Ecol Res (2014) 29: 45–53
DOI 10.1007/s11284-013-1097-y

ORIGINAL ARTICLE

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Responses of carbon and oxygen stable isotopes in rice grain (*Oryza sativa* L.) to an increase in air temperature during grain filling in the Japanese archipelago

Received: 19 September 2012 / Accepted: 4 November 2013 / Published online: 28 November 2013

Abstract Stable isotopic compositions of carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) in plants reflect growth conditions. Therefore, these isotopes might be good indicators of changes in environmental factors, such as variations in air temperature caused by climate change. It is predicted that climate change will lead to a greater increase in minimum air temperatures (primarily during the night) than in maximum air temperatures (primarily during the day) in many parts of Japan. In the present study, we investigated whether the $\delta^{13}C$ and $\delta^{18}O$ of the rice grain Koshihikari (*Oryza sativa* L.) from the northern latitudes (30.49°–37.14°) of Japan reflect variations in air temperature during grain filling and are related to the yield and proportion of first-grade rice (< 15 % transparency, roundness, and cracking) as an indicator of quality. We revealed that rice $\delta^{13}C$ was not correlated with mean maximum or minimum air temperatures for each prefecture. By contrast, rice $\delta^{18}O$ was positively correlated with mean minimum air temperature, suggesting that rice $\delta^{18}O$ reflects changes in night air temperature. We further showed that an increase in the mean minimum air temperature during grain filling had a negative effect on rice yield and quality. Our findings indicate that the $\delta^{18}O$ of rice grain may be a good indicator of physiological changes in response to minimum air temperatures during grain filling.

Keywords Carbohydrate · Climate change · Koshihikari rice · Minimum air temperature · Oxygen isotope discrimination

Electronic supplementary material The online version of this article (doi:10.1007/s11284-013-1097-y) contains supplementary material, which is available to authorized users.

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Introduction

The Intergovernmental Panel on Climate Change (IPCC 2007) has developed future climate scenarios based on expected increases in temperature and atmospheric concentrations of greenhouse gases such as carbon dioxide (CO_2), methane, and nitrous oxide. Increased concentrations of CO_2, coupled with higher temperatures, are likely to have a considerable impact on the future dynamics of water and nutrients, and also on the structure and function of ecosystems (Cramer et al. 2001; Henry et al. 2005). This, in turn, will lead to substantial changes in agricultural crops (Peng et al. 2004; Yoshimoto et al. 2005; Masutomi et al. 2010), which are widely distributed and grown using the same agricultural management practices.

The Japanese archipelago extends over a distance of approximately 2,000 km, and its climate ranges from subtropical to subarctic (Japan Meteorological Agency 2005). There are concerns that climate change in the region will affect not only the production but also the quality of rice (*Oryza sativa* L.)—one of the most important basic food resources in Japan. The impact of climate change on rice production has been investigated extensively using crop models and climate change scenarios (Matthews et al. 1997; Aggarwal and Mall 2002; Tao et al. 2008). The results suggest that global warming will enhance rice production in northern Japan, but reduce production by at least 15–30 % in the south-central and southwestern parts of the country (Horie et al. 1995). In addition, high temperatures during the grain-filling period may reduce rice quality, as measured by grain size, transparency, roundness, and cracking (Yamakawa et al. 2007; Morita 2008; Hasegawa et al. 2011). However, the way in which high air temperature, especially at night, decreases the fertility of rice grains remains unclear (Cheng et al. 2009).

Carbon isotope discrimination ($\Delta^{13}C$) occurs in C_3 plants, owing to series of isotope fractionations along the photosynthesis pathway. As a consequence, plant carbon is invariably depleted in ^{13}C in respect to the isotopic composition of atmospheric CO_2. The parameter $\Delta^{13}C$ is an important indicator of all those conditions affecting the photosynthetic set point, i.e., the internal to atmospheric CO_2 concentration ratio (Ci/Ca) (Farquhar et al. 1989). The $\delta^{13}C$ of plants depends on fractionation during diffusion of CO_2 into the leaf, and also on subsequent photosynthetic metabolism and water-use efficiency (WUE) independently (Farquhar et al. 1982; Kume et al. 2003; Hanba et al. 2010; Ma et al. 2012). Most carbon stored in mature rice grains originates from CO_2 assimilation during the grain filling period, with the flag leaf as the most photosynthetically active (Murchie et al. 1999). Factors such as the photosynthesis rate of the flag leaf during this period could potentially determine grain yield (Dingkuhn et al. 1989). Furthermore, the plastic response of WUE to water availability has been demonstrated using instantaneous gas-exchange measurements, by analyses of plant $\delta^{13}C$ (Ehleringer and Cooper 1988; Toft et al. 1989; Centritto et al. 2009). Therefore, rice $\delta^{13}C$ may reflect an integration over time of photosynthesis and plant water status (with related factors such as sunshine hours, precipitation, humidity, and air temperature), as this is affected by water availability and evapotranspiration demand by the atmosphere.

