	0.6	-27.4	0.2	22.7	0.8			1.30	40.24	6.76	51.70	30.95
Ibaragi	3	4.6	1.2	-27.3	0.3	22.1	1.0							
Shimane	4	1.7	1.0	-27.3	0.3			(-94)	6	0.95	38.63	6.54	53.88	40.66
Total	103													
Average		3.5		-27.4		21.6		(-91)		1.05	39.82	6.80	52.34	38.64
California, United States of America														
2005SF	6	3.8	1.5	-25.7	0.4	27.3	0.7	(-85.7)	nd	0.93	39.19	6.53	53.36	42.68
2005SM	2	6.1	0.1	-26.3	0.2					1.00	38.76	6.72	53.52	38.76
2005MF	8	1.3	0.5	-26.8	0.5					0.95	39.36	6.66	53.04	41.65
2005MM	2	7.7	2.3	-26.9	0.4					0.89	39.32	6.69	53.10	44.18
2005LF	3	4.1	1.4	-27.0	0.4					1.25	39.28	6.76	52.71	31.42
2006SF	7	1.1	0.4	-26.1	0.5	24.9	1.2							
2006SM	2	4.3	0.3	-26.2	0.1	24.1	nd			1.08	39.70	6.91	52.31	36.76
AK, L <sup>a</sup>	8			-26.3	0.6	26.5	0.9							
MS, L <sup>b</sup>	6			-25.6	1.5	25.6	0.9							
TX, L <sup>c</sup>	6			-26.8	0.6	26.6	0.8							
Total	50													
Average		4.1		-26.4		25.8		(-85.7)		1.02	39.27	6.71	53.01	39.24
Australia														
2006S	17	5.4	0.4	-27.1	0.3	35.0	1.4	(-72.6)	nd	1.05	40.61	6.80	51.53	39.18
2006M	4					32.2	0.6			1.08	40.00	6.51	52.42	38.72
Total	21													
Average		5.4		-27.1		32.6		(-72.6)		1.06	40.31	6.66	51.97	38.95
Thai														
2006S	2	3.2	0.8	-27.6	0.6	25.0	1.8			0.93	40.19	6.67	52.22	43.21
Vietnam														
2006S	1			-26.6	nd	24.5	nd			1.18	40.16	6.87	51.79	34.06
China														
2006S	6	3.9	nd	-26.3	nd	25.3	1.6			1.32	39.13	6.63	52.92	29.64
India														
Kelly2002 <sup>d</sup>	23			-27.7	0.6	22.1	2.6							
Pakistan														
Kelly2002 <sup>d</sup>	5			-26.1	0.4	23.3	1.6							
France														
Kelly2002 <sup>d</sup>	3			-24.1	1.9	25.1	1.9							
Italy														
Kelly2002 <sup>d</sup>	17			-25.7	0.4	24.5	1.0							
Spain														
Kelly2002 <sup>d</sup>	5			-25.5	0.4	28.4	1.5							

All isotopic data were shown as their average values with their standard deviations, and the elemental compositions were presented as the averages of samples measured in this study. s.d., Standard deviation; nd, not determined. Besides Japanese samples, the sample name indicates the cultivation year, grain size (S, short; M, middle; L, long grain), and kinds of soil nutrition (F, fertilizer; M, manure); *i.e.*, the 2005MM means a middle grain rice grown with manure cultivated in the year of 2005. The  $\delta\text{D}$  values in parentheses were analytical results on one or two sample sizes with a large uncertainty of each analysis (see text). a. Long grain rice samples from Arkansas. b. Long grain rice samples from Missouri. c. Long grain rice samples from Texas. d. Data in literature.<sup>8</sup>

data in the literature<sup>8</sup> is drawn in Fig. 2. The rice samples from Japan, Australia, and USA, are apparently distinct from each other. There is no intersection between any pair out of the three countries. Moreover, no dependence of rice species, grain size, or soil nutrition on the  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  diagram is observed. European rice samples, which are colonized at around  $\delta^{13}\text{C} = -25.5\text{‰}$  and  $\delta^{18}\text{O} = 25\text{‰}$ , are close to Californian rice samples whereas they are different from the

