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Rice varietal differences in responses of stomatal gas exchange to supplemental nitrogen application

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Abstract

In the field, supplemental application of N fertilizer to rice (*Oryza sativa*) shortly before the beginning of heading stage increases leaf N content and enhances photosynthesis during the grain-filling period. In search of varietal differences in leaf gas exchange in response to supplemental N application, we examined 13 rice varieties grown in the field during two successive years. The varieties included *japonica* and *indica* varieties, both of which are widely grown in Japan. The response to supplemental N application could not be separated clearly between variety groups; some of the *japonica* varieties, but none of the *indica* varieties, exhibited significant increase in stomatal conductance (g_s) after supplemental N application. Supplemental N was more effective to increase stomatal aperture in the varieties with inherently lower g_s. Varieties that showed greater response of g_s to supplemental N application might be able to adjust their stomatal aperture with appropriate N control. Although the internal-to-ambient CO_2 mole fraction ratio and the leaf carbon isotopic composition ($\delta^{13}C$) differed among varieties as a result of variations in stomatal aperture and the CO_2 requirement of mesophyll, supplemental N application barely influenced these parameters, because it only moderately affected stomatal aperture. Since $\delta^{13}C$ tended to increase with increasing number of days from transplantation to heading stage in *japonica* varieties, $\delta^{13}C$ values were more sensitive to differences in growth rate between years than to N application.

Additional key words: carbon discrimination; genotype; leaf nitrogen; nitrogen fertilization; stomatal conductance; yield.

Introduction

Supplemental N fertilizer applied shortly before the beginning of the heading stage (emergence of the panicle tip from the flag leaf sheath) increases leaf N content without increasing leaf area, resulting in enhanced net photosynthetic rate (P_N) during the grain-filling period (Yoshida 1981). In recently improved varieties, supplemental N fertilizer applied earlier, at the panicle formation stage, also increases leaf N content without increasing leaf area, and suppresses the reduction in leaf area after heading (Taylaran *et al*. 2011). N status is an important constraint that influences all physiological processes involved in rice growth and development. Leaf g_s is enhanced with increasing N availability. For example, there is a strong influence of basal N application during early growth stages on g_s (Sasaki and Ishii 1992, Kondo *et al*. 2004), and g_s differs in plants grown continuously under N-sufficient and N-deficient conditions (Sarkar *et al*. 2002, Kumagai *et al*. 2009). But at later growth stages, the effect of supplemental N on photosynthetic parameters has received little attention, even though photosynthesis depends on leaf N. Leaf g_s in rice generally decreases from the time of basal fertilization to the time of additional fertilization (Maruyama and Kuwagata 2008, Ono *et al*. 2013), and recovers after supplemental N application (Shimoda 2012). P_N in rice is closely associated with the content of Rubisco during ripening (Makino *et al*. 1985). As the relationship between the N and Rubisco contents in leaves is very similar in several *japonica* and *indica* types (Hirasawa *et al*. 2010, Taylaran *et al*. 2011), it is possible

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Abbreviations: C_i/C_a – ratio of intercellular to ambient CO_2 mole fraction; g_s – stomatal conductance; P_N – net photosynthetic rate; $\delta^{13}C$ – carbon isotope composition.

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to determine how photosynthetic traits vary among varieties by comparing their responses to changes in leaf N. Although differences in the relationship of P_N to leaf N between *japonica* and *indica* types have been identified (Weng and Chen 1987), the effects of additional N on other photosynthetic traits during the growth period have not been examined.

The contribution of stomatal regulation to P_N can depend on the variety, even at identical concentrations of leaf N (Hirasawa *et al.* 2010). In principle, an increase in g_s, which regulates the exchange of both CO_2 and water, can allow plants to increase their CO_2 assimilation. Ishihara *et al.* (1978) reported that high leaf N content enlarged stomatal aperture in one *japonica* variety. To evaluate varietal differences of the effects of supplemental N on stomatal aperture and gas exchange traits, it is necessary to determine differences during the grain-filling period and to clarify the relationship of g_s to leaf N content.

