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Effect of topdressing on individual leaf photosynthesis at different position in direct-sown rice with non-woven fabric mulch system

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Abstract

Direct sowing with non-woven fabric mulch is the new organic rice cultivation system. We studied the effect of topdressing on individual leaf photosynthesis at different position and grain yield in rice plants cultivated by this system. Leaf photosynthetic rate at the different leaf position per plant ($P_{N\text{-}LP}$) of the third and fourth to lower leaves was higher when the topdressing amount was increased. Without topdressing or in no-fertilizers plots, the $P_{N\text{-}LP}$ values of lower leaves were very low. The leaf photosynthetic rate per unit leaf area ($P_{N\text{-}LA}$) decreased gradually as the leaf position became lower. Again, the $P_{N\text{-}LA}$ values of the top-dressed plots at the lower leaves were higher than that of plots without topdressing or without fertilizers. The lower leaves maintained a higher P_N because of a higher rate of nitrogen accumulation due to topdressing. The higher rate of photosynthesis in these leaves resulted in better root activity, which contributed to a better ripening percentage and ultimately higher rice grain yield.

Additional key words: leaf nitrogen; organic farming; *Oryza sativa*.

Introduction

Products of plants grown with fewer or no pesticides are increasingly preferred by consumers because the use of agrochemicals increases price of the product and causes health hazards. The use of natural resources in accordance with suitable agronomic management practices for sustainable agricultural production is most demanding and will certainly receive attention in future (Ghosh *et al.* 2004).

Direct-sown culture with non-woven fabric mulch (renewable cotton) is a new organic rice production system that was developed for an effective weed control and better rice grain yield without use of any agrochemical. Each mulch roll measures 100 m in length and 1 m in width. Seeds are placed between two sheets of the mulch cloth like in a sandwich. Water is drained two weeks after spreading (mulching) the sheets in the field (Sugimoto *et al.* 2003). In a previous study, we observed the efficacy of nitrogen (N) fertilizer in this production system (Hossain *et al.* 2005).

The photosynthetic capacity of a rice community depends on three factors: the photosynthetic capacity per unit leaf area, leaf area index (LAI, the ratio of total upper leaf surface of a crop divided by surface area of the land on which the crop grows; it is used to predict the photosynthetical primary production and as a reference tool for crop growth), and canopy structure (Murata *et al.* 1957).

Most studies of the relationship between photosynthetic rate under saturating irradiance (P_{max}) and leaf N content used pot-grown plants in soil or culture solution in the greenhouse growth chamber, and P_{max} was often determined under artificial irradiation provided by incandescent lamps (Makino *et al.* 1988). Field conditions, however, differ greatly from greenhouse environments. The differences between environments may result in different growth and development characteristics between plants grown in the field and those grown in greenhouses. In this study, we measured leaf photosynthetic rates at different leaf position ($P_{N\text{-}LP}$) of field-grown rice plants using the closed chamber method (Sugimoto and Koesmaryono 2001). Peng *et al.* (2002) measured the single-leaf net photosynthetic rates (P_N) with portable photosynthesis systems (*LI-6200* and *LI-6400*). Matsushima (1973) stated that N topdressing (the

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application of fertilizers after the crop has been established) at the full heading stage helped improve the percentage of ripened grains, prevented lodging (fall over of crops due to rain or wind), improved leaf thickness,and increased grain yield. Although earlier studies confirmed the efficacy of topdressing on the rice leaf P_N at the grain filling stage, however, the P_N of individual leaves as well as the contribution of the lower leaves to the total leaf P_N have not yet been well characterized. In this study we checked the effect of topdressing on P_N and explored the underlying mechanism by assessing the rate of photosynthesis at individual leaf positions (particularly lower leaves).

Materials and methods

Plants and growth conditions: The field experiments were conducted in 2004 in Matsuyama City, south-western Japan (33°50.6`N latitude, 132°46.6`E longitude, 32 m a.s.l.). The pH of the soil was 6.24 and the electrical conductivity was 0.028 mS cm^{-1}. Rice (*Oryza sativa* L. cv. Koshihikari) seeds with a sowing rate of 4 g m^{-2} were used for the experiment.

