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Araki, Takuya
Faculty of Agriculture, Kyushu University

Yasuoka, Mika
Faculty of Agriculture, Kyushu University

Kubota, Fumitake
Faculty of Agriculture, Kyushu University

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Cultivaral Difference in Growth Sustainability of Rice Species Grown in Low Nitrogen–Input Condition

Takuya ARAKI*, Mika YASUOKA[1] and Fumitake KUBOTA

Laboratory of Plant Production Physiology, Division of Soil Science and Plant Production,
Department of Plant Resources, Faculty of Agriculture,
Kyushu University, Fukuoka 812–8581, Japan
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The experimental materials used here were 12 rice cultivars released and grown in the past one century in the southern area of Japan and two African cultivars. With plants water–cultured under standard and low nitrogen applications, their dry matter weight, leaf area and net assimilation rate (NAR), rubisco content and chlorophyll content in leaves were investigated. Results obtained were as follows: By reducing the nitrogen concentration of culture–solution to 10% of the standard, the average plant dry matter weight and leaf area of the 14 cultivars were decreased to 25% and 20%, respectively. To evaluate the growth sustainability of cultivars grown under the low nitrogen application, the sustainability index (SIL) was used here. SIL of dry matter weight (SIL–DM), leaf area (SIL–LA) and NAR (SIL–NAR) were significantly different among cultivars. For these three parameters, large values were found in Koshihikari, one of the high quality cultivars widely grown in Japan today, and the small ones were observed in old, traditional cultivars, such as Asahi and Shinriki, and in G174 (*O. glaberrima*). A significant cultivaral difference in response to nitrogen application was also found in the rubisco and chlorophyll contents and their SILs.

INTRODUCTION

The realization of high and stable productivity of rice has so far chiefly depended on the high–input cultivation system in Japan. Excessive fertilizer applications aggravate environmental conditions, leading to more serious environment destructions in the future. In order to resolve this problem, it is essential to develop new cultivars having a sustainable growth in fertilizer–saving cultivations. However, there are not so many studies on the growth and yield sustainability of rice cultivars.

Nitrogen is one of the essential nutrients for plant growth. In a rice plant, 80% of the nitrogen contained in the leaf is located in chloroplasts (Morita, 1980), and 30% of that was delivered to rubisco (Makino *et al.*, 1985; Evans, 1989). The rubisco content is regarded as one of the main photosynthetic determinants (Mae *et al.*, 1983), and a close, positive relationship was frequently found between leaf nitrogen content and photosynthetic rate (Evans, 1983; Field and Mooney, 1987; Terashima and Evans, 1988; Makino *et al.*, 1992; Tagawa *et al.*, 2000). Rubisco is an important factor as a determinant of the sustainability of photosynthetic production.

As a step toward developing new cultivars with a sustainable growth, it is important

[1] Laboratory of Plant Production Physiology, Department of Agronomy, Faculty of Agriculture, Kyushu University.
* Corresponding author (E–mail; araki@agr.kyushu–u.ac.jp)

to make screenings of growth response features over the existing rice genotypes. Then, in this study, the materials chosen from the new and traditional rice cultivars were solution–cultured at low and standard nitrogen concentrations, and their responses in growth parameters, and chlorophyll and rubisco contents were examined using a sustainability index (SIL).

MATERIALS and METHODS

Materials and cultivation

The experimental materials used were 12 rice cultivars having a cultivation record in the past one century in the northern area of Kyushu Island in Japan, and two cultivars from West Africa. The names of the 12 cultivars (*O. sativa*) were Asahi, Shinriki, Sirobeniya, Okute–shirasasa, Koshihikari, Norin–18, Reiho, Houyoku, Nipponbare Hinohikari, Nishihomare and Yumetsukushi, and the two cultivars from Africa were S70 (*O. sativa*) and G174 (*O. glaberrima*). Seeds of these cultivars were sown in nursery boxes on July 9, 2002 and grown for three weeks. Young shoots were transplanted into two water–culture bathes with standard and low nitrogen concentrations. Forty two plants (three plants / one cultivar) were transplanted and grown in a pot with a capacity of 500L. Yoshida's solution (Yoshida *et al.*, 1971) was used here. The nitrogen concentration in solution was set at two levels: the standard level was 2.9 mmolL^{-1} and the low level was 0.29 mmolL^{-1} (10% of the standard). The former plot was termed SN and the later was LN. The concentration of nutrients other than nitrogen was prepared at the standard level in both plots. The culture solution in SN and LN was renewed at 10–day intervals and its pH was controlled within a range of 5.0 to 5.5.