To determine such varietal differences, we carried out field experiments with 13 rice varieties during two years. The values of g_s and of the ratio of intercellular to ambient CO_2 mole fraction (C_i/C_a) indicate a physiological condition of the plant at the time of measurement. The stable carbon isotope composition ($\delta^{13}C$) of leaf dry matter, which indicates the discrimination against $^{13}CO_2$ in the surrounding air, provides a time-integrated record of the C_i/C_a of the plant carbon fixation over the growth period (Farquhar *et al.* 1982). $\delta^{13}C$ thus provides an index of mesophyll conductance (Hanba *et al.* 1999). During photosynthesis, CO_2 moves from the ambient air through the stomata to the intercellular spaces, and from there through the leaf mesophyll to the site of carboxylation inside the chloroplast stroma (Flexas *et al.* 2008). The change in the chloroplast CO_2 (C_c) to ambient CO_2 mole fraction is small in relation to C_i/C_a, although C_c is not constant during leaf development and senescence (Miyazawa and Terashima 2001). Thus, values of $\delta^{13}C$ are influenced much more by environmental and physiological conditions than by C_i/C_a. Previous studies suggest that a positive correlation between N application and $\delta^{13}C$ indicates stomata closure in response to added N due to increased demand for water (Kondo *et al.* 2004, Cabrera-Bosquet *et al.* 2007). We determined whether or not supplemental N application was effective to improve stomatal gas exchange during the grain-filling period in 13 *japonica* and *indica* genotypes.

Materials and methods

The experimental site was an irrigated, paddy field with gray lowland soil in Fukuyama, Hiroshima, Japan (34°30′N, 133°23′E; 2 m a.s.l.). The mean annual temperature at this site was 16.1°C in 2010 and 15.6°C in 2011. The summer of 2010 was exceptionally warm in Japan (Kobayashi *et al.* 2011); the high temperatures caused faster growth in 2010 than that in 2011. Thirteen rice varieties were tested (Table 1). The mean time between transplantation and the heading stage was 65.3 d in 2010 and 70.1 d in 2011 (Table 1). Water-soaked seeds of each variety were sown in the middle of May. In early June, three weeks after sowing, the young plants were transplanted, three to a hill. Hills were spaced 0.15 or 0.20 m apart in rows 0.3 m apart, in plots consisting of four rows of nine hills each. Basal fertilizer was applied to the field at the rate of 4 g(N) m^{-2}, 2.6 g(P) m^{-2}, and 3.3 g(K) m^{-2} before the plants were transplanted. Rice plants (36 hills of each variety) were grown either without (treatment UN) or with supplemental N fertilization [4 g(N) m^{-2}; treatment SN] in each of concrete block frames (1.8 × 22.4 m; six or seven varieties by two supplemental N treatments per frame) maintained under submerged water management. Three replicates of each experiment were performed. In western Japan, the usual practice is to apply supplemental N fertilization twice, at about 25 and 10 d before heading. The earlier supplemental N fertilization affects primarily the number of panicles and canopy structure. In this study, supplemental N fertilization was applied only once, at about 10 d before heading, thus the morphological traits were not changed by the earlier N application.

Table 1. The number of days between transplantation and heading in the 13 rice cultivars tested. Twelve cultivars were grown in two successive years, and Takanari and Mizuhochikara were each grown only in one of the years.