Mulch sheet, mulching, and culturing details: Non-woven (cloth) mulching sheet was acquired from the Marusan Company, Ozu-shi, Ehime-ken, Japan. The mulching (sowing) was done on June 10, 2004. Organic fertilizers (rapeseeds and poultry manure) were applied at the rate of 6 g(N) m^{-2} as a basal fertilizer (F6) at plowing time with or without topdressing. Three combinations of basal fertilizer and topdressing were used in this experiment: (*1*) basal 6 g(N) m^{-2} + no topdressing = F6+0, (2) basal 6 g(N) m^{-2} + topdressing [3 g(N) m^{-2}] = F6+3, and (*3*) basal 6 g(N) m^{-2} + topdressing [6 g(N) m^{-2}] = F6+6; control treatment was without fertilizer (NF). Topdressing was done at 13 d before the heading stage (growth stage of the rice plant marked by emergence of the panicle from the boot). The experiment was done using a Randomized Complete Block (kind of statistical design for conducting field experiments) design with two replications.

Plant sampling: Heading (emergence of the panicle from the boot) was observed at 70 DAS (d after sowing). Plants were harvested on September 28. To calculate the grain yield, six samples (portions) were taken, each 1 m in length. These samples were also used for the calculation of yield components. The grains were divided into filled grains and unfilled grains using salt water with a specific gravity of 1.06, and the percentage of filled grains was determined. A 1 000-grain mass of brown rice was measured, and the grain mass was calculated to the value corresponding to a 14 % moisture content.

Measurement of photosynthesis: P_N was measured by means of a closed chamber system that enables measurement of photosynthesis of entire plant (Sugimoto and Koesmaryono 2001) around noon on sunny days in the middle of September at the mid-ripening stage. Sasaki *et al.* (2005) examined the translocation and partitioning of ^{13}C at the early grain-filling stage. At the beginning, we uprooted the rice plants from the field and maintained them in plastic pots for a week. Three or four plants were placed in a single pot. For every treatment three pots were maintained for the measurement of photosynthesis. In an aluminum-framed chamber (70×60×130 cm) covered with a transparent vinyl film two pots were set. Three fans were used to circulate the air in the chamber, and the air temperature was controlled by passing tap water through a cooling system installed in the chamber. The air temperature in the chamber varied from 28 to 32 °C according to the outside temperature. Due to dehumidification by the cooling system, dew did not form inside the chamber wall. An infra-red gas analyzer (*ZFP5*, *Fuji Electric*, Japan) was set in the chamber to determine the change in CO_2 concentration per unit of time.

The photosynthetic rate of all plants in the chamber, $P_{N\text{-}AP}$ [μmol(CO_2) $plant^{-1}$ s^{-1}] was determined based on the volume of the chamber (the volume of the plants and equipment was deduced) and the decrease in CO_2 concentration during the measurement period. Exhaust CO_2 was supplied by the CO_2 gas cylinder (5 %) expiration into the chamber. Photosynthetically active radiation (PAR) was monitored using a quantum sensor (*LI-190SB*, *LI-COR*, USA). Air and soil temperatures were recorded using copper-constantan thermocouples.

After the determination of $P_{N\text{-}AP}$, in order to estimate the photosynthetic rate at the different leaf position per plant ($P_{N\text{-}LP}$) and per unit leaf area ($P_{N\text{-}LA}$), the plants were fed with $^{13}CO_2$ gas with a PAR above 1 500 μmol(photon) m^{-2} s^{-1}. The $^{13}CO_2$ gas formed by the addition of an appropriate amount of 3 % lactic acid to ^{13}C-labelled barium carbonate ($Ba^{13}CO_3$, 99 % atom ^{13}C) was supplied into the chamber using a circulating air pump for 30 min. Immediately after the termination of $^{13}CO_2$ feeding, the plants were harvested. Soil respiration was determined within minutes of cutting the plants. The cut plants were then separated into leaves in accordance with the leaf position (starting from the top of plant), *e.g.* first leaves followed by second, third, and fourth to lower leaves and other parts. Leaf area (LA) was measured using an area meter (*Hayashi Denko*, *AAM-7*). Plant samples were oven-dried at 85 °C to almost constant mass (3 d), then weighed (Sugimoto *et al.* 2005), and ground to a fine powder. The ^{13}C content of the samples was determined using a ^{13}C analyzer (*Integra CN*, Isotope Ratio Mass Spectrometer, *SerCon*, UK). The N and carbon contents of the samples were determined using a N/C analyzer (*Sumitomo*, *NC-80*).