Oxygen isotope discrimination ($\Delta^{18}O$) in C_3 plants occurs mainly during transpiration at the leaf relating to the atmospheric to internal vapor pressure ratio (Barbour 2007). Although the oxygen stable isotopic composition ($\delta^{18}O$) of xylem water reflects purely soil water taken up by roots (Dawson and Ehleringer 1991), there are complex phenomena of remixing and back diffusion of the enriched water with xylem water (i.e., metabolic water) arriving to the leaves and permeating the chloroplasts, consequently determining the $\delta^{18}O$ of metabolic water (Farquhar and Lloyd 1993). Additionally, the $\delta^{18}O$ of soil water is normally closely linked to precipitation, which becomes enhanced with $\delta^{18}O$ as the temperature increases at lower latitudes and altitudes (Dansgaard 1964; Mizota and Kusakabe 1994). The metabolic water retains the environmental signal (both soil water and transpiration effects) and is detectably affected by air temperature, which imprints the isotopic signature of the organic matter by means of isotopic exchange on the gem-diole groups of triose-phosphates (Samuel and Silver 1965; Sternberg et al. 1986; Roden and Farquhar 2012). Maximum air temperature (primarily during the day) has the potential to affect both photosynthesis and transpiration, whereas minimum air temperature (primarily at night) may affect transpiration, which promotes translocation in mature rice grains. Therefore, it is expected that the $\delta^{13}C$ and $\delta^{18}O$ of organic matter in rice grains will reflect physiological changes in response to warming impacts. If the $\delta^{13}C$ and $\delta^{18}O$ of rice grains reflect an increase in maximum and/or minimum air temperatures, they may provide direct indicators for changes in rice yield and quality under conditions of climate change.

The aim of the present study was to investigate whether stable carbon and oxygen isotopes of rice organic matter reflect variations in air temperature during grain filling, and are related to the yield and quality of rice. We analyzed the $\delta^{13}C$ and $\delta^{18}O$ of rice grains from the northern latitudes (30.49°–37.14°) of Japan in relation to mean maximum and minimum air temperatures, yield, and quality—as defined by the content of first-grade rice.

Materials and methods

Rice and rice-field water samples

A total of 205 samples of the *japonica* rice cultivar Koshihikari (*Oryza sativa* L.) were obtained from 114 paddies within 21 prefectures in September 2007, and 91 paddies within 13 prefectures in September 2008, on the Japanese islands of Honshu, Shikoku, and Kyusyu (detailed in Fig. 1; Table 1). We selected and polished

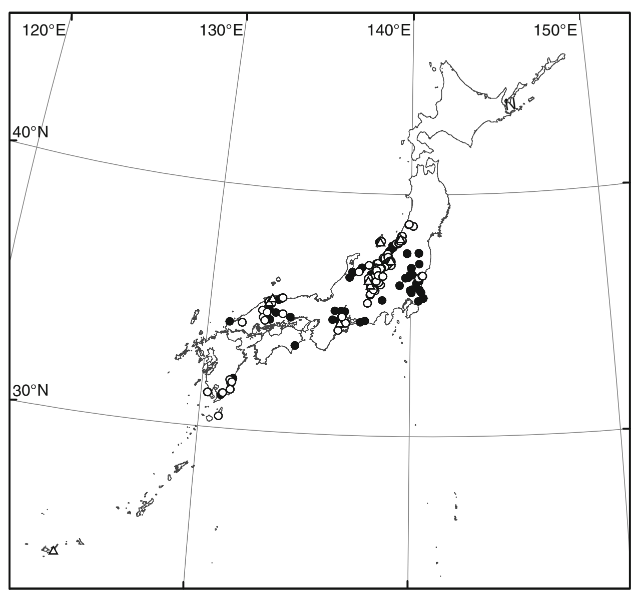

Fig. 1 Sampling locations of Koshihikari rice (*Oryza sativa* L.) and rice-field water in Japan. *Closed* and *open circles* represent locations of rice samples in 2007 and 2008, respectively. *Open triangles* represent locations of rice-field water samples in 2007

Table 1 Yield, proportion of first-grade rice, $\delta^{13}C$, and $\delta^{18}O$ of Koshihikari rice (*Oryza sativa* L.), and sunshine hours, precipitation, and mean maximum and minimum air temperatures during grain filling in 2007 and 2008