Group	Cultivar	2010	2011
japonica	Koshijiwase	57	62
japonica	Akitakomachi	57	64
japonica	Sasanishiki	58	64
japonica	Hatsuboshi	61	66
japonica	Koshihikari	61	66
japonica	Hitomebore	61	66
japonica	Fukumirai	62	67
japonica	Tennotsubu	63	67
japonica	Hinohikari	76	81
japonica	Nikomaru	77	81
indica	IR36	76	78
indica	Hokuriku 193	74	79
indica	Takanari	72	
japonica-indica	Mizuhochikara		83

Measurements of g_s were performed on fully expanded topmost and second leaves at the heading period for each variety using a portable photosynthesis system (*LI-6400*, *Li-COR*, *Inc.*, Lincoln, NE, USA). Gathered data included g_s and C_i/C_a. Two leaves from each of four different plants of each variety were measured between 09:00 and 13:00 h of the local time on clear days under a constant saturated light of 1,000 μmol(photon) m^{-2} s^{-1} provided by red/blue light-emitting diodes. The leaf chamber temperature was 35.7 ± 0.3°C in 2010 and 35.1 ± 0.1°C in 2011, the reference CO_2 concentration was maintained at 380 μmol mol^{-1}, and the relative humidity was 61.2 ± 0.5% in 2010 and 64.0 ± 0.2% in 2011. Gas-exchange parameters were recorded as soon as the topmost expanded leaf was enclosed in the chamber and the system software indicated that CO_2, H_2O, and air flow in the chamber was stabilized. After the above measurements were completed, the leaves were harvested and their $\delta^{13}C$ and N content were determined as described below.

The harvested leaves were oven-dried at 80°C for at least 48 h and then ground into fine powder in a vibration mill (*Heiko Co.*, *Ltd.*, Tokyo, Japan) for the analysis of leaf N and $\delta^{13}C$. Approximately 2 mg of leaf sample was weighed into a tin cup and combusted in an elemental analyzer (*Flash EA1112*, *Thermo Finnigan*, Bremen, Germany) coupled to an isotope ratio mass spectrometer (*Delta Plus Advantage*, *Thermo Finnigan*, Bremen, Germany) by a *Conflo III* interface.

One-way analysis of variance was used to evaluate the effects of both N application and year on gas exchange and $\delta^{13}C$ of each variety. *Tukey*'s Honestly Significant Difference test was used to test differences in photosynthetic traits between UN and SN plants of varieties Takanari in 2010 and Mizuhochikara in 2011. *Pearson*'s correlation test was used to assess the significance of correlation coefficients. Statistical analyses were conducted with *R* statistical software (*R v. 2.14.2*, *R Foundation for Statistical Computing*, Vienna, Austria).

Results

In UN plants, the leaf N ranged from 1.0 to 1.5 g m^{-2} in 2010 and 2011, respectively. In SN plants, the values ranged from 1.4 to 2.0 in 2010 and 1.3 to 2.1 in 2011 (Table 2). All varieties exhibited higher leaf N in SN plants than in UN plants. The g_s values were lower in *japonica* types than in *indica* types. In 2011, *japonica* varieties, such as Koshijiwase, Akitakomachi, and Sasanishiki, showed g_s ≥0.44 mmol(H_2O) m^{-2} s^{-1} in UN plants (Table 2). Four *japonica* varieties and all three *indica* varieties showed no significant difference in g_s between SN and UN plants. The leaf C_i/C_a values of the *japonica* plants ranged from 0.71 to 0.80, while those of *indica* and *indica-japonica* varieties ranged from 0.76 to 0.84 (Table 3). Within a variety and N treatment, differences in $\delta^{13}C$ between years were ≤0.6‰ (Table 3). Koshiji-wase, Akitakomachi, and Sasanishiki showed higher g_s in 2011 (Table 2) and lower $\delta^{13}C$ (Table 3) in both years compared with other *japonica* varieties except for Hitomebore in SN. Supplemental N application did not affect either C_i/C_a or $\delta^{13}C$ except in one variety. Although there was little variation in the leaf N between years (Table 2), nine varieties showed significant variations in $\delta^{13}C$ between years. Six varieties showed the significant decrease of $\delta^{13}C$, while Koshihikari, Hinohikari, and Nikomaru showed significant increases (Table 3). $\delta^{13}C$ tended to be higher in *japonica* types than in *indica* types. Values of g_s, C_i/C_a, and $\delta^{13}C$ in the *indica-japonica* variety, Mizuhochikara, were intermediate between those of *japonica* and *indica* varieties. Supplemental N application affected total biomass and the grain yield in all *indica* types (Table 4). Although there was little variation in total biomass between years, ten varieties showed significant variations in the grain yield between years (Table 4).

