

Analytical Methods

# Geographical origin of polished rice based on multiple element and stable isotope analyses

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Received 18 January 2007; received in revised form 26 December 2007; accepted 28 December 2007

## Abstract

We determined carbon and nitrogen contents (C and N contents) and stable carbon, nitrogen, and oxygen isotopic compositions ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{18}\text{O}$ ) of polished rice in order to develop a simple method to discriminate its geographical origin. As a first attempt, we examined a single cultivar, Koshihikari rice, from 14 different cultivation areas including Australia ( $n = 1$ ), Japan ( $n = 12$ ), and USA ( $n = 1$ ). For all rice samples, C and N contents and the isotopic compositions are consistent with those of general plant materials, being 37.2–40.0% (C content), 0.8–1.4% (N content),  $-27.1$  to  $-25.4\%$  ( $\delta^{13}\text{C}$ ),  $+0.4$  to  $+9.0\%$  ( $\delta^{15}\text{N}$ ), and  $+18.8$  to  $+22.9\%$  ( $\delta^{18}\text{O}$ ). However, its cultivated area is clearly distinguished by a pentagonal radar plot based on the elemental and isotopic compositions. Thus, the comparison of C and N contents and  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{18}\text{O}$  values would potentially be useful for rapid and routine discrimination of geographical origin of the polished rice.

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**Keywords:** Rice; Geographical origin;  $\delta^{13}\text{C}$ ;  $\delta^{15}\text{N}$ ;  $\delta^{18}\text{O}$ ; C and N contents; Koshihikari

## 1. Introduction

In Japan, polished rice, when packaged, requires labels indicating cultivar, cultivation area, and year of production in accordance with the Japanese Agricultural Standard (JAS) Law. However, the geographical location or rice cultivar cannot be distinguished by image analysis, leading to rice authenticity problems such as mislabeling and addition of inferior rice to premium rice. In particular, Koshihikari, a cultivar of Japanese rice is the most famous premium rice and has sometimes been targeted for the adulteration. Moreover, Koshihikari is cultivated not only in Japan but also in various countries such as Australia and USA. Thus, rice cultivar and cultivation area are important factors in determining the market value of polished rice.

A simple analytical method which identifies them is required to resolve these rice authenticity problems.

Recently, rice cultivars have been identified powerfully by gene analysis using polymerase chain reaction (PCR) (Ohtsubo, Nakamura, & Imamura, 2002). On the other hand, the relationship between chemical compositions and cultivation areas has been evaluated using strontium and boron isotope ratios (Kawasaki, Oda, & Hirata, 2002; Oda, Kawasaki, & Hirata, 2002), and fatty acid compositions (Kitta et al., 2005). However, they require numerous purification steps, thus increasing the risk of sample contamination and loss of analyte. Trace element analysis has also been used as a rapid tool for discrimination of cultivation areas (Yasui & Shindoh, 2000).

Stable isotope analysis has widely been used to trace the origin of organic materials in various fields, such as geochemistry, biochemistry, archaeology, and petroleum chemistry (e.g., Ambrose & DeNiro, 1986; Hayes, Freeman, Popp, & Hoham, 1990; Rozanski, Araguás-Araguás,

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& Gonfiantini, 1992). It has also become increasingly important as a solution tool for food authenticity problems. Generally, the isotopic compositions of plant materials reflect various factors such as isotopic compositions of source materials (e.g., CO<sub>2</sub>, H<sub>2</sub>O, NH<sub>4</sub>, and NO<sub>2</sub>) and their assimilation processes as well as growth environments. For example, the carbon isotopic composition of plant materials strongly depends on the carbon fixation process such as the C<sub>3</sub> or C<sub>4</sub> cycle (Smith & Epstein, 1971). The nitrogen isotopic composition mainly depends on soil nutrition (Kohl, Shearer, & Commanes, 1973; Meints, Shearer, Kohl, & Kurtz, 1975; Shearer & Legg, 1975). The oxygen isotopic composition mainly reflects that of local groundwater such as precipitation and meltwater (Craig, 1961; Gray & Thompson, 1976). Therefore, characteristics of the isotopic compositions have been used to investigate the authenticity of food materials (Kelly, Heaton, & Hoo-gewerff, 2005). For example, the adulteration of honey, juice, and wine can be identified by differences in the carbon, nitrogen and/or oxygen isotopic compositions between authentic and adulterated products (Padovan, De Jong, Rodrigues, & Marchini, 2003; Rossmann et al., 1999; Simpkins, Patel, Harrison, & Goldberg, 2000). Moreover, the geographical origin of meat (Schmidt et al., 2005), dairy products (Ritz et al., 2005; Rossmann et al., 2000), wine (Baxter, Crews, Dennis, Goodall, & Anderson, 1997), and cereal crops (Kelly et al., 2002) can be traced by using natural variations of the isotopic compositions.