Measurement of matter production

The plants grown for 47 days after transplanting were sampled and separated into three parts: leaf, culm and root. They were dried at 80 °C in an oven for three days and weighed. Leaf area was measured with an automatic area meter (AAM–8, Hayashi–denko, Japan). Net assimilation rate (NAR) was given here as a value per plant.

Determination of chlorophyll content

Using a cork borer with a diameter of 6 mm, three leaf discs were sampled from top expanded leaves on the same sampling day mentioned above. The sampled leaf discs were soaked in 3 mL ethanol solution to extract chlorophyll. The chlorophyll content of leaf was calculated from the absorbance at 649 and 665 nm spectra with a spectrometer (UV–1200, Shimadzu, Japan), according to the method reported by Wintermans *et al.* (1965).

Determination of the content of rubisco (ribulose–1,5–bisphosphate carboxylase/oxygenase) [EC4.1.1.39]

Leaf discs were sampled from the same leaves at the same time described above. Directly after the sampling, the leaf discs were frozen in liquid nitrogen, and stocked at −80 °C. Just before the measurement, stocked leaves were powdered in liquid nitrogen, and the rubisco content was determined with a SDS–polyacrylamid gel electrophororesis, according to the method reported by Makino (1985). The quantitative analysis of soluble protein was carried out through the method by Bradford (1976).

Calculation of a sustainability index (SIL)

In order to evaluate a cultivaral difference in sustainability against the reduction of nitrogen application, the sustainability index (SIL) was calculated from the equation (1).

$$SIL = P_{LN} \cdot (1/R) / P_{SN} \qquad (1)$$

where P_{SN} and P_{LN} are parameter values measured with plants grown in the standard and low nitrogen concentrations, respectively, and R is a ratio of the solution nitrogen concentration of LN to that of SN. The value of R is 0.1 here. A higher SIL means a higher sustainability. SIL was calculated for five growth parameters: dry matter weight (SIL–DM), leaf area (SIL–LA), NAR (SIL–NAR), rubisco content (SIL–rub) and chlorophyll content (SIL–chl).

RESULTS

Table 1 shows the growth parameters, plant dry matter weight, leaf area and NAR, determined with the cultivars grown in SN and LN, and SIL calculated from the equation (1). The average of plant dry matter weight of the 12 Japanese cultivars was 15.3 g in SN, and it decreased to 3.8 g in LN. Leaf area also varied with nitrogen level: the average was 770 cm^2 in SL and 157 cm^2 in LN. The largest values of both parameters in SN were found in Shinriki; while in LN, Koshihikari, Houyoku, and Yumetsukushi had large values in dry matter weight, and a large leaf area was shown in Houyoku. A cultivaral difference was

Table 1. Values of dry weight, leaf area and NAR under the conditions of SL and LN.

Cultivar	Dry weight (g)			Leaf area (cm^2)			NAR ($g\ cm^{-2}$)		
	SN	LN	SIL	SN	LN	SIL	SN	LN	SIL
Asahi	8.7 f	1.6 e	1.77 e	408 i	64 g	1.58d	214ab	241 b	11.3 cd
Shinriki	22.2 b	3.7 cd	1.65 e	995 c	157 e	1.58d	223ab	232 b	10.4 d
Shirobeniya	19.1 c	4.2 cd	2.22 d	807 de	174 cde	2.16ab	236a	243 b	10.3 d
Okute–shirasasa	15.9 d	4.1 cd	2.60 bc	872 d	171 de	1.95c	182cd	242 b	13.3 b
Norin-18	17.2 cd	4.4 c	2.56 cd	946 c	189 cd	2.00bc	181cd	232 b	12.8 bc
Koshihikari	11.5 e	4.6 c	3.99 a	755 ef	178 cde	2.36a	153e	258 b	16.9 a
Reiho	14.9 d	4.1 cd	2.74 bc	865 d	166 e	1.92c	172d	245 b	14.3 ab
Houyoku	17.3 cd	4.6 c	2.74 bc	943 c	195 c	2.06abc	183c	238 b	13.0 b
Nipponbare	15.6 d	3.5 d	2.27 d	728 fg	158 ef	2.17ab	214ab	224 b	10.5 d
Hinohikari	14.7 d	3.2 d	2.19 d	651 g	140 f	1.99c	227ab	249 b	11.0 cd
Nishihomare	11.2 e	3.5 d	3.09 ab	583 h	143 f	2.45a	192cd	242 b	12.6 bc
Yumetsukushi	16.0 d	4.6 c	2.87 bc	681 g	156 ef	2.28a	234a	294 a	12.5 bc
Ave. of domestic cultivar	15.3	3.8	2.56	770	157	1.89	201	245	12.4
S70	25.1 b	7.0 b	2.78 b	1517 b	287 b	1.89c	161de	244 b	14.7 ab
G174	76.6 a	8.8 a	1.15 e	3753 a	428 a	1.14e	203bc	206 c	10.2 d

* Same letters in the column represent not significant at 5% level.

also found in SIL–DM, SIL–LA and SIL–NAR; in particular, Koshihikari had high values for all these three SIL parameters.