Table 2. Leaf N and g_s of flag leaves of 13 rice cultivars grown in 2010 and 2011 without (UN) or with supplemental N fertilization (SN). Significance levels were determined for differences between supplemental N fertilization (N), years (Y), and the product of the two (Y × N). g_s – stomatal conductance; ns – not significant; *,**, and *** – significant at 5, 1, and 0.1%, respectively. *n* = 3.

Group	Cultivar	Leaf N [g m^{-2}]							g_s [mol(H_2O) m^{-2}s^{-1}]						
		2010		2011		Significance			2010		2011		Significance		
		UN	SN	UN	SN	Year	N	Y × N	UN	SN	UN	SN	Year	N	Y × N
japonica	Koshijiwase	1.2	1.7	1.5	1.8	ns	***	ns	0.41	0.48	0.54	0.54	**	ns	ns
japonica	Akitakomachi	1.5	2.0	1.5	2.1	ns	***	ns	0.38	0.45	0.47	0.54	ns	ns	ns
japonica	Sasanishiki	1.2	1.4	1.2	1.8	**	***	*	0.36	0.40	0.44	0.48	*	ns	ns
japonica	Hatsuboshi	1.1	1.4	1.4	1.6	*	***	ns	0.36	0.45	0.42	0.47	ns	*	ns
japonica	Koshihikari	1.3	1.7	1.5	1.6	ns	***	ns	0.30	0.43	0.39	0.47	ns	*	ns
japonica	Hitomebore	1.2	1.6	1.4	1.7	ns	***	ns	0.36	0.41	0.42	0.49	ns	ns	ns
japonica	Fukumirai	1.3	1.6	1.4	1.6	ns	***	ns	0.34	0.46	0.40	0.46	ns	**	ns
japonica	Tennotsubu	1.2	1.7	1.5	1.7	ns	***	*	0.35	0.43	0.38	0.46	ns	**	ns
japonica	Hinohikari	1.3	1.7	1.4	1.8	ns	***	ns	0.45	0.52	0.39	0.44	*	*	ns
japonica	Nikomaru	1.1	1.6	1.0	1.3	ns	**	ns	0.40	0.57	0.31	0.37	***	**	ns
indica	IR36	1.1	1.5	1.3	1.4	ns	**	ns	0.57	0.62	0.60	0.62	ns	ns	ns
indica	Hokuriku 193	1.1	1.7	1.1	1.6	ns	**	ns	0.69	0.73	0.65	0.67	ns	ns	ns
indica	Takanari	1.1	1.5				*		0.57	0.62				ns	
jap.-ind.	Mizuhochikara			1.3	1.7		**				0.51	0.55		ns	

For all 13 varieties combined, the linear regression of g_s of UN plants *vs*. g_s of SN plants was less steep than the 1:1 line (Fig. 1), indicating that N supplementation had greater effects on g_s in varieties with inherently lower values for that trait. The linear regression equation was g_s (in SN) = 0.81 × g_s (in UN) + 0.15. The values of g_s in *indica* varieties were higher than those in *japonica* varieties at all concentrations of leaf N (Fig. 2). The value of g_s was significantly correlated with leaf N in *japonica* types (Fig. 2; $r^2 = 0.52$, $P<0.05$), but not in *indica* types ($r^2 = 0.24$, not significant). The g_s intercept of the g_s-N relationship was higher in the *indica* types ($g_s = 0.10 \times$ leaf N + 0.50) than in the *japonica* types ($g_s = 0.19 \times$ leaf N + 0.15). Although $\delta^{13}C$ did not change after supplemental N, it increased with increasing number of days from transplantation to heading in *japonica* varieties (Fig. 3; $r^2 = 0.46$, $P<0.05$), but not in *indica* varieties ($r^2 = 0.00$, not significant).