Arkansas-Missouri-Texas rice samples. Indian rice samples cover all of the Japanese rice samples and Arkansas-Missouri-Texas rice samples. Pakistan rice samples were closer to European-Californian rice samples. Remarkably, Australian rice samples are significantly heavier in  $\delta^{18}\text{O}$ , which American rice samples have slightly heavier  $\delta^{18}\text{O}$  values than the Japanese samples. This may be explained by referring to the GNIP/ISOHIS databases from International Atomic Energy

Agency (IAEA), by which the  $\delta^{18}\text{O}$  value of the precipitant at any place in the whole world is known (GNIP/ISOHIS database, 2002).<sup>26</sup> For instance, the  $\delta^{18}\text{O}$  value of precipitation in New South Wales during the rice cultivation period (November to March) is in a range from  $-4$  to  $-2\text{‰}$ . That in California during the cultivation period (May to September) is generally very high ( $-4$  to  $0\text{‰}$ ). However, during the period, California is very dry and has only a chance of rain. Californian rice uses surface water supplied from a dam, which stores meteoric water precipitated during wintertime. Such water has very light  $\delta^{18}\text{O}$  values as  $-14$  to  $-6\text{‰}$ . In Japan, the  $\delta^{18}\text{O}$  of meteoric water ranges from  $-10$  to  $-6\text{‰}$  during the period between May and September. The  $\delta^{18}\text{O}$  value of the Japanese irrigation water could be lower than this when the water includes snowmelt water, which is much lighter than the usual precipitate. Thus, a trend along the  $\delta^{18}\text{O}$  scale in Fig. 2 is likely explainable as follows: Australian  $\delta^{18}\text{O}$  > American  $\delta^{18}\text{O}$  > Japanese  $\delta^{18}\text{O}$ . As a summary, according to the  $\delta^{18}\text{O}$  classification, the  $\delta^{18}\text{O}$  values of the rice samples indicate those of the regional irrigation water.

#### Comparisons of the Asian rice samples to the three majors

We determined the  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{18}\text{O}$  values of the rice samples from Thailand, Vietnam, and China. In Fig. 1, one sample of China is indistinguishable from the USA-F group, whereas at least it does not belong to either Japanese or Australian group. In Fig. 2, one sample of Vietnam is clearly isolated and belongs to none of the groups. Two samples from Thailand are much more distinctive in Fig. 2 than Fig. 1. As seen in Figs. 1 and 2, this multi-isotopic classification could be useful to distinguish those Asian rice samples from Japanese rice samples, only if a sufficient number of data verifies it.

#### Application of the principal component analysis to the rice samples in this study

Figure 3 shows a diagram of two major principal components representing three variable quantities,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{18}\text{O}$  values of rice samples. The first principal component expressed as  $Z_1 = 0.521 \times (\delta^{15}\text{N}) + 0.175 \times (\delta^{13}\text{C}) + 0.612 \times (\delta^{18}\text{O})$  is drawn along the horizontal axis, whereas the second principal component expressed as  $Z_2 = -0.452 \times (\delta^{15}\text{N}) + 0.791 \times (\delta^{13}\text{C}) + 0.160 \times (\delta^{18}\text{O})$  is along the vertical axis. As can be seen in the equations, the  $\delta^{18}\text{O}$  has largest factor among three variables of  $Z_1$  and the  $\delta^{13}\text{C}$  has that of  $Z_2$ . For the  $\delta^{15}\text{N}$ , remarkably, the absolute value of the  $Z_1$  is nearly equal to that of the  $Z_2$ , although the directions are opposite. The  $Z_1$  score increases with increasing both the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values together, whereas the  $Z_2$  score increases with decreasing the  $\delta^{15}\text{N}$  and increasing the  $\delta^{13}\text{C}$  value, synchronously. The  $Z_1$  may thus indicate a change of soil nutrition from artificial fertilizer to natural manure or irrigation water originating from isotopically distinct sources. Similarly, the  $Z_2$  may represent a reverse change of the soil nutrition and a change of the humidity from humid to arid cultivation environments. If this is the case, Fig. 3 would show agrochemical and climatic classification of the rice samples employed in this study, although the meaning of the axes is still under discussion. It is clear that all of Japanese rice samples are closely gathered at the left side of the lower part of Fig. 3. They are obviously distinctive from the Australian rice samples, and moderately distinguishable from American rice samples with an outlier, as Suzuki *et al.*<sup>10,11</sup> pointed out earlier. Thai rice samples ( $n = 2$ ) are only included in this analysis, showing that one out of the two samples is clearly discriminated from any of the groups.