Year	Prefecture	Rice yield (t ha^{-1})	Proportion of first-grade rice (%)	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)	Sunshine hours (h)	Precipitation (mm)	Mean maximum air temperature (°C)	Mean minimum air temperature (°C)	n
2007	Fukushima	5.39	94	−27.6 ± 0.3	20.8 ± 0.6	195 ± 26	153 ± 16	30.6 ± 0.1	20.3 ± 0.5	9
	Ibaraki	5.06	91	−27.4 ± 0.4	22.5 ± 0.7	229 ± 14	55 ± 36	32.2 ± 0.5	22.8 ± 0.2	8
	Tochigi	5.31	92	−27.6 ± 0.3	21.4 ± 0.7	164 ± 11	167 ± 32	30.9 ± 1.3	21.0 ± 0.9	13
	Gunma	4.87	65	−28.4 ± 0.0	21.5 ± 0.6	202 ± 0	33 ± 0	34.5 ± 0.0	24.3 ± 0.0	2
	Chiba	5.11	92	−27.4 ± 0.2	21.6 ± 0.4	276 ± 19	10 ± 0	30.6 ± 0.9	24.1 ± 0.3	6
	Niigata	5.31	83	−27.4 ± 0.3	21.8 ± 1.0	208 ± 13	221 ± 58	31.0 ± 0.8	22.3 ± 1.0	22
	Toyama	5.20	90	−27.7 ± 0.4	21.0 ± 0.6	237 ± 13	133 ± 16	32.0 ± 0.9	23.2 ± 0.5	5
	Yamanashi	5.31	86	−28.4	22.0	237	62	34.0	23.6	1
	Nagano	6.27	97	−26.8 ± 0.5	21.1 ± 0.5	222 ± 21	81 ± 27	30.9 ± 1.5	19.4 ± 1.2	11
	Shizuoka	4.97	78	−27.2 ± 0.1	24.0 ± 0.7	252 ± 0	29 ± 0	32.4 ± 0.0	24.9 ± 0.0	2
	Aichi	5.03	46	−27.7	22.5	144	71	33.9	24.6	1
	Mie	4.66	65	−27.5 ± 0.5	22.4 ± 0.5	236 ± 12	73 ± 32	32.4 ± 0.4	24.3 ± 1.0	11
	Shiga	4.91	65	−28.0	21.7	208	72	33.4	22.6	1
	Tottori	4.67	62	−27.2 ± 0.4	22.3 ± 0.8	256 ± 4	307 ± 116	31.9 ± 1.4	23.7 ± 0.8	3
	Shimane	4.78	60	−27.6 ± 0.2	24.1 ± 0.5	204 ± 0	325 ± 0	32.1 ± 0.0	23.0 ± 0.0	3
	Okayama	5.09	60	−28.2	23.8	181	74	32.7	22.3	1
	Hiroshima	5.04	84	−27.4 ± 0.3	22.5 ± 0.2	186 ± 10	142 ± 10	31.1 ± 0.3	21.1 ± 0.3	5
	Yamaguchi	4.86	61	−28.2	21.3	240	295	31.3	24.3	1
	Tokushima	4.54	62	−28.0 ± 0.1	21.7 ± 0.6	233 ± 0	119 ± 0	31.4 ± 0.0	24.2 ± 0.0	3
	Miyazaki	2.05	0	−27.2 ± 0.1	20.3 ± 0.3	190 ± 0	297 ± 0	31.0 ± 0.0	22.8 ± 0.0	3
	Kagoshima	2.98	10	−27.9 ± 0.2	21.2 ± 0.7	148 ± 29	701 ± 223	30.5 ± 0.4	23.5 ± 0.5	3
2008	Yamagata	5.55	96	−27.7 ± 0.3	23.2 ± 1.4	178 ± 5	389 ± 203	28.3 ± 0.5	20.5 ± 0.3	3
	Ibaraki	5.32	89	−27.7 ± 0.4	20.6 ± 0.1	118 ± 0	166 ± 0	29.4 ± 0.0	20.7 ± 0.0	3
	Niigata	5.45	87	−27.4 ± 0.6	21.6 ± 0.9	177 ± 21	216 ± 39	29.2 ± 1.0	21.3 ± 0.9	40
	Toyama	5.48	87	−27.4	23.1	172	262	30.3	22.5	1
	Nagano	6.35	98	−27.3 ± 0.4	21.8 ± 0.9	182 ± 14	164 ± 22	28.8 ± 1.5	19.3 ± 1.2	20
	Mie	5.09	30	−27.5 ± 0.4	21.2 ± 0.7	186 ± 8	199 ± 31	31.5 ± 0.8	23.2 ± 0.4	4
	Shimane	5.10	65	−27.9 ± 0.5	20.5 ± 0.5	145 ± 7	238 ± 12	30.1 ± 0.2	21.3 ± 0.1	4
	Tottori	5.12	65	−27.5	21.4	187	262	29.4	22.9	1
	Okayama	5.40	70	−27.4	20.8	141	76	32.6	22.6	1
	Hiroshima	5.37	78	−27.7 ± 0.3	20.4 ± 0.6	160 ± 6	171 ± 56	34.5 ± 3.2	21.4 ± 0.1	3
	Yamaguchi	4.97	55	−28.1	21.5	145	209	29.4	20.9	1
	Miyazaki	5.05	60	−27.1 ± 0.4	23.9 ± 0.7	268 ± 18	37 ± 17	32.4 ± 0.5	24.3 ± 0.5	5
	Kagoshima	4.67	54	−27.3 ± 0.3	23.5 ± 0.2	257 ± 28	66 ± 18	32.3 ± 1.0	24.6 ± 1.1	5