Table 3. C_i/C_a and $\delta^{13}C$ of flag leaves of 13 rice cultivars grown in 2010 and 2011 without (UN) or with supplemental N fertilization (SN). Significance levels were determined for differences between supplemental N fertilization (N), years (Y), and the product of the two (Y × N). C_i/C_a – ratio of intercellular to ambient CO_2 mole fraction; $\delta^{13}C$ – carbon isotope composition. ns – not significant; *,**, and *** – significant at 5, 1, and 0.1%, respectively. *n* = 3.

Group	Cultivar	C_i/C_a							$\delta^{13}C$ [‰]						
		2010		2011		Significance			2010		2011		Significance		
		UN	SN	UN	SN	Year	N	Y × N	UN	SN	UN	SN	Year	N	Y × N
japonica	Koshijiwase	0.76	0.75	0.76	0.74	ns	ns	ns	−29.1	−29.6	−29.2	−29.5	*	ns	ns
japonica	Akitakomachi	0.74	0.75	0.75	0.74	ns	ns	ns	−29.2	−29.1	−29.0	−29.3	ns	ns	ns
japonica	Sasanishiki	0.75	0.73	0.73	0.73	ns	ns	ns	−29.1	−29.3	−29.0	−29.4	***	ns	ns
japonica	Hatsuboshi	0.75	0.74	0.74	0.73	ns	ns	ns	−28.6	−28.9	−28.9	−28.9	***	ns	ns
japonica	Koshihikari	0.71	0.73	0.73	0.74	ns	ns	ns	−28.7	−28.6	−28.5	−28.4	**	ns	ns
japonica	Hitomebore	0.73	0.73	0.73	0.74	ns	ns	ns	−28.2	−28.4	−28.6	−29.0	***	**	ns
japonica	Fukumirai	0.75	0.75	0.73	0.74	ns	ns	ns	−28.7	−28.5	−28.3	−29.0	*	ns	ns
japonica	Tennotsubu	0.74	0.74	0.73	0.74	ns	ns	ns	−28.4	−28.4	−28.8	−28.8	ns	ns	ns
japonica	Hinohikari	0.80	0.79	0.73	0.74	***	ns	*	−28.4	−28.3	−27.8	−28.1	***	ns	ns
japonica	Nikomaru	0.78	0.80	0.73	0.72	***	ns	ns	−28.5	−28.6	−28.3	−28.7	***	ns	ns
indica	IR36	0.83	0.81	0.80	0.77	**	ns	ns	−29.7	−29.8	−29.6	−29.6	ns	ns	ns
indica	Hokuriku 193	0.84	0.84	0.78	0.78	***	ns	ns	−29.5	−29.6	−29.7	−29.7	**	ns	ns
indica	Takanari	0.83	0.81				ns		−29.7	−29.8				ns	
jap.-ind.	Mizuhochikara			0.76	0.76		ns				−29.3	−29.4		ns	

Table 4. Total biomass and the grain yield of 13 rice cultivars grown in 2010 and 2011 without (UN) or with supplemental N fertilization (SN). Significance levels were determined for differences between supplemental N fertilization (N), years (Y), and the product of the two (Y × N). ns – not significant; *,**, and *** – significant at 5, 1, and 0.1%, respectively. *n* = 3