For further investigation, rice samples collected from the USA, Australia, and other regions are certainly required.

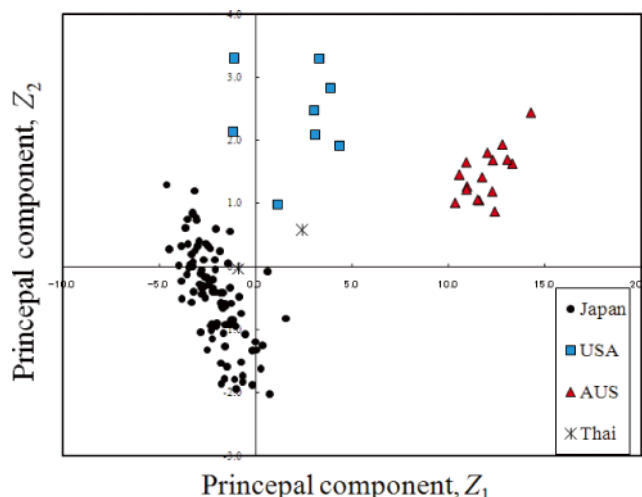


Fig. 3 Two dimensional plot generated by the principal component analysis on the C, N, and O isotopic compositions of the rice samples. The horizontal and vertical axes represent the score points of the principal component ( $Z_1$ ) and those of the  $Z_2$ , respectively. The origin of the coordinate axes is equivalent to the average values of the two scores.

## Conclusions

Stable isotopes of the light elements, H, C, N, and O, were analyzed in rice samples, mostly Koshihikari, taken from three different farming countries such as Japan ( $n = 103$ ), United States of America ( $n = 30$ ), and Australia ( $n = 21$ ), in addition of rice samples from Thailand ( $n = 2$ ), Vietnam ( $n = 1$ ), China ( $n = 6$ ). Major remarks are as follows.

- 1) The  $\delta^{15}\text{N}$ - $\delta^{13}\text{C}$  diagram could explain a difference in climate and in source of fertilizer.
- 2) According to the  $\delta^{18}\text{O}$  classification, the  $\delta^{18}\text{O}$  values of the rice samples indicate those of the regional irrigation water.
- 3) The multi-isotopic classification could be useful to distinguish those Asian rice samples from Japanese rice samples, only if a sufficient number of data verifies it.
- 4) By performing the principal component analysis, we could factorize the rice samples into two dimensions: along a  $Z_1$ -axis, the score increases with increasing both the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values taken together; along a  $Z_2$ -axis, the score increases with decreasing the  $\delta^{15}\text{N}$  and increasing the  $\delta^{13}\text{C}$  value, synchronously.

## Acknowledgements

The authors thank an anonymous reviewer for the constructive comments, and T. Ichimiya for his enormous efforts to obtain mass spectrometry results, and acknowledge those who kindly donated rice samples: I. Yamagata (JRMA), Nagano Agricultural Experiment Station, Zen-Noh Pearl Rice Corp., DAFF of Ibaragi Prefecture, Mie Rice Corp., and Shokkyo Corp. This work was financially supported by Bio-oriented Technology Research Advancement Institution, Japan.

## Supporting Information

The details of the analytical results of the rice samples from

Japan (JPN), the United States of American (USA), Australia (AUS), Thailand, Vietnam, and China, together with published data of those from India, Pakistan, France, Italy, Spain, and three States (Arkansas, Missouri, and Texas) of the USA,<sup>8</sup> are summarized in Tables S1 and S2. These materials are available free of charge on the web at <http://www.jsac.or.jp/anasci/>.