This is also applied to a rice provenance study. Kelly et al. (2002) demonstrated that long-grain rice from USA, Europe, India, and Pakistan is distinguished by boron and magnesium contents and by oxygen isotopic composition.

In this study, we determined carbon and nitrogen contents (C and N contents) and stable carbon, nitrogen, and oxygen isotopic compositions ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{18}\text{O}$ ) of polished Koshihikari rice cultivated in Australia (New South Wales), Japan (12 different cultivation areas), and USA (California) by using elemental analyzer/isotope ratio mass spectrometry (EA/IRMS), in order to develop a simple and rapid method to discriminate geographical origin of the rice. Because multiple element and stable isotope analyses will potentially discriminate cultivation areas of rice and the recent spread of EA/IRMS facilitates rapid and routine analysis of the elemental and stable isotopic compositions of organic materials, this method will become an improvement tool for discriminating the geographical origin of polished rice.

## 2. Materials and methods

### 2.1. Samples

Fourteen samples of polished Koshihikari rice were collected from Australia (1 sample from New South Wales), Japan (12 samples from different cultivation areas), and

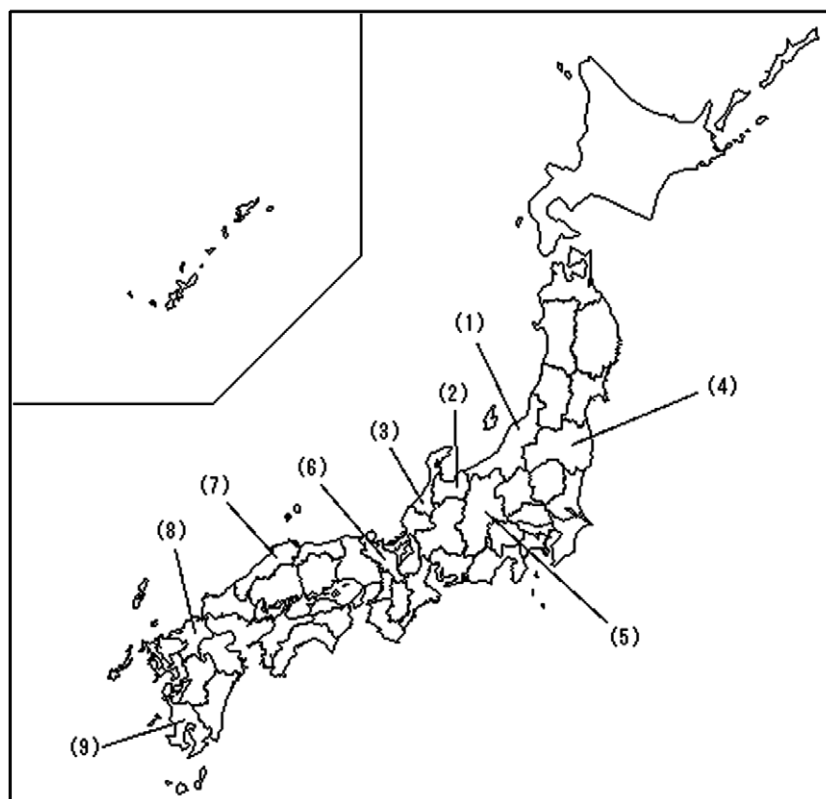


Fig. 1. Location map for Japanese rice (Niigata (1), Toyama (2), Ishikawa (3), Fukushima (4), Nagano (5), Kyoto (6), Shimane (7), Fukuoka (8), and Kagoshima (9)).