Data are presented as mean ± SD. Rice yield and proportion of first-grade rice are mean data only on the agricultural statistics rice crops for which heading and flowering occurred between July and August. Each polished rice grain sample (50 g) was freeze-dried and ground into a powder for stable isotope analysis. In May 2007, water samples were filtered by a membrane filter (nominal pore size: 0.45 μm, ADVANTEC, Tokyo, Japan) and collected in 100 ml polyethylene bottles from the irrigation ditch of one rice field in each of the following locations (within the region 24.35–38.17°N): Sado (Niigata), Uonuma (Niigata), Murakami (Niigata), Matsusaka (Mie), Matsukawa (Nagano), Matsumoto (Nagano), Matsue (Shimane), Unnan (Shimane), and Ishigaki (Okinawa) in order to consider geographical changes in isotopic composition of source water (Fig. 1). The rice crops were irrigated and fertilized according to conventional management practices for rice farming in Japan. Intermittent irrigation was applied from approximately 1 month after transplanting to maintain wet soil conditions. Irrigation was stopped from approximately 1 month after ear emergence until harvest, because precipitation water provided sufficient water levels for crop production (e.g., Chapagain and Yamaji 2010).

Meteorological and rice statistical data collection

Meteorological data (mean maximum and minimum air temperatures, precipitation, and sunshine hours during the grain-filling period) were obtained from local meteorological stations within a 15-km radius of each sampling point (Japan Meteorological Agency 2012). Where possible, these data were collected for the grain-filling period of Koshihikari rice, determined according to statistical reports (Ministry of Agriculture, Forestry and Fisheries 2012). Rice yield and grade data for 2007 and 2008 were collected from statistical reports (Ministry of Agriculture, Forestry and Fisheries 2012). We used the characteristics of first-grade rice (< 15 % transparency, roundness, and cracking) as indicators of quality.

Stable isotope analysis

The $\delta^{13}C$ of rice grain ($\delta^{13}C_{rice}$) was determined using an elemental analyzer/isotope ratio mass spectrometer (EA/IRMS, Finnigan Delta V Advantage, interfaced with FlashEA 1112 HT; Thermo Fisher Scientific, Bremen, Germany) (Suzuki et al. 2008). Subsamples of rice (1.0 mg) were placed into tin capsules (5 × 9 mm) and loaded into the auto-sampler of the EA/IRMS. The obtained CO_2 was separated on a PorapakQS column (length, 3 m) at 40 °C. The δ notation defined in Eq. (1) was used to describe the isotopic composition.

$$\delta(‰) = (R_{sample}/R_{standard} - 1) \times 1000 \qquad (1)$$

where R_{sample} is the isotope ratio of the sample, and $R_{standard}$ is the isotope ratio of the international standard, Vienna Pee Dee Belemnite. Each sample was determined in duplicate, and analytical errors in $\delta^{13}C$ had a standard deviation (SD) < 0.1 ‰.

The $\delta^{18}O$ of rice grain ($\delta^{18}O_{rice}$) and rice-field water ($\delta^{18}O_{water}$) were determined by EA/IRMS in the pyrolysis mode. Subsamples of rice and rice-field water (0.5 mg and 1.0 μL, respectively) were placed into silver capsules (5 × 3 mm). Care was taken to avoid atmospheric contamination of the samples. All rice samples were dried overnight by lyophilization before isotope measurement. Pyrolysis was performed in a ceramic tube of glassy carbon at 1,450 °C under a continuous flow of He at 100 ml min^{-1}. The gas obtained was separated from residual gases by molecular sieving through a 5-Å column (length, 3 m) at 50 °C (Werner et al. 1996; Böhlke et al. 2003; Leuenberger and Filot 2007). $\delta^{18}O$ was calculated according to Eq. (1) and expressed relative to that of Vienna Standard Mean Ocean Water (V-SMOW). The measured $\delta^{18}O$ values were obtained using known isotope benzoic acid standards (+ 71.4, + 23.2 ‰) purchased from Indiana University (Indianapolis, IN). The accuracy of the obtained $\delta^{18}O$ values was checked independently against V-SMOW and Vienna Standard Light Antarctic Precipitation standards distributed by the International Atomic Energy Agency. Four working standards—dibenzo-24-crown-8 (−15.7 ‰), dibenzo-18-crown-6 (+ 1.7 ‰), β-D-galactose pentaacetate (+ 12.7 ‰), and D-(+)-sucrose octaacetate (+ 26.8 ‰)—were determined every six samples to confirm the reproducibility of the measurements (Nakashita et al. 2008). The analytical error of the isotope measurements had a SD < 0.3 ‰, with a minimum sample amount of 10 μmol oxygen.

