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Ontogenetic changes of photosynthetic and dark respiration rates in relation to nitrogen content in individual leaves of field crops

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Abstract

Ontogenetic changes of rates of photon-saturated photosynthesis (P_{sat}) and dark respiration (R_D) of individual leaves were examined in relation to nitrogen content (Nc) in rice, winter wheat, maize, soybean, field bean, tomato, potato, and beet. P_{sat} was positively correlated with Nc as follows: P_{sat} = Cf Nc + P_{sat0}, where Cf and P_{sat0} are coefficients. The value of Cf was high in maize, medium in rice and soybean, and low in field bean, potato, tomato, and beet, of which difference was not explained by ribulose-1,5-bisphoshate carboxylase/oxygenase (RuBPCO) content. R_D was explained by P_{sat} and/or Nc, however, two models must be applied according to plant species. R_D related linearly with P_{sat} and Nc in maize, field bean, and potato as follows: R_D = a P_{sat} + b, or R_D = a'Nc + b', where a, a', b and b' are coefficients. In other species, the R_D/P_{sat} ratio increased exponentially with the decrease of Nc as follows: R_D/P_{sat} = a exp(b Nc), where a and b are coefficients. Therefore, R_D in these crops was expressed as follows: ln(R_D) = ln(a P_{sat}) + b Nc, indicating that R_D in these crops was regulated by both P_{sat} and Nc.

Additional key words: carbon-nitrogen interaction; growth respiration; leaf mass per area; maintenance respiration.

Introduction

Ingestad (1977) reported that the relative growth rate (RGR) showed a close correlation with nitrogen content (Nc) in whole tree seedlings. This concept has been widely applied to develop models for RGR as a function of Nc of whole plants (Hirose 1988, Pons *et al.* 1994, Wilkström and Ågren 1995), which indicate that the C and N metabolisms are interrelated in plants. The C-N balance was elucidated not only in whole plant, but also at single leaf level. A positive correlation between Nc and the leaf photon-saturated net photosynthetic rate (P_{sat}) has often been reported (Gulmon and Chu 1981, Field and Mooney 1986, Hirose and Kitajima 1986, Evans 1989). Moreover, P_{sat} was linearly correlated with leaf Nc for a wide range of C_3 species (Field and Mooney 1986). Greenwood *et al.* (1991), who reported that P_{sat} is limited by Nc, stated that along with leaf senescence and under shade, N is translocated from older leaves to younger ones. This enables such maintenance of active photosynthesis in the younger or less shaded leaves that the relationship between P_{sat} and leaf Nc remains constant. However, the P_{sat}-Nc relation varies with growth stages (Murata 1961, Hayami 1982) and species (Evans and Seemann 1989). Thus P_{sat} is regulated not only by Nc, but also by other factors such as ageing, leaf longevity, leaf structure, *etc.* For example, the surface area of mesophyll cells regulates the diffusion of CO_2 (Koike 1988), or the maximum P_{sat} is negatively correlated with leaf longevity (Chabot and Hicks 1982, Koike 1988).

Leaf dark respiration rate (R_D) is also an important factor in the regulation of the C balance in leaves. Plant R_D can be divided into growth respiration used for the growth process (*e.g.*, starch, protein, and cell wall synthesis) and maintenance respiration used for the maintenance process (*e.g.*, protein turnover, ion uptake – Penning de Vries 1975). Therefore both growth respiration and maintenance respiration are probably related to N nutrition in the leaves. R_D can be related to Nc of leaf tissue (Connor *et al.* 1993), but Byrd *et al.* (1992) showed that R_D is not correlated with Nc. P_{sat} is generally in a positive correlation with R_D (Murata 1961, McCree 1970, Tanaka and Hara 1970, Sato and Kim 1980, André *et al.* 1982, Azcón-Bieto *et al.* 1983). Thus the respiratory process is closely related to Nc and P_{sat}. According to McCree (1974), R_D of whole plants can be

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Abbreviations: LMA, leaf mass per area; Nc, nitrogen content per dry matter unit; P_G, gross photosynthetic rate; P_{sat}, leaf net photosynthetic rate; R_D, leaf dark respiratory rate; RGR, relative growth rate; RuBPCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBPCO-N, content of nitrogen as RuBPCO per dry matter unit.