## References

1. K. Ohtsubo, S. Nakamura, and T. Imamura, *Nippon Nogeikagaku Kaishi*, **2002**, 76, 388.
2. A. Kawasaki, H. Oda, and T. Hirata, *Soil Sci. Plant Nutrition*, **2002**, 48, 635.
3. H. Oda, A. Kawasaki, and T. Hirata, *Anal. Sci.*, **2002**, 17, i1627.
4. K. Kitta, M. Ebihara, T. Iizuka, R. Yoshikawa, K. Isshiki, and S. Kawamoto, *J. Food Comp. Anal.*, **2005**, 18, 269.
5. A. Yasui and K. Shindoh, *Bunseki Kagaku*, **2000**, 49, 405.
6. E. Wada and Y. Handa, *Seikagaku (Biochemistry, in Japanese)*, **1994**, 66, 15.
7. J. Hoefs, "Stable Isotope Geochemistry," **2004**, Springer.
8. S. Kelly, M. Baxter, S. Chapman, C. Rhodes, J. Dennis, and P. Brereton, *Eur. Food Res. Technol.*, **2002**, 214, 72.
9. S. Kelly, K. Heaton, and J. Hoogewerff, *J. Trends Food Sci. Technol.*, **2005**, 16, 555.
10. Y. Suzuki, Y. Chikaraishi, N. O. Ogawa, N. Ohkouchi, and T. Korenaga, *Food Chem.*, **2008a**, 109, 470.
11. Y. Suzuki, R. Nakashita, F. Akamatsu, and T. Korenaga, *Nippon Shokuhin Kagaku Kogaku Kaishi*, **2008b**, 55, 250.
12. G. J. Padovan, D. De Jong, L. P. Rodrigues, and J. S. Marchini, *Food Chem.*, **2003**, 82, 633.
13. A. Roßmann, F. Reniero, I. Moussa, H. L. Schmidt, G. Versini, and M. H. Merle, *Eur. Food Res. Technol.*, **1999**, 208, 400.
14. W. A. Simpkins, G. Patel, M. Harrison, and D. Goldberg, *Food Chem.*, **2000**, 70, 385.
15. O. Schmidt, J. M. Quilter, B. Bahar, A. P. Moloney, C. M. Scrimgeour, I. S. Begley, and F. J. Monahan, *Food Chem.*, **2005**, 91, 545.
16. P. Ritz, P. Gachon, J. P. Garel, J. C. Bonnefoy, J. B. Coulon, and J. P. Renou, *Food Chem.*, **2005**, 91, 521.
17. A. Rossmann, G. Haberhauer, S. Hölzl, P. Horn, F. Pichlmayer, and S. Voerkelius, *Eur. Food Res. Technol.*, **2000**, 211, 32.
18. M. J. Baxter, H. M. Crews, M. J. Dennis, I. Goodall, and D. Anderson, *Food Chem.*, **1997**, 60, 443.
19. B. N. Smith and S. Epstein, *Plant Physiol.*, **1971**, 47, 380.
20. D. H. Kohl, G. B. Shearer, and B. Commones, *Soil Sci. Soc. Am. J.*, **1973**, 37, 888.
21. V. W. Meints, G. Shearer, D. H. Kohl, and L. T. Kurtz, *Soil Sci.*, **1975**, 119, 421.
22. G. Shearer and J. O. Legg, *Soil Sci. Soc. Am. J.*, **1975**, 39, 896.
23. H. Craig, *Science*, **1961**, 133, 1702.
24. J. Gray and P. Thompson, *Nature*, **1976**, 262, 481.
25. Y. Chikaraishi, K. Matsumoto, N. Ogawa, H. Suga, H. Kitazato, and N. Ohkouchi, *Phytochemistry*, **2005**, 66, 911.
26. GNIP/ISOHIS Databases, IAEA, **2004**, <http://isohis.iaea.org>.