USA (1 sample from California). The 12 Japanese samples were collected from the following nine prefectures: Niigata (1), Toyama (2), Ishikawa (3), Fukushima (4), Nagano (5), Kyoto (6), Shimane (7), Fukuoka (8), and Kagoshima (9) (Fig. 1). For Nagano, Niigata, and Shimane, they were collected from two different location within a single prefecture: Azumino and Iiyama in Nagano, Arai, and Uonuma

in Niigata, and Matue and Okuizumo in Shimane. All samples were obtained in the year 2004.

## 2.2. Measurement

Dried rice (10–15 g) was ground to a fine powder before analysis. For carbon and nitrogen, the powdered rice was

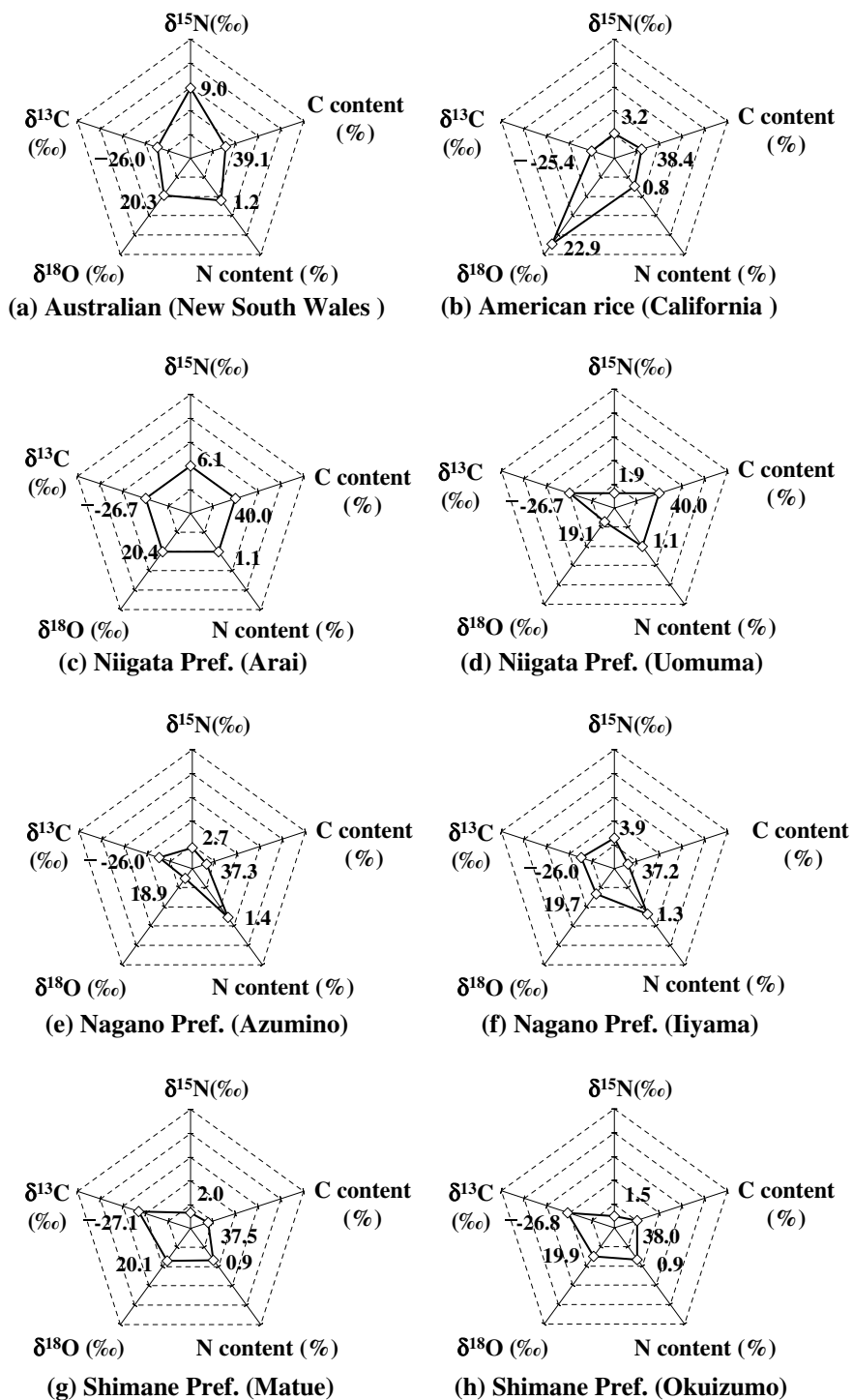


Fig. 2. Distributions of C and N contents, and carbon, nitrogen, and oxygen isotopic compositions of rice from (a) New South Wales (Australia), (b) California (USA) and 12 different cultivation areas in Japan ((c) Niigata (Arai), (d) Niigata (Uonuma), (e) Nagano (Azumino), (f) Nagano (Iiyama), (g) Shimane (Matue), (h) Shimane (Okuizumo), (i) Fukushima, (j) Kyoto, (k) Ishikawa, (l) Toyama, (m) Kagoshima, and (n) Fukuoka).

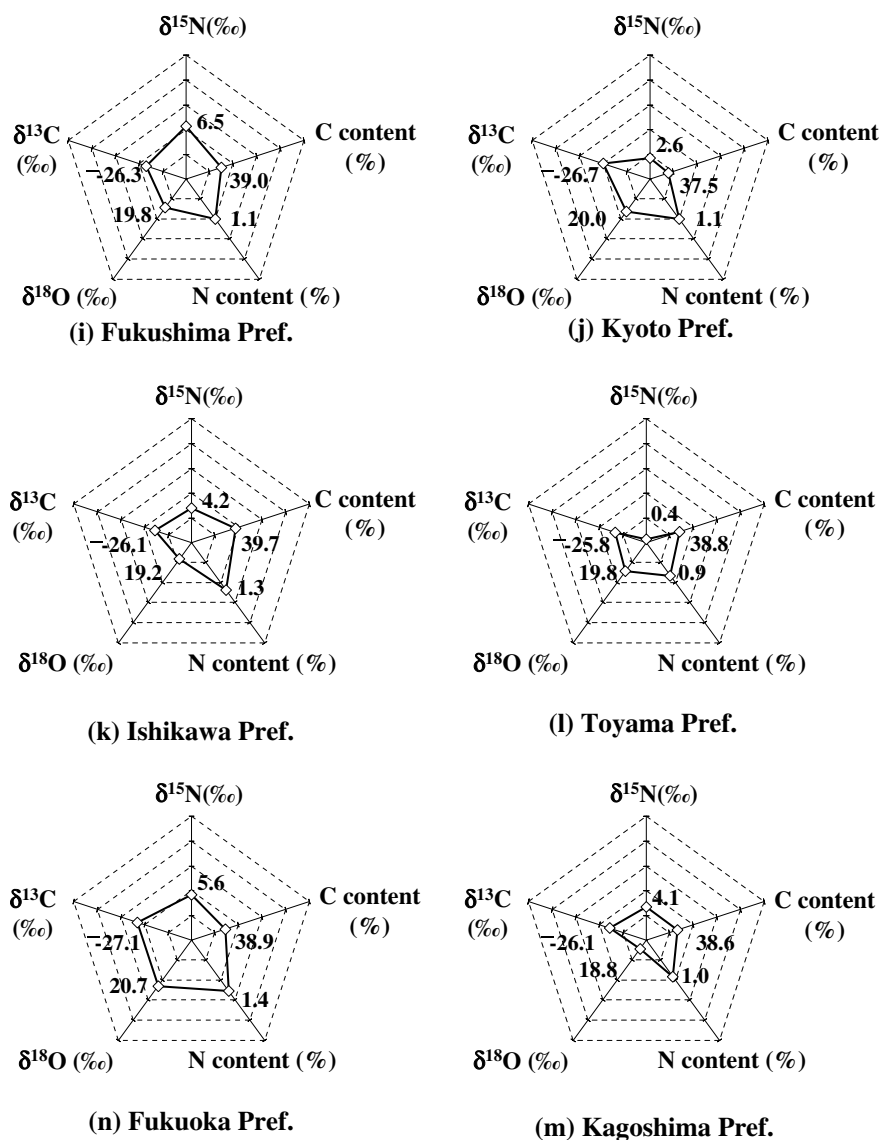


Fig. 2 (continued)