Statistical analysis

Correlations between rice ($\delta^{13}C$, $\delta^{18}O$, yield, and quality) and meteorological data (mean maximum and minimum air temperatures, precipitation, and sunshine hours during the grain-filling period) were tested independently using Pearson's product-moment coefficient with the average values for each prefecture. We excluded from our statistical analyses the rice yield and quality of 2007 data from Kagoshima and Miyazaki prefectures, because of typhoon damage. Correlations between the $\delta^{18}O_{water}$ and the $\delta^{18}O_{rice}$, and also between the mean minimum air temperature during grain filling and the $\delta^{18}O_{rice}$ and the $\delta^{18}O_{water}$ with global mean ^{18}O enrichment of carbohydrate relative to the source water (+ 27 ‰, Sternberg 1989), were tested using Pearson's product-moment coefficient. In this study, the ^{18}O enrichment of rice grain to source water was defined as $\delta^{18}O_{rice}$–$\delta^{18}O_{water}$ (e.g. Matsuo et al. 2013). We compared the theoretical values ($\delta^{18}O_{water}$ + global mean ^{18}O enrichment) with measured $\delta^{18}O_{rice}$, because the difference between the theoretical and measured ^{18}O enrichments may reveal physiological changes such as rice translocation and evaporative demands. For all tests, an α value of 0.05 was used to indicate statistical significance. All analyses were conducted using R ver. 2.15.0 (R Development Core Team 2012).

Results

Rice yields ranged from 2.05 to 6.27 t ha^{-1} in 2007, and from 4.67 to 6.35 t ha^{-1} in 2008 (Table 1). The proportion of first-grade rice in the rice yield (rice quality) ranged from 0 to 97 % in 2007, and from 30 % to 98 % in 2008. There was a positive correlation between yield and quality (Table 2). Sunshine hours during grain filling ranged from 144 to 276 h in 2007, and from 118 to 268 h in 2008. Sunshine hours were not correlated with yield or quality of rice (Table 2). Precipitation during grain filling ranged from 10 to 701 mm in 2007, and from 37 to 389 mm in 2008. Precipitation was also not correlated with yield or quality of rice (Table 2).

Rice $\delta^{13}C$ ranged from −28.4 to −26.8 ‰ in 2007, and from −28.1 to −27.1 ‰ in 2008 (Table 1). Rice $\delta^{13}C$ was not correlated with yield, quality, sunshine hours, or precipitation (Table 2). $\delta^{18}O_{rice}$ ranged from 20.8 to 24.1 ‰ in 2007, and from 20.4 to 23.9 ‰ in 2008 (Table 1). $\delta^{18}O_{rice}$ was not correlated with sunshine hours, yield, quality or precipitation (Table 2). The $\delta^{18}O_{rice}$ was not correlated with the $\delta^{13}C$ (Table 2).

The mean maximum air temperatures ranged from 30.6 to 34.5 °C in 2007, and from 28.3 to 34.5 °C in 2008 (Table 1). The maximum air temperature was negatively correlated with rice yield (Fig. 2a), quality (Fig. 2b), but not with $\delta^{13}C$ (Fig. 2c), or $\delta^{18}O_{rice}$ (Fig. 2d).

The mean minimum air temperatures ranged from 19.4 to 24.9 °C in 2007, and from 19.3 to 24.6 °C in 2008 (Table 1). The minimum air temperature was negatively correlated with rice yield (Fig. 2e) and quality (Fig. 2f), positively correlated with $\delta^{18}O_{rice}$ (Fig. 2h), but not correlated with rice $\delta^{13}C$ (Fig. 2g).

There was a positive correlation between the $\delta^{18}O_{water}$ and $\delta^{18}O_{rice}$ (Fig. 3). The ^{18}O enrichment of rice relative to rice-field water decreased significantly with an increase in the mean minimum air temperature during grain filling (Fig. 4).