Both cultivars, S70 and G174, from West Africa were more sensitive to nitrogen: the response in dry matter weight and leaf area in the two cultivars to nitrogen was larger than that of Japanese cultivars (Table 1).

The relationships between SIL–DM and SIL–LA, and between SIL–DM and SIL–NAR are shown in Fig. 1. Significant positive coefficients, $r^2=0.695^{***}$ and 0.709^{***}, were found in both relationships, respectively. A cultivar with a large value in SIL–LA or/and SIL–NAR had a tendency to show a high SIL–DM.

The relationship between SIL–LA and SIL–NAR is shown in Fig. 2. Though there was a cultivar like Koshihikari that had a large value in both SIL–LA and –NAR, yet no significant relationship was detected between these two parameters through the 14 cultivars.

In Fig. 3–A and –B, the relationships between SIL–NAR and SIL–chl, and between SIL–NAR and SIL–rub are shown, respectively. NAR was predicted to have a close relationship with the chlorophyll and rubisco contents, but a significant relationship was not found here. Then, we tried to classify the distributing data into four groups from the quadrant I to IV. The cultivar included in the quadrant–I (Fig. 3–B) was judged to have a higher value in both SIL–NAR and SIL–rub than the average values. In koshihikari, both parameters were included in the quadrant–I, showing highest values among the 14 cultivars. No cultivars were found in the quadrant–III of Fig. 3–B. Only S70 was placed in the quadrant–III (Fig. 3–A). Two old, traditional Japanese cultivars, Asahi and Shinriki, had low values in SIL–NAR, SIL–chl and SIL–rub (Fig. 3–A and –B).

Fig. 4–A and –B illustrate the relationships between SIL–chl and chlorophyll content, and between SIL–rub and rubisco content, respectively. The significant relationships were found here, and the regression equations were given as $y=12.94e^{-1.2047x}$ and $y=12.725e^{-0.3889x}$, respectively. The cultivar with a higher value in chlorophyll or rubisco con-

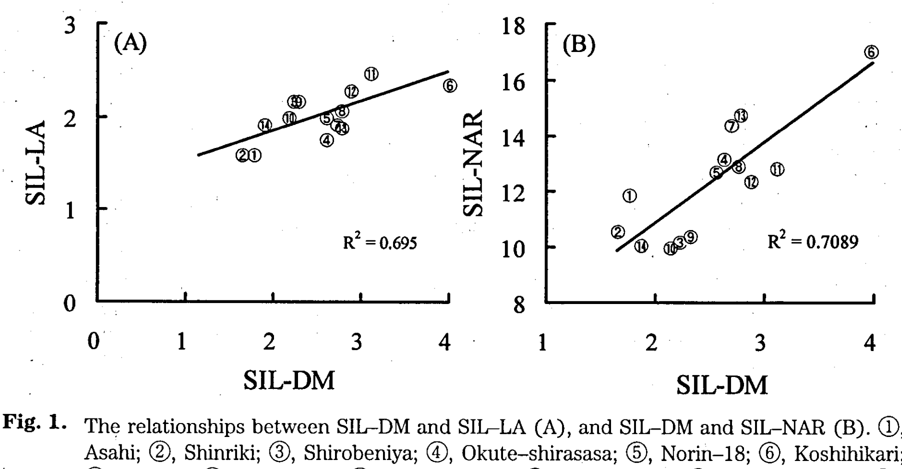

Fig. 1. The relationships between SIL–DM and SIL–LA (A), and SIL–DM and SIL–NAR (B). ①, Asahi; ②, Shinriki; ③, Shirobeniya; ④, Okute–shirasasa; ⑤, Norin–18; ⑥, Koshihikari; ⑦, Reiho; ⑧, Houyoku; ⑨, Nipponbare; ⑩, Hinohikari; ⑪, Nishihomare; ⑫, Yumetsukushi, ⑬, S70; ⑭, G174.