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divided into two components—growth respiration and maintenance respiration—as indicated in the equation: $R_D = k\ P_G + cM$, where P_G is the gross photosynthetic rate, M is the dry mass, and k and c are growth and maintenance respiration coefficients, respectively. According to Thornley (1970) and McCree (1974), respiratory substances are used only for maintenance respiration when plants are exposed to darkness for a long time (more than 2 d). To test this hypothesis, Shinano *et al.* (1996) grew rice and soybean plants under natural irradiation and under darkness for 4 d (corresponding to maintenance condition), then ^{14}C-[U]-sucrose was introduced at the tip of the leaf. At 24 h after the introduction of the ^{14}C-[U]-compounds, ^{14}C distribution to organic acids, free amino acids, proteins, sugars, polysaccharides, and respiration was analysed. If the above hypothesis is correct, the ^{14}C-compounds introduced were distributed mainly to the fraction of organic acids and respiration under maintenance status. However, when ^{14}C-sucrose was introduced to rice leaf, the ^{14}C was distributed to sugars, proteins, and polysaccharides even in maintenance conditions (darkness). In other experiment it was assumed that the maintenance metabolism was dominant in the lower (older) leaves, however, the ^{14}C-distribution ratio was similar to that in the upper leaves (new growing). Based on the above results, we suggest that since the ^{14}C-distribution ratio into each chemical component did not change regardless of irradiation or leaf age, it was impos-sible to distinguish the components of growth and main-tenance respiration. Moreover, we did not find a relationship between R_D and P_{sat}, which indicated that the model of McCree does not apply to analysis of long term ontogenetic changes of P_{sat} and R_D in current paper.

In general, it is hypothesised that P_{sat} and R_D are functions of Nc and P_{sat}, respectively. In the present paper, we tried to find the C-N relationship in individual leaves throughout the growth stages in plants. To this end, data on the ontogenetic changes of P_{sat}, R_D, Nc, and saccharide contents were collected and the relationships of these factors were analysed in individual leaves.

Materials and methods

Plants: Rice (*Oryza sativa* L.), winter wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), soybean [*Glycine max* (L.) Merr.], field bean (*Phaseolus vulgaris* L.), potato (*Solanum tuberosum* L.), fodder beet (*Beta vulgaris* L. var. *crassa* Alef.), and tomato (*Lycopersicon esculentum* L.) were planted in duplicate with a complete random design in a field belonging to Hokkaido University at Sapporo, Japan (located at the northern part of Japan, 43°03'N, 141°20'E, altitude 17 m, alluvial soil). Crops were cultivated by conventional farmer's method, of which outline is shown in Table 1.

Measurement of rates of photosynthesis and respiration: To determine leaf position, individual leaves were marked counting from ground level. CO_2 gas exchange rate was measured in individual leaves of one plant by one- or two-week intervals according to crop. P_N was measured by placing an individual attached leaf in a transparent plastic chamber varying in size and connected to differential-type infrared gas analyser (model *LIA-2*, *Hitachi-Horiba*, Tokyo, Japan, for potato, tomato, and field bean; and *KIP 9010*, *Koito Seisakusyo*, Tokyo, Japan, for the remaining crops). P_{sat} was measured at photon saturation: namely, at [μmol m^{-2} s^{-1}] 1 000 to 1 500 (rice, winter wheat, maize, soybean, and fodder beet), 740 (field bean), 1 300 (tomato), and 740 (potato). Leaf was irradiated by a reflection lamp for field bean, tomato, and potato, and by a halogen lamp (*Kenko Co.*, *KTS-100R*) for the remaining crops. In maize, though the photosynthesis was saturated at around 3 000 μmol m^{-2} s^{-1}, due to the limit of the facility, P_{sat} was estimated at around 1 500 μmol m^{-2} s^{-1}, at which P_{sat} is about 90 % of the value at photon saturation. Thus, for all crops examined, P_{sat} was estimated at or near the maximum rate. In the chamber, air temperature was 20 to 25 °C, relative humi-dity 40-50 %, and CO_2 concentration 350-370 g m^{-3}. The air flow rate was 16.7 cm^3 s^{-1} for *LIA-2* and 8.3 cm^3 s^{-1} for *KIP 9010* for the measurement of P_{sat} and R_D. R_D was measured by covering the leaf with aluminium foil after P_{sat} had been measured, and the rate was adjusted at 25 °C, assuming that the Q_{10} value was 2 (James 1953).

The measured whole single leaf blade was sampled. Leaf area was determined using a leaf area meter (*Hayashi Denki Co.*, model *AAC-400*). Then it was dried in an air-forced oven at 80 °C for 48 h to determine leaf dry mass and N content. Leaf mass area (LMA) was calculated as leaf dry mass per leaf area.