Discussion

In the present study, the $\delta^{18}O_{rice}$ was correlated positively with the $\delta^{18}O_{water}$ (Fig. 3), confirming that the oxygen atoms in the organic compounds of rice grain were derived mainly from the region-specific ambient water. The $\delta^{18}O$ of precipitation and groundwater (i.e., source water for rice plants) varied in accordance with climatic and geographical changes relating to amount and thermal effects (Dansgaard 1964; Rozanski et al. 1992), and becomes enhanced at lower latitudes and altitudes in Japan (e.g., Mizota and Kusakabe 1994). Therefore, similar to variations in the $\delta^{18}O$ of organic compounds (e.g., cellulose, sucrose, etc.) in trees to

Table 2 Results of Pearson's product–moment coefficient with the average values for each prefecture between rice grain (yield, quality, $\delta^{13}C$, and $\delta^{18}O$) and meteorological data (sunshine hours, precipitation, and maximum and minimum air temperatures)

	Yield	Quality	$\delta^{13}C$ of rice grain	$\delta^{18}O$ of rice grain	Sunshine hours	Precipitation	Maximum air temperature	Minimum air temperature
Yield (n = 32)	–	< 0.001	0.055	0.121	0.072	0.699	0.021	< 0.001
Quality (n = 32)	0.642*	–	0.168	0.335	0.996	0.999	0.037	< 0.001
$\delta^{13}C$ of rice grain (n = 34)	0.343	0.250	–	0.436	0.668	0.668	0.138	0.364
$\delta^{18}O$ of rice grain (n = 34)	−0.280	−0.176	0.138	–	0.349	0.349	0.373	0.042
Sunshine hours (n = 34)	−0.322	0.001	0.229	0.401	–	0.030	0.107	0.001
Precipitation (n = 34)	0.071	0	−0.076	−0.166	−0.371*	–	0.005	0.365
Maximum air temperature (n = 34)	−0.406*	−0.371*	−0.260	0.158	0.281	−0.469*	–	< 0.001
Minimum air temperature (n = 34)	−0.752*	−0.556*	−0.161	0.350*	0.533*	−0.161	0.565*	–

Lower left and upper right sides of the matrix show correlation coefficients and probabilities, respectively. The rice yield and quality of Kagoshima and Miyazaki prefectures in 2007 were excluded because of typhoon damage