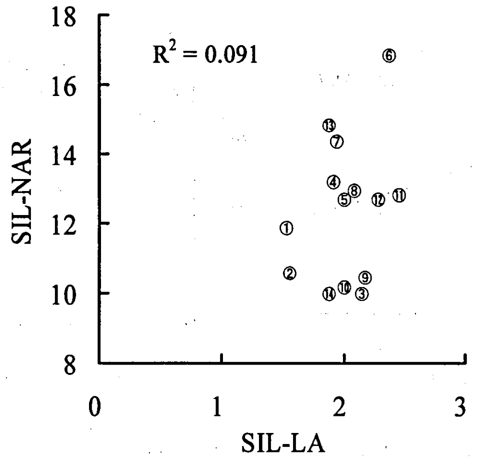

Fig. 2. The relationships between SIL–LA and SIL–NAR. See Fig. 1 for encircled numbers.

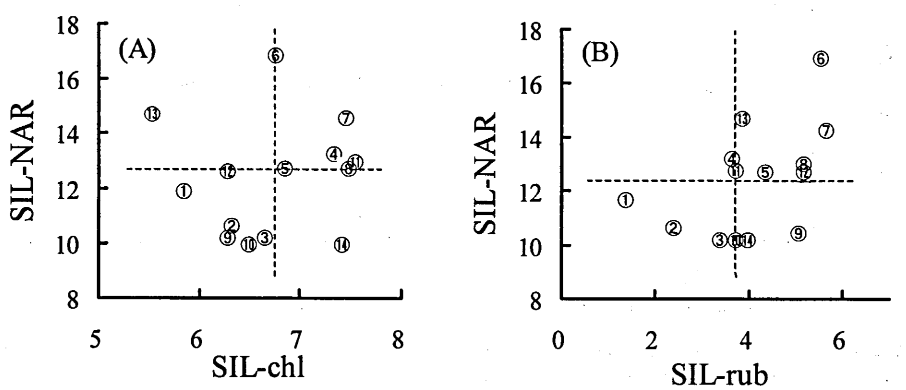

Fig. 3. The relationships between SIL–NAR and SIL–chl (A), and between SIL–NAR and SIL–rub (B). See Fig. 1 for encircled numbers. Broken lines show the average of each parameter.

tent in SN tended to decrease in SIL–chl and SIL–rub.

The equation (1) is re-written as $P_{LN}=SIL \cdot R \cdot P_{SN}$, and each item is re-placed as $Y=P_{LN}$, $R=0.1$, $SIL=y$, or $y=12.94e^{-1.2047x}$, $P_{SN}=x$. The equation, $Y=x\ 1.294e^{-1.2047x}$, in Fig. 5–A gives the relationship in chlorophyll content between SN and LN. Similarly, the relationship in rubisco content between SN and LN is shown as the equation, $Y=x\ 12.725e^{-0.3889x}$, in Fig. 5–B. This equation showed a clear peak: as shown in Fig. 5–B, the cultivar having a rubisco content of 2.57 gm^{-2} in SN was predicted to have the highest value of rubisco content (1.20 gm^{-2}) in LN. Of the materials used here, such a cultivar as

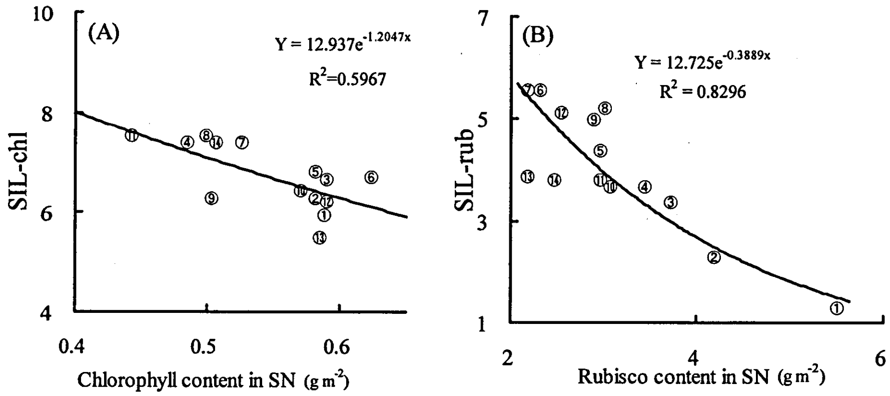

Fig. 4. The relationships between SIL–chl and chlorophyll content (A), and between SIL–rub and rubisco content (B). See Fig. 1 for encircled numbers.