weighed 1.1–1.2 mg into a tin capsule (5 × 9 mm). Then, each sample was analyzed by elemental analyzer/isotope ratio mass spectrometry (EA/IRMS) using a Finnigan Delta plus XP (Thermo Electron Corporation) interfaced with a Flash EA 1112 (Thermo Electron Corporation) to determine C and N contents and carbon and nitrogen isotope ratios. For oxygen, the powdered rice was weighed 0.2–0.3 mg into a silver capsule (5 × 9 mm). Then, oxygen isotope analysis was carried out by thermo-combustion EA/IRMS (TCEA/IRMS) using a Finnigan Delta plus XP interfaced with a Finnigan TCEA (Thermo Electron Corporation).

The  $\delta$  notation is used to describe the isotopic difference between the sample and an international standard, which was defined as a following Eq. (1),

$$\delta(\text{‰}) = (R_{\text{sam}} - R_{\text{std}}) / R_{\text{std}} \cdot 1000 \quad (1)$$

where  $R_{\text{sam}}$  is the isotope ratio (i.e.,  $^{13}\text{C}/^{12}\text{C}$ ,  $^{15}\text{N}/^{14}\text{N}$ , and  $^{18}\text{O}/^{16}\text{O}$ ) of the sample, and  $R_{\text{std}}$  is the isotope ratio of the international standards: for carbon: Pee Dee Belem-

nite (PDB); for nitrogen: Air; and for oxygen: Standard Mean Ocean Water (SMOW). Isotope value is given per mil (‰). Carbon, nitrogen, and oxygen isotopic compositions can normally be measured with an analytical precision of  $\pm 0.2$ – $0.3\%$  (e.g. Ohkouchi et al., 2005). C and N contents are given % of dry w/w.

### 3. Results and discussion

For all samples, C and N contents and  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{18}\text{O}$  values are contributed from 37.2–40.0%, 0.8–1.4%,  $-27.1$  to  $-25.4\%$ ,  $+0.4$  to  $+9.0\%$ , and  $+18.8$  to  $+22.9\%$ , respectively. These C and N contents and isotopic compositions are consistent with those of general plant materials (Kelly et al., 2002; Yoneyama, Fujiwara, & Wilson, 1998). However, Californian rice has a higher  $\delta^{18}\text{O}$  value ( $+22.9\%$ ) than other samples, which is clearly distinguished from New South Wales ( $+20.3\%$ ) and Japanese rice samples ( $+18.8\%$  to  $+20.7\%$ ). In general, oxygen isotopic

composition of plant materials mainly reflects that of precipitation. In fact, based on the GNIP/ISOHIS Databases from International Atomic Energy Agency (IAEA) monthly weighted average data on the oxygen composition of precipitation, the  $\delta^{18}\text{O}$  of precipitation in California ( $-6\%$  to  $-2\%$ ) during the rice cultivation period is higher than that in New South Wales ( $-9\%$  to  $-6\%$ ) and Japan ( $-10\%$  to  $-6\%$ ) (GNIP/ISOHIS databases, 2004). These data suggest that the high  $\delta^{18}\text{O}$  value of precipitation in California may be reflected in the high  $\delta^{18}\text{O}$  value of the Californian rice. Moreover, New South Wales rice has a higher  $\delta^{15}\text{N}$  value ( $+9.0\%$ ) than other rice samples, which is clearly discriminated from both Californian ( $+3.2\%$ ) and Japanese rice samples ( $+0.4$  to  $+6.1\%$ ). Nitrogen isotopic composition of rice is thought to depend mainly on soil nutrition, where the rice is cultivated. Generally, organic fertilizers increase  $^{15}\text{N}$  content in soil and plants, whereas the utilization of artificial fertilizers decreases it (Kohl et al., 1973; Meints et al., 1975; Shearer & Legg, 1975). In fact, in Australia, many farmers rotate rice crops with pasture crops during several years. In the turn of pasture crop, cattle and sheep graze on the pasture and their dung is put into the soils. They could increase  $\delta^{15}\text{N}$  value of the soils. Thus, the agricultural cycle in Australia may be consistent with the high  $\delta^{15}\text{N}$  value of the Australian rice in this study.