* $P < 0.05$

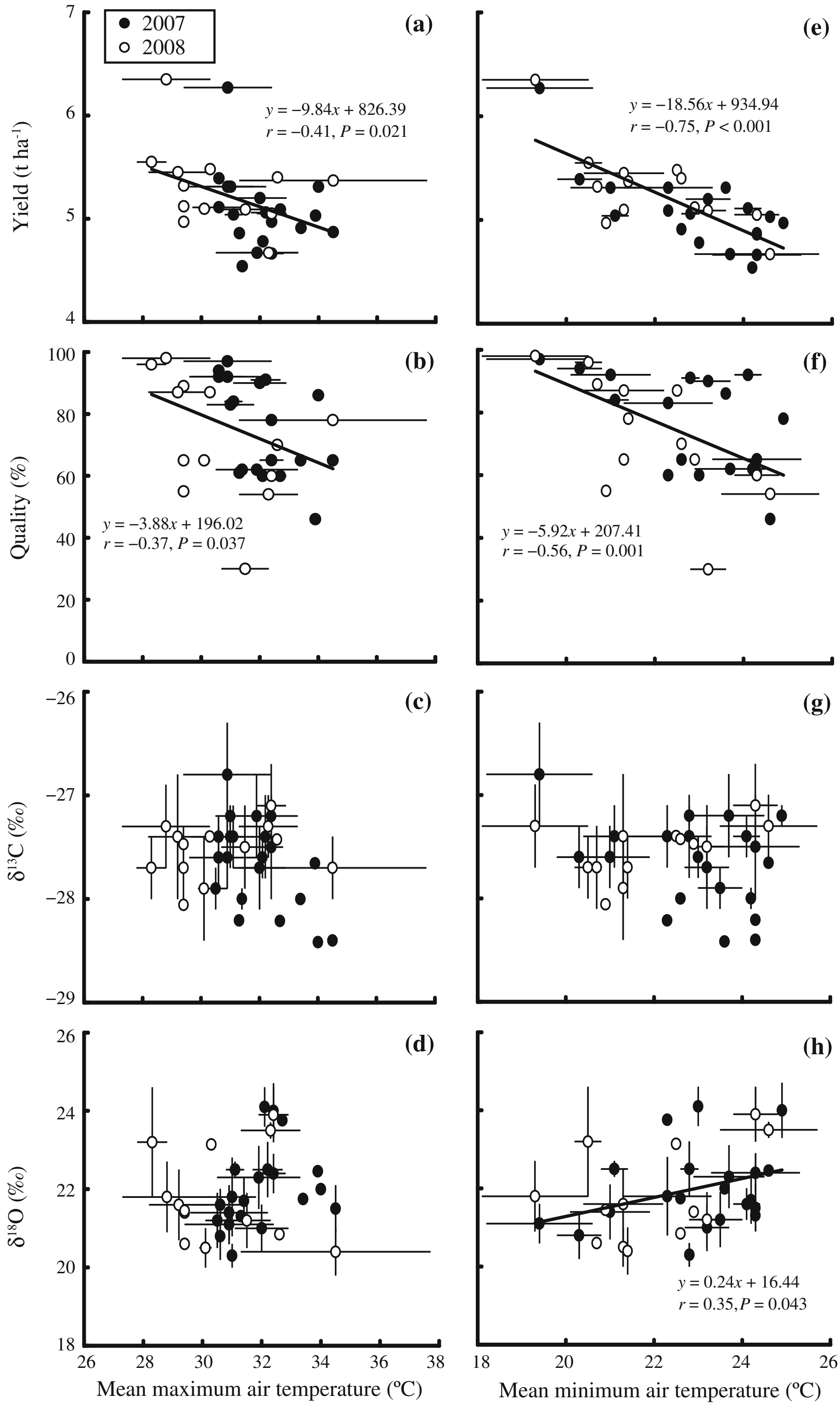

Fig. 2 Relationships among mean maximum and minimum air temperatures during grain filling, and yield, quality, δ^{13}C, and δ^{18}O of Koshihikari rice (*Oryza sativa* L.). **a** Yield versus mean maximum air temperature, **b** quality versus mean maximum air temperature, **c** δ^{13}C versus mean maximum air temperature, **d** δ^{18}O versus mean maximum air temperature, **e** yield versus mean minimum air temperature, **f** quality versus mean minimum air temperature, **g** δ^{13}C versus mean minimum air temperature, **h** δ^{18}O versus mean minimum air temperature. Data are represented as mean ± SD

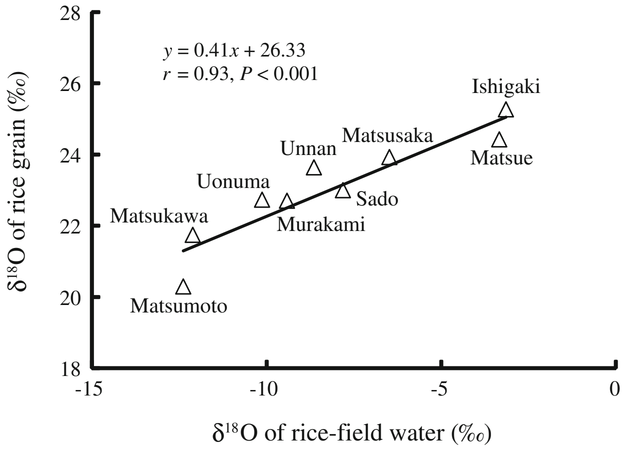

Fig. 3 Relationship between the δ^{18}O of rice-field water and Koshihikari rice (*Oryza sativa* L.) in different regions of Japan

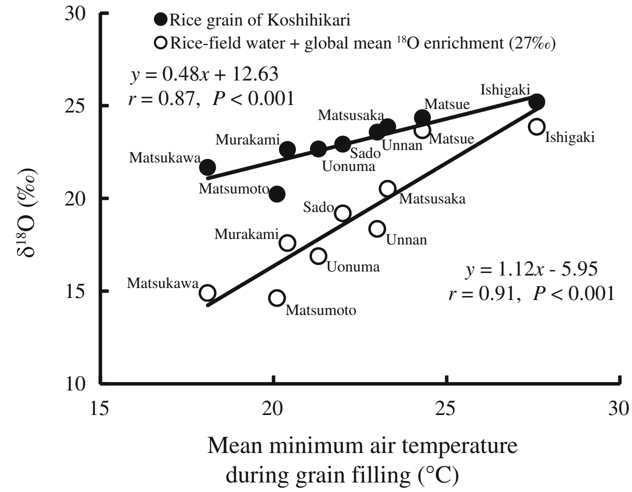

Fig. 4 Relationship between mean minimum air temperature during grain filling and the δ^{18}O of Koshihikari rice (*Oryza sativa* L.) and rice-field water with global mean ^{18}O enrichment of carbohydrate relative to the source water (+27 ‰)

climate gradients in air temperature, rainfall, sunshine hours, and water availability during the growing season (Libby and Pandolfi 1974; Cernusak et al. 2003; Li et al. 2011), the $\delta^{18}O_{rice}$ may reflect temperature-induced changes in the $\delta^{18}O_{water}$. However, the δ^{18}O of irrigation water may not fully reflect precipitation δ^{18}O in some locations, because irrigation water is obtained from various sources—not only rivers, but also ground water and springs far away from the paddy fields. For instance, water used in paddy fields may be derived from precipitation in the mountains either directly or via ground water or springs. As a result, sources of water used in different paddy field regions need to be examined in order to reflect temperature-induced changes.