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Group	Cultivar	Total biomass [kg m^{-2}]							Grain yield [kg m^{-2}]						
		2010		2011		Significance			2010		2011		Significance		
		UN	SN	UN	SN	Year	N	Y × N	UN	SN	UN	SN	Year	N	Y × N
japonica	Koshijiwase	1.2	1.1	1.2	1.3	ns	ns	ns	0.43	0.44	0.51	0.57	**	ns	ns
japonica	Akitakomachi	0.9	1.1	1.2	1.4	***	**	ns	0.31	0.38	0.49	0.54	***	*	ns
japonica	Sasanishiki	1.3	1.3	1.3	1.5	ns	ns	ns	0.47	0.51	0.57	0.66	**	ns	ns
japonica	Hatsuboshi	1.2	1.3	1.3	1.3	ns	ns	ns	0.39	0.47	0.57	0.54	**	ns	ns
japonica	Koshihikari	1.3	1.4	1.4	1.4	ns	ns	ns	0.45	0.55	0.63	0.63	*	ns	ns
japonica	Hitomebore	1.1	1.3	1.3	1.4	ns	*	ns	0.45	0.50	0.59	0.63	***	*	ns
japonica	Fukumirai	1.2	1.2	1.3	1.4	ns	ns	ns	0.41	0.49	0.60	0.61	**	ns	ns
japonica	Tennotsubu	1.2	1.3	1.3	1.3	ns	ns	ns	0.39	0.50	0.53	0.56	**	ns	ns
japonica	Hinohikari	1.4	1.4	1.4	1.5	ns	ns	ns	0.54	0.55	0.59	0.59	ns	ns	ns
japonica	Nikomaru	1.4	1.4	1.4	1.5	ns	ns	ns	0.51	0.58	0.56	0.61	ns	ns	ns
indica	IR36	1.4	1.6	1.4	1.5	***	*	ns	0.68	0.82	0.64	0.67	***	*	*
indica	Hokuriku 193	1.3	1.4	1.5	1.7	ns	**	ns	0.49	0.55	0.70	0.80	**	**	ns
indica	Takanari	1.4	1.7				*		0.68	0.87				***	
jap.-ind.	Mizuhochikara			1.3	1.5		*				0.62	0.70		**	

Discussion

Change in g_s after N supplementation: The g_s was higher in the *indica* types than in the *japonica* types at the equal leaf N content (Fig. 2), and increased significantly with the leaf N content only in the latter types (Fig. 2). However, the response could not be separated clearly between variety groups (Table 2). One report concluded that varietal differences in the relationship between g_s and leaf N content depend on genotype (Hirasawa *et al.* 2010), but another concluded that they do not (Ohsumi *et al.* 2008). The slope and positive intercept of g_s in SN *vs.* g_s in UN indicated that N supplementation was more effective in increasing stomatal aperture in the varieties with inherently lower g_s, which in this case were the *japonica* varieties with lower g_s (Fig. 1). Supplemental N did not affect the stomatal aperture of Koshijiwase, Akitakomachi, or Sasanishiki (Table 2). Some reports suggest that the responses of each are not likely to classify together into phenotypic groups (Kanemura *et al.* 2007). This indicates that the responses of g_s after supplemental N depended on g_s of the variety. N control did not lead to improvement of stomatal aperture in the groups with the higher g_s, namely the *indica* varieties and the *japonica* varieties with higher g_s. Supplemental N increased total biomass and the grain yield without improving g_s in the *indica* varieties (Tables 2, 4). It was inconsistent with the expected results. Some varieties invest more biomass and N into cell walls, leading to a high leaf mass per unit area and improved toughness, allowing them to survive certain conditions (Hikosaka 2004). The resultant variations in leaf thickness do not affect g_s or C_i on an area basis, but do affect leaf mesophyll and $\delta^{13}C$ (Hanba *et al.* 1999). Supplemental N might be then allocated to leaf and shoot mass, allowing accumulated carbohydrates to increase the grain yield in the *indica* varieties without improving g_s.