Measurement of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO): RuBPCO was extracted according to Osaki *et al.* (1993). 200 mg of lyophilised leaves were homogenised in 100 mM Tris-HCl (pH 7.8), containing 1 mM EDTA, 10 mM mercaptoethanol, 10 mM $MgCl_2$, 1 mM monoiodoacetate, 10 μM leupeptin, and 12 500 cm^3 m^{-3} glycerine, in a chilled mortar with a pestle with acid-washed quartz sand. The homogenate was centrifuged at 3 000×*g* for 15 min at 4 °C. The precipitate was re-extracted two times with 5 cm^3 of the same buffer. Obtained supernatants were mixed, made up to 25 cm^3 with the same buffer, and 1 cm^3 of it was centrifuged at 15 000×*g* for 20 min at 4 °C. Polypeptides in the extracted sample were further separated by SDS-PAGE according to Laemmli (1970). The gel was dried and then the RuBPCO concentration was determined by a densitometric method using *NIH* image software after the gel image was obtained by scanner (*EPSON 7000G*) with purified spinach RuBPCO (*Sigma*) as standard.

Chemical analysis: Nc was determined by the Kjeldahl method (Hind 1993). Sugar was extracted from 200 mg of lyophilised sample by the addition of 25 cm^3 of 80 % hot ethanol, then centrifuged at 6 000×g, and starch was extracted by 30 cm^3 of 30 % perchloric acid using the residue after sugar extraction. Each procedure was repeated 3 times. The solution that contained sugar was concentrated on a water bath (100 °C) to remove ethanol. The contents of sugar and starch were determined colorimetrically by an anthron method (Trevelyan and Harrison 1952), and expressed on a glucose basis.

Statistical analysis: All values were collected primarily by using the duplicate plantings, and were expressed as the average value. Nc after full leafing was adapted in the current paper by the following equation at 1 % significance level:

$$\mathrm{Nc} = (\mathrm{Nc}_0 - \mathrm{Ncd}) \exp(-\mathrm{Nf}\, t) + \mathrm{Ncd} \qquad (1)$$

where Nc$_0$ is the initial value of Nc, Ncd is the Nc of dead leaves, Nf is a coefficient, and t is the number of days after leafing. All values were subjected to statistical analysis by SPSS (1994).

Table 1. Outline of the cultivation conditions for estimating the photosynthetic rate. Data collectors were undergraduate and graduate students in the Laboratory of Plant Nutrition, Faculty of Agriculture, Hokkaido University. Exceptions: *Date of transplanting. **High-yielding cultivars or experimental lines; other cultivars were standard yield cultivars for farmers. ***Ammonium sulfate + coated urea (commercial name: LP coat) + coated N, P, and K (commercial name: Long). Tomato was transplanted in mid June to the field. In field bean additional top dressing with N was used.

Species	Cultivar or line	Hill spacing [cm]	Plant number per hill	Fertiliser N : P_2O_5 : K_2O	Planting date	Year 1992	Collector
Rice	Joiku 404**	30×15	3	(40+80+160)***:200:200	May 26*	1992	T. Kaneda
Winter wheat	Tsukisamu**	20	270 grains per m^2	(20+0+160)***:200:200	Sep. 15 (1991)	1992	T. Kaneda
Maize	Pioneer 3540**	40×30	1	(40+110+200)***:200:200	May 12	1992	T. Kaneda
Soybean	Tsurumusume**	50×20	2	(40+0+160)***:214.5:200	May 18	1992	T. Kaneda
Field bean	Gintebou	25×25	2	(40+20):32:40	May 21	1973	K. Kikuchi
Potato	Norin 1	70×40	1	10:10:10	Apr. 28	1977	K. Kimijima
Tomato	Fukuju No. 2	90×50	1	30:30:30	mid Apr.	1972	K. Kikuchi
Fodder beet	Sugarmangold**	50×25	1	(40+160+200)***:200:200	Apr. 28*	1992	T. Kaneda

Results

Ontogenetic changes of Nc, P_{sat}, and R_{D}: After leaf emergence, Nc decreased gradually with growth, and subsequently Nc decreased exponentially. As this Nc tendency was similar to our previous finding (Osaki 1995), we do not show the values here. Nf (estimated from Eq. 1) was high in maize and soybean, low in tomato, potato, and fodder beet (Table 2).

Table 2. Variation of Nf and Cf among species. Nf of individual leaves was estimated from the Eq. 1 (Nc$_0$ and Nf are constants, significant at 1 % level in all the crops), Cf from Eq. 2 (Cf and P_{sat0} are constants, significant at 1 % level in all the crops). Different letters indicate significant difference (Duncan's test at 5 % level).