Photosynthetic rates were calculated as follows:

$P_{\text{N-LP}}$ [μmol(CO$_2$) plant^{-1} s^{-1}] = $P_{\text{N-AP}}$ [μmol(CO$_2$) plant^{-1}s^{-1}] A [mg(individual leaf)]/B [mg(all leaves)]

$P_{\text{N-LA}}$ [μmol(CO$_2$) m^{-2} s^{-1}] = $P_{\text{N-LP}}$ [μmol(CO$_2$) plant^{-1} s^{-1}]/LA [m^2 plant^{-1}]

where A is the ^{13}C content of the individual leaf at different position, B is the ^{13}C content in all leaves in the chamber, and LA relates to individual leaf at different position.

Statistical analysis: Data of the plant growth, yield, and other yield components were statistically analyzed using ANOVA and data means were tested at $p \leq 0.05$ according to Fisher's least significant difference test (LSD).

Results

LA, $P_{\text{N-LP}}$, and $P_{\text{N-LA}}$ of the individual leaves in the mid ripening period are shown in Fig. 1. The first and second leaf values were higher in all plots in comparison to the F6+6 plot. But in the third leaf and below, LA in the NF and F6+0 plots was very small, almost zero. The highest LA of the third leaf and below was found in the F6+6 plot followed by the F6+3 plot.

$P_{\text{N-LP}}$ of the first leaf was higher in F6+0 [0.18 μmol (CO$_2$) plant^{-1} s^{-1}] than in other plots, but $P_{\text{N-LP}}$ values of the second leaf were similar in the F6+3 and F6+6 plots and were higher than in the non-topdressed (F6+0 and NF) plots. $P_{\text{N-LP}}$ values of the third and fourth to lower leaves were highest in the F6+6 plot, followed by the F6+3 plot, but those of in the F6+0 and NF plots were very low, and almost zero below the third leaf.

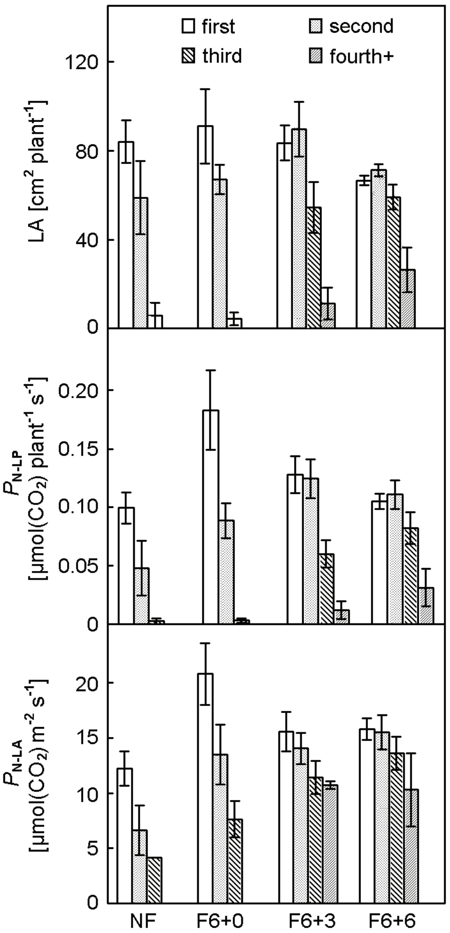

Fig. 1. Leaf area (LA), leaf photosynthetic rate at the different leaf position per plant ($P_{\text{N-LP}}$) and per unit leaf area ($P_{\text{N-LA}}$) of the individual leaf. Fourth +: fourth to onward leaves. For treatments see the text.

$P_{\text{N-LA}}$ gradually decreased as the leaf position became lower. Moreover, these values of the top-dressed plots at the third leaf and lower were higher than those of the NF and F6+0 plots, which were near to zero.

N-leaf and ALM of the individual leaf (Fig. 2): The N-leaf in the third and fourth to onward leaves was higher in the F6+3 and F6+6 plots than in the F6+0 and NF plots. A similar trend was observed for ALM among the plots.