For all Japanese rice samples, C and N contents and the isotopic compositions ranged from 37.2–40.0% (C content), 0.9–1.4% (N content),  $-27.1$  to  $-25.8\%$  ( $\delta^{13}\text{C}$ ),  $+0.4$  to  $+6.5\%$  ( $\delta^{15}\text{N}$ ), and  $+18.8$  to  $+20.7\%$  ( $\delta^{18}\text{O}$ ). It is difficult to discriminate between the regional origins within Japan from a single factor of them. However, these cultivation locations are distinguished by a pentagonal radar plot based on the elemental and isotopic compositions (Fig. 2). Moreover, in Shimane prefecture, a quite similar pattern on the pentagonal radar plot between Matue and Okuizumo suggests that rice from very similar growth condition shows almost the same pattern on the plot. On the other hand, distinct pattern on the radar plots are observed between different locations within Nagano and Niigata prefectures. In Nagano prefecture, the radar plots between Azumino and Iiyama shows different  $\delta^{18}\text{O}$  signatures. The Azumino rice has a lower  $\delta^{18}\text{O}$  value ( $18.9\%$ ) than the Iiyama one ( $19.7\%$ ), probably reflecting a lower  $\delta^{18}\text{O}$  value of precipitation in Azumino than Iiyama because the former locates at higher latitude than the later. Thus, a small (but important) difference in growth condition is probably reflected in the factors on the radar plot. Interestingly, the radar plot of the polished rice from Uonuma is quite different from Arai as well as other Japanese rice samples, characterized by lower values in both  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ . Since Uonuma rice is the most premium rice in Japan, it has been always targeted for adulteration. In this study, however, Uonuma rice is distinguishable from all other Japanese rice samples and thus our method could particularly be useful as a diagnostic tool for the Uonuma rice.

#### 4. Conclusion

We determined carbon and nitrogen contents (C and N contents) and stable carbon, nitrogen, and oxygen isotopic compositions ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{18}\text{O}$ ) of polished Koshihikari rice from various cultivated areas (Australia, Japan, and USA) in order to develop a simple method to discriminate its geographical origin. In this study, rice from New South Wales (Australia) and California (USA) are clearly distinguished from Japanese rice samples by high  $\delta^{15}\text{N}$  or high  $\delta^{18}\text{O}$  values. Also, 12 Japanese rice show the characteristic pentagonal radar plot based on the elemental and isotopic compositions, which probably reflect even a small difference between growth environments within Japan. Particularly, Uonuma rice, the most premium rice in Japan, is clearly distinguished from all other rice samples by the radar plot. Thus, comparison of C and N contents and  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{18}\text{O}$  values would be potentially useful for rapid and routine analyses of geographical origin of polished rice.

However, it is still uncertain whether or not the observed difference in elemental and isotopic compositions is generally applicable to all Koshihikari rice as well as other cultivars. Particularly, in this study, it was difficult to assess the natural variability on Australia and USA rice samples. In fact, Kelly et al. (2002) reported  $\delta^{18}\text{O}$  variation by as much as 4% for long-grain rice within USA. Moreover, little information is available for annual variations on the elemental and isotopic compositions of rice. Thus, further statistical investigation based on a large number of samples is required. However, such requirement could hardly be problematic for the developed method of this study because EA/IRMS method allows rapid and routine analysis of the elemental and stable isotopic compositions even for a larger number of samples. Combined with such further investigation, we anticipate that the developed method will become a strong tool to discriminate geographical origin of polished rice.

#### Acknowledgments

This work was supported by a research fellowship of the Japan Society for the Promotion of Science for Young Scientists and the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN).

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