We found negative effects of mean minimum air temperature during grain filling on the $\delta^{18}O_{rice}$ (Fig. 2h), but no significant effects of mean maximum air temperature (Fig. 2d). Throughout many parts of Japan, global warming is predicted to lead to a greater increase in minimum air temperatures than in maximum air temperatures (Japan Meteorological Agency 2005). Therefore, the $\delta^{18}O_{rice}$ is potentially a good indicator for climate-change-induced variations in minimum air temperature. The mean enrichment of $\delta^{18}O_{rice}$ relative to source water was +31 ± 2 ‰, it is 4 ‰ higher than the global mean ^{18}O enrichment of carbohydrate relative to source water of +27 ± 4 ‰ (Sternberg 1989). The oxygen isotope enrichment of rice grain decreased as the minimum air temperature increased and the difference between measured and global mean ^{18}O enrichments decreased with increase in the minimum temperature (Fig. 4). A rise in minimum air temperature forecast to cause increase in respiration and translocation (Morita 2008), having the potential for increase in δ^{18}O of the rice grain with degradation of starch. Additionally, the increase of minimum air temperature may induce an increase in soil and metabolic water temperatures, and may act to promote of smaller isotope enrichment during transpiration and photosynthesis compared to low water temperatures, because high water temperature increases stomatal conductance and leaf photosynthesis (Shimono et al. 2004). In this study, although we did not accumulate data for other environmental factors such as humidity, altitudes, and nutrients, they may help to demonstrate the effects of minimum temperature on $\delta^{18}O_{rice}$. The δ^{18}O of rice carbohydrate is also dependent on the actual metabolic status of plants as reported by Saurer et al. (1997).

Many physiological studies have indicated that decreases in grain size and quality, such as transparency, roundness, and cracking, are caused by abnormal growth of the endosperm under high temperatures (e.g., Morita 2008; Hasegawa et al. 2011). For example, rice grain size was shown to decrease when night air temperatures exceeded 21 °C during grain filling (Matsushima and Tsunoda 1957; Aimi et al. 1959). In the present study, we detected stronger negative effects from mean minimum air temperature during grain filling on rice yield and quality than from mean maximum air temperature (Fig. 2). Yamakawa et al. (2007) reported that several starch synthesis–related genes, such as granule-bound starch synthase *I* (*GBSSI*), branching enzymes (especially *BEIIb*), and a cytosolic pyruvate orthophosphate dikinase, are downregulated by high temperatures, whereas genes for starch-consuming α-amylases and heat shock proteins are upregulated. Therefore, an increase in minimum air temperature during grain filling can cause changes in the processes involved in amylose composition and translocation, and might lead to changes in the $\delta^{18}O_{rice}$. Minimum air temperature could therefore be useful for predicting the yield and quality of rice, with changes in the $\delta^{18}O_{rice}$ reflecting the physiological conditions during grain filling.

In contrast to the $\delta^{18}O_{rice}$, the $\delta^{13}C_{rice}$ was not correlated with minimum or maximum air temperatures, suggesting that the $\delta^{13}C_{rice}$ might not be a direct indicator of changes in air temperature. The $\delta^{13}C$ of rice is known to be a good indicator of WUE (Kondo et al. 2004; Xu et al. 2009). In the present study, we observed a relatively small difference in rice $\delta^{13}C$ among the prefectures surveyed (1.6 ‰), suggesting nearly constant water-use efficiency. The $\delta^{13}C_{rice}$ might reflect the nearly constant wet soil conditions maintained under the unified irrigation and fertilization practices of conventional rice farming in Japan, irrespective of the differences in plant-experienced atmospheric vapor demand, namely the leaf-to-air vapor pressure difference caused by different atmospheric temperatures, hours of sunshine, and precipitation. We determined no effects of precipitation on the yield and quality of rice; this finding could also be derived from cultivation under constantly wet soil conditions.

Acknowledgments We thank Dr. T. Yanagihara, Dr. S. Yanagihara, Dr. Y. Iizumi, Mr. D. Tabei, Mr. I. Yamagata, Mr. H. Yamaguchi, and members of Syokukyo CORP, Yaeyama Branch of the Okinawa Prefectural Agricultural Experiment Station, Japan Rice Millers Association, ZEN-NOH Pearl Rice East Japan Corp., and the Agricultural Cooperative Association for supporting the collection of certified rice and water samples. This work was supported by the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN).

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