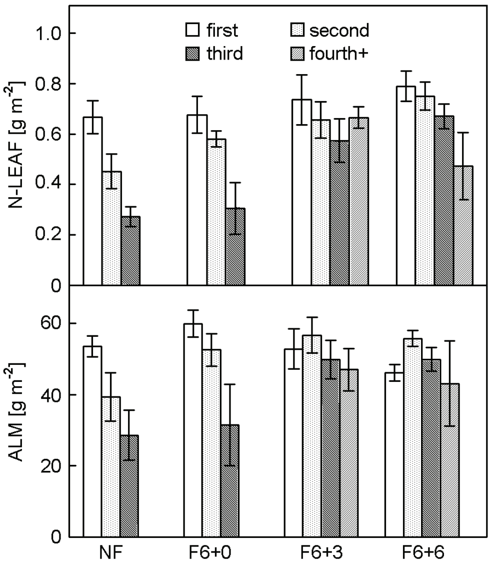

Fig. 2. Leaf nitrogen content (N-leaf) and area leaf mass (ALM) of the individual leaves. For abbreviations see Fig. 1.

Relation between $P_{\text{N-LA}}$ and N-leaf and between $P_{\text{N-LA}}$ and ALM: A strong positive correlation (r = 0.768) was observed between $P_{\text{N-LA}}$ and N-leaf. A similar strong positive correlation (r = 0.770) was observed between $P_{\text{N-LA}}$ and ALM (Fig. 3).

Lower leaf ratio shows the contribution ratio of lower leaves (below the third) to the total $P_{\text{N-LP}}$ of each plot (Fig. 4). This ratio became higher as the topdressing

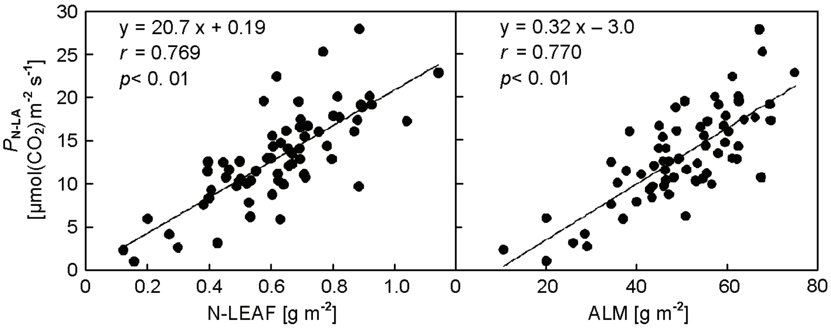

Fig. 3. Relationships between leaf nitrogen content (N-leaf) or area leaf mass (ALM) with leaf photosynthetic rate at the different leaf position per plant per unit leaf area ($P_{N\text{-}LA}$).

amount increased. The lower leaf ratio was highest in the F6+6 plot (0.32) followed by the F6+3 plot (0.22), but it was very low in the NF plot.

Yield components (Table 1): The best rice grain yield was obtained in the F6+6 plot followed by the F6+3 plot. The highest grain yield in the F6+6 plot was due to significantly higher ripening percentage and a higher thousand grain mass in comparison to the F6+3 plot. Again, the top-dressed plots produced a noticeably higher rice grain yield than the plots without topdressing and the NF plot due to differences in the number of panicles, the grain number, and the thousand grain mass.

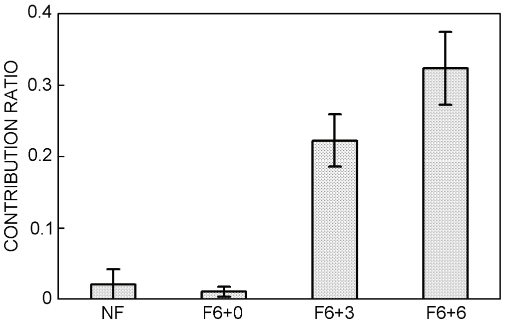

Fig. 4. Contribution ratio of lower leaves (below the third) to total leaf photosynthetic rate at the different leaf position per plant ($P_{N\text{-}LP}$).

Discussion

We analyzed the photosynthetic activity of individual leaves (as per position) together with the partitioning of photosynthetic activity labelled with ^{13}C in order to estimate the contribution of the reserves. At least 90 % of the biomass of higher plants is derived from CO_2 assimilated through photosynthesis (Zelitch 1982), and as a result crop yield is largely influenced by photosynthesis.

Table 1. Effect of topdressing on rice yield and yield components. Mean values. For treatments see the text.