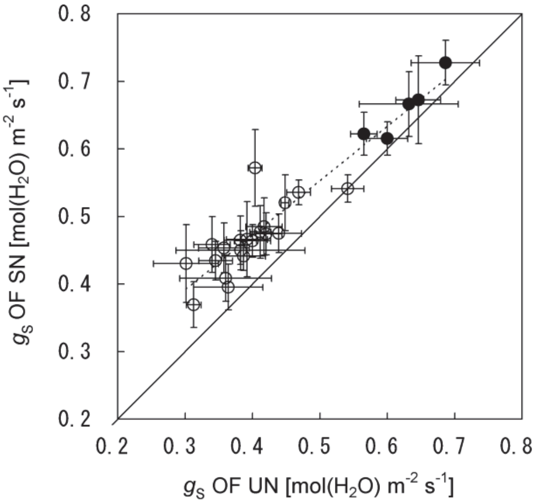

Fig. 1. Stomatal conductance (g_s) of control (UN) *vs.* g_s of plants with additional N (SN) in *japonica* (○) and *indica* cultivars (●). The *dotted line* indicates the linear regression between UN and SN for all cultivars [g_s (in SN) = 0.81 × g_s (in UN) + 0.15]. The bars show SEM (n = 3). The *solid line* indicates the 1:1 line.

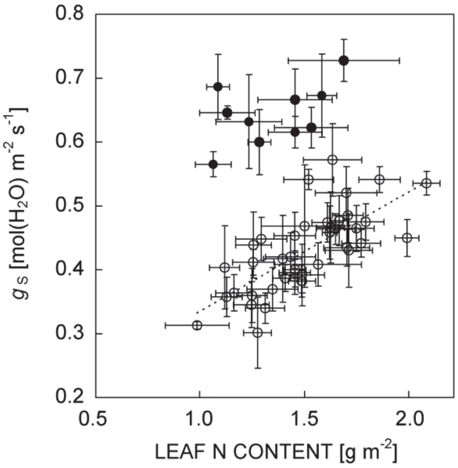

Fig. 2. Relationship between g_s and leaf N in *japonica* (○) and *indica* cultivars (●). The *dotted lines* indicate the linear regressions between leaf N and g_s for each group. The bars show SEM (n = 3).

No change in C_i/C_a and $\delta^{13}C$ after N supplementation: Previous studies reported a positive correlation between N application and $\delta^{13}C$ (Kondo *et al.* 2004, Cabrera-Bosquet *et al.* 2007). Greater shoot growth due to improved N availability may induce stomata closure due to increased demand for water (Kondo *et al.* 2004, Cabrera-Bosquet *et al.* 2007). However, in our study, where water was always readily available, supplemental N application was not related to changes in any morphological trait (Shimoda 2012) and did not significantly alter the $\delta^{13}C$ of any variety except Hitomebore (Table 3). $\delta^{13}C$ is determined by the balance between g_s and carboxylation efficiency. Because the abundant water supply maintained g_s, the balance between g_s and carboxylation efficiency maintained both C_i/C_a and $\delta^{13}C$. It is generally accepted that in photosynthesizing leaves, g_s is correlated with P_N and is coordinated with the CO_2 requirement of the mesophyll, such that the C_i/C_a ratio may be maintained at a constant value (*e.g.* Wong *et al.* 1979). Under abundant water supply, the application of supplemental N alone could have increased both the stomatal aperture and the carboxylation reaction in some *japonica* varieties, and thus it could have enhanced the stomatal aperture without changing C_i/C_a.