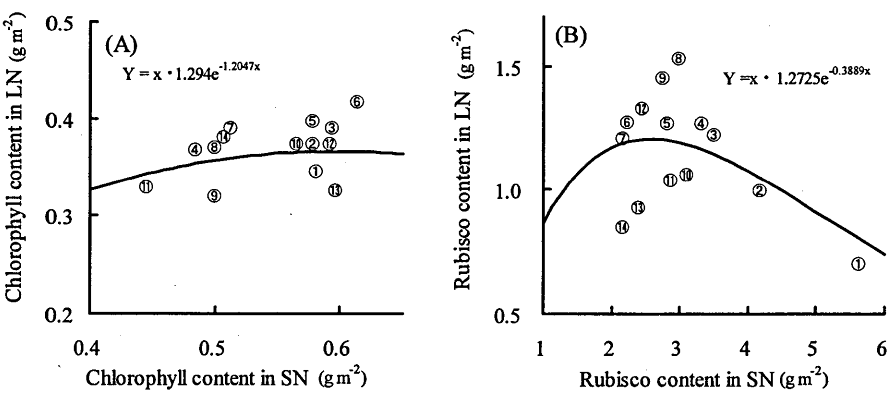

Fig. 5. The relationships in chlorophyll content (A) and rubisco content (B) between SN and LN. See Fig. 1 for encircled numbers.

Koshihikari, Reiho and Yumetsukushi belonged to this type. On the other hand, no clear peak was found in SIL–chl as shown in Fig. 5–A.

DISCUSSION

The protection and remediation of the natural environment have been taken up as one of the important subjects in the agricultural area today. As the first step to solve this problem, it is important to develop a new cultivation system with an adequately saved fertilization, and also to create new cultivars suitable to such a cultivation system. As a process toward establishing the fertilizer low–input system in the paddy, Hasegawa and

Horie (1994) pointed out the importance of the utilization of inorganic nitrogen in paddy soil, and estimated the amount of unorganized nitrogen by simulation. Until today, a lot of studies on nitrogen and rice production have been carried out (Kumura, 1955; Cho and Murata, 1980; Uchida *et al.*, 1982), but there are not so many studies concerning the effects of nitrogen savings on the yield and production of rice cultivars.

In our experiment, a significant cultivaral difference was found in SIL–DM, SIL–LA and SIL–NAR (Table 1). As the values of SIL–DM are compared among Japanese cultivars, the lowest one is found in Asahi and Shinriki. Since theses two cultivars were ever widely grown in the less–intensive fertilization system in the past, they have been predicted to have a higher sustainability in low nitrogen applications as one of the obtained genetic characteristics, but such a feature is not found here. While the recent cultivars such as Koshihikarin, Nishihomare and Yumetsukushi show a higher SIL–DM. It is the important evidence that Koshihikari, one of the most popular cultivars with high quality grown today in Japan, is characterized by a high productivity and sustainability under the low nitrogen input (Table 1 and Fig. 1). In addition, Koshihikari is high in SIL–LA and SIL–NAR (Fig. 1 and 2).

As compared to Japanese cultivars, both cultivars, S70 and G174, from Africa have a considerably large values of dry matter weight and leaf area, though their SIL–DM and SIL–LA were not high (Table 1). The large biomass production is regarded as one of the beneficial genetic potentials in both cultivars.

It has been well known that there is a close relationship between photosynthetic rate and nitrogen content in a leaf (Evans, 1983; Field and Mooney, 1987; Terashima and Evans, 1988; Makino *et al.*, 1992). We examined the mutual relationships between NAR, chlorophyll content and rubisco content. But no significant relationships were found between them, though there was a trend that cultivars with a high SIL–NAR had a high SIL–chl and SIL–rub (Fig. 3). Of the cultivars with a high SLI–NAR, Koshihikari and Reiho had a high value in both SLI–chl and SIL–rub. On the other hand, there is a cultivar like S70 showed a high SLI–rub and a low SLI–chl. These facts may suggest that there is a high possibility of selection of genotypes with a high sustainability in both chlorophyll and rubisco contents in leaves. It is an interesting fact that such an old traditional cultivar as Asahi and Shinriki has a considerably higher rubisco–content than the cultivars recently grown and their SIL–rub is low.

The regression equation indicates that a cultivar with 2.75 g m^{-2} in rubisco content in SN is predicted to have the largest rubisco content in LN (Fig. 5). This might suggest that there is an optimum selection point for the sustainability improvement of leaf rubisco content of rice. More detailed experimental sets using various rice cultivars including a wide genetic range are necessary in order to conduct strict evaluations for the growth and production sustainability in rice species.

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