Treatment	Plants m^{-2}	Panicles [m^{-2}]	Spiklets [$panicle^{-1}$]	Grain [m^{-2}]	Ripening ratio [%]	1 000 grain mass [g]	Grain yield [g m^{-2}]
NF	94.8	259	61.4	15 680	77.5	24.6	295.5
F 6+0	99.5	352	55.0	19 895	76.2	24.4	367.6
F 6+3	95.3	355	60.3	21 885	77.6	25.0	424.2
F 6+6	94.3	371	62.5	22 249	83.6	25.8	479.6

Generally, the upper leaves had the features of sun-leaves and received full sunlight. The lower leaves had features typical of shade-leaves, namely a smaller ALM (thin leaves) and lower N content, and therefore less P_N occurred in them (Šesták 1985, Evans 1989). Leaf position is important for determining the effects of N nutrition on photosynthetic activity and photosynthate production (Shinano *et al.* 2006a,b). Most of photosynthesis occurred in the top two leaves, particularly the first leaf, an observation that is supported by the results of Li *et al.* (1998). In our experiment, the features of lower leaves were different due to the application of topdressing, and plants maintained a higher leaf N content during the ripening stage in accordance with the amount of topdressing. Delayed leaf senescence is an important factor in the high production of dry matter and grain. Likewise, in many crops the leaf P_N is high during the ripening stage, and higher dry matter and higher grain yield are produced in the plants with slower leaf senescence (Jiang *et al.* 1988).

In the current experiment we observed less dry matter (data not shown) when there was no topdressing or a smaller application of the topdressing. This finding supports that of Peng *et al.* (1995), who stated that low dry matter production is often caused by low solar irradiation and low leaf N content at later growth stages.

$P_{\text{N-LA}}$ values are usually higher during the grain filling stage after heading than during the vegetative growth stage (Hayami 1982). According to that P_{N} is often larger in the grain-filling stage when topdressing is applied, although study does not evaluate single leaf photosynthesis or the contribution of lower leaves to total leaf photosynthesis.

We observed a close relationship between $P_{\text{N-LA}}$ and N-leaf, a finding that was supported by Cook and Evans (1983) and Peng *et al*. (1995). We also observed a close positive correlation between the ALM and $P_{\text{N-LA}}$. Peng (2000) also stated that the single-leaf P_{N} of rice plants was greatly affected by leaf N content and ALM. Sakai *et al.* (2006) found a decrease in canopy photosynthetic capacity when leaf N content decreased. Kuroda and Kumura (1989) stated that the degree of depression in P_{N} of single leaves was larger in lower leaves than in upper leaves because the depression in stomatal conductance was larger in the lower leaves.

Tsuno and Toryu (1987) stated that the sugar content of roots was closely related to the respiration rate, and Tanaka (1958) observed that photosynthates tranfered to roots originate largely in the lower leaves. Therefore, to keep root activity high, the rice plants must have higher leaf-N and ALM in the lower leaves. As a result, the higher P_{N} of lower leaves increases the root activities. This higher root activity stimulated the production of major yield components, namely the ripening percentage and thousand-grain mass, which ultimately contributed to the higher rice grain yield (Table 1). Youn and Ota (1973) evaluated the degree of senescence of leaves based on the ratios of chlorophyll content of the lower leaves to that of the upper leaves and concluded that the higher the root activity, the slower the leaf senescence. In addition, Arikado (1975) stated that the lower leaves can supply O_2 to the roots more easily than the upper leaves, and that withered leaves loose the function of O_2 intake. As a result, it is important to keep the lower leaves alive as long as possible so as to provide the roots with not only photosynthesis but also with O_2; the application of topdressing facilitates this.

Growth and development processes are associated with higher grain yields of rice. Nutrient release rate is largely dependent upon the C:N ratio of the source, which is a soil property. In order to increase rice yield, attention should be paid to elevating the leaf photosynthesis, and such an improvement could be achieved through erect upper leaves and higher P_{N} in lower leaves obtained by increasing the leaf N amount using topdressing.

We conclude that the lower leaves of rice plants maintained a higher P_{N} mainly because of a higher N accumulation due to topdressing. Thus, a higher P_{N} in the lower leaves resulted in better root activity, which contributed to a better ripening percentage, and finally a better rice grain yield.

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