Crop	Nf	Cf
Rice	0.0288±0.0038^{a}	0.0298±0.0006^{d}
Winter wheat	0.0291±0.0032^{b}	0.0190±0.0032^{a}
Maize	0.0470±0.0042^{d}	0.0606±0.0030^{e}
Soybean	0.0463±0.0090^{e}	0.0253±0.0019^{b}
Field bean	0.0360±0.0043^{c}	0.0188±0.0010^{a}
Tomato	0.0326±0.0013^{b}	0.0200±0.0025^{c}
Potato	0.0227±0.0013^{a}	0.0166±0.0010^{a}
Fodder beet	0.0175±0.0009^{a}	0.0149±0.0007^{a}

Generally, P_{sat} was low at the leafing, and increased with growth until the full leaf expansion was reached (Fig. 1). After P_{sat} had reached its maximum value, or after the first measurement of P_{sat}, it decreased continuously with growth. For example, P_{sat} was low at high Nc (at a very early stage) at L_1, L_3, and L_6 in field bean; at L_{12} and L_{18} in fodder beet; and at L_{10}, L_{15}, and L_{20} in potato.

On the other hand, the value of R_{D} was generally high in the early growth stage of leaves, then decreased (Fig. 2). However, in some crops or at some leaf positions, R_{D} increased slightly at a late growth stage of leaves. Thus, the changes of P_{sat} were significantly different from the changes of R_{D}, indicating that it is difficult to derive a simple relationship between P_{sat} and R_{D} in individual leaves.

Relationship between P_{sat} and Nc or saccharide content: At the very early leaf expanding stage, P_{sat} was negatively correlated with Nc (values in parentheses in Fig. 3). However, after full leaf expansion, P_{sat} was positively correlated with Nc as follows:

$$P_{\mathrm{sat}} = \mathrm{Cf}\,\mathrm{Nc} + P_{\mathrm{sat0}} \qquad (2)$$

where Cf and P_{sat0} are coefficients.

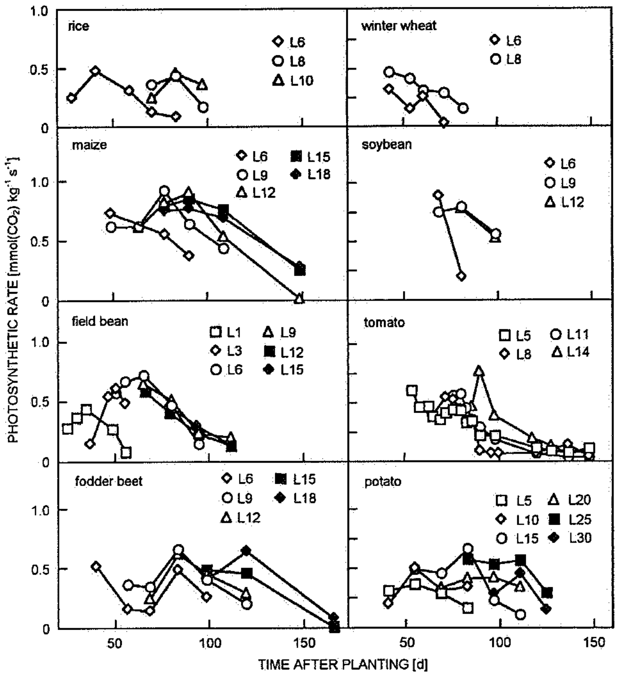

Fig. 1. Changes in photosynthetic rate of individual leaves at successive growth stages. The L numbers in the figure indicate leaf position counted from the bottom. Time after transplanting in rice, and time after wintering in winter wheat.

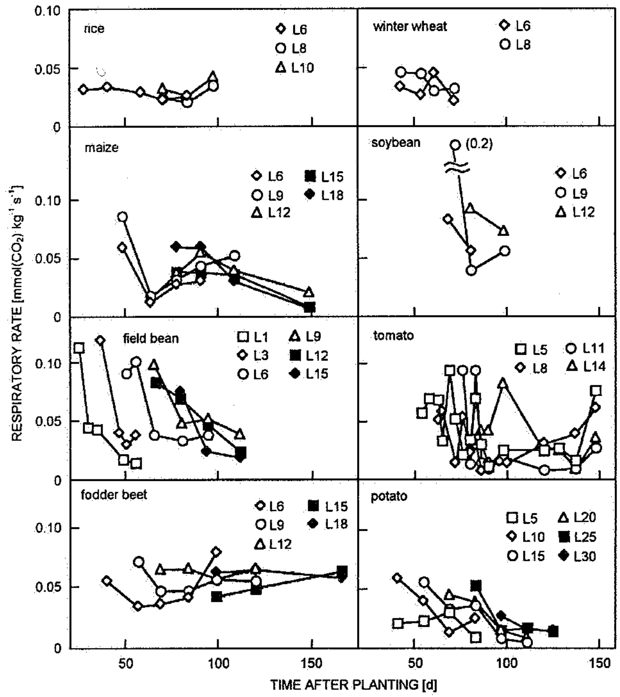

Fig. 2. Changes in respiratory rate of individual leaves at successive growth stages. The L numbers in the figure indicate leaf position counted from the bottom. Time after transplanting in rice, and time after wintering in winter wheat.