Interannual variation: The values of g_s and C_i/C_a showed differences between both years in four varieties. The values reflect the instantaneous physiological condition, which is often influenced by the growth conditions. Interannual differences in gas exchange are larger at later

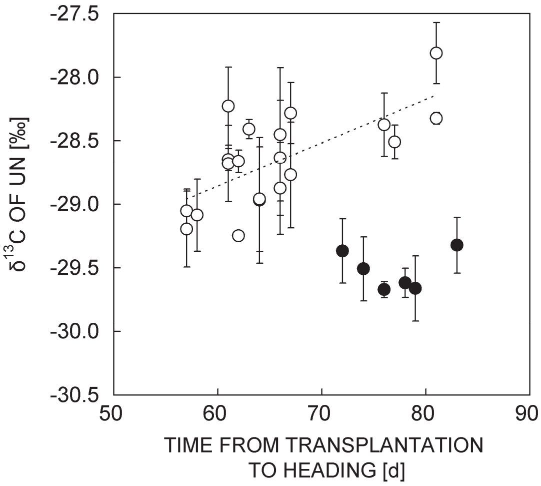

Fig. 3. Relationship between δ^{13}C in and time from transplantation to heading in control (UN) *japonica* in 2010 (○) and 2011 (□), and *indica* cultivars in 2010 (●) and 2011 (■). The *dotted lines* indicate the linear regressions between δ^{13}C and time from transplantation to heading for *japonica* type. The bars show SEM (n = 3).

growth stages even under the same management (Takai *et al.* 2006). Nikomaru and Hinohikari exhibited significant differences in g_s, C_i/C_a, and δ^{13}C between years (Tables 2, 3). These varieties had longer growth periods than other *japonica* varieties (Table 1), and therefore their stomatal aperture and gas-exchange parameters were more sensitive to differences in growth conditions between years. The values of δ^{13}C exhibited significant differences between years in most varieties, regardless of the slight differences in the leaf N (Tables 2, 3). δ^{13}C is often associated with growth speed in a wide range of crops (Hall *et al.* 1994, Takai *et al.* 2009). Here, δ^{13}C tended to increase with an increasing number of days from transplantation to heading in *japonica* varieties (Fig. 3). The mean time between transplantation and heading was 4.8 d shorter in 2010 than that in 2011 (Table 1), owing to the higher air temperatures in 2010, when the hot summer caused rapid senescence (Shimoda 2011). In normally developing leaves, δ^{13}C reflects the time-integrated photosynthetic ability of the plant during leaf expansion (Scartazza *et al.* 1998, Shimoda 2011). Since changes in δ^{13}C are associated with the growth rate, supplemental N application only slightly decreased δ^{13}C. The depletion of δ^{13}C indicates that high temperatures both before and after heading caused rapid senescence. In contrast, δ^{13}C in Koshihikari, Hinohikari, and Nikomaru was significantly higher in 2010 than in 2011, but there were no significant variations in the grain yield between years. Interannual variations in the grain yield might be associated with δ^{13}C values through the leaf mesophyll variation.

The g_s increased significantly with the leaf N content only in some *japonica* varieties. N supplementation was more effective to increase stomatal aperture in the varieties with inherently lower g_s, which in this case were the *japonica* varieties with lower g_s. These results suggest that the stomatal aperture of those *japonica* varieties could be improved by N management during the growth period. Recent breeding improvement has enhanced P_N by increasing the leaf N content under heavy N application. The relationship between g_s and the leaf N in each genotype might demonstrate the potential for altering stomatal aperture to increase further the yield in elite varieties. Interannual variation in g_s and C_i/C_a indicated that climatic conditions affected physiological condition regardless of N control. Although stomatal parameters (g_s, C_i/C_a, and δ^{13}C) in Nikomaru and Hinohikari differed significantly between years, the improvement of stomatal aperture was not associated with the grain yield (Tables 2–4). High temperatures reduce grain quality and yields in Japan. Some *japonica* varieties have been released recently to secure grain quality and the yield even at high temperatures (Morita and Nakano 2011). It is necessary to investigate their g_s responses to supplemental N application in order to improve further rice physiological condition by N control under variable climatic conditions.

We found that supplemental N application increased g_s of the *japonica* varieties with lower g_s. The value of δ^{13}C could indicate both growth rate and plant physiological conditions, but it was not sensitive to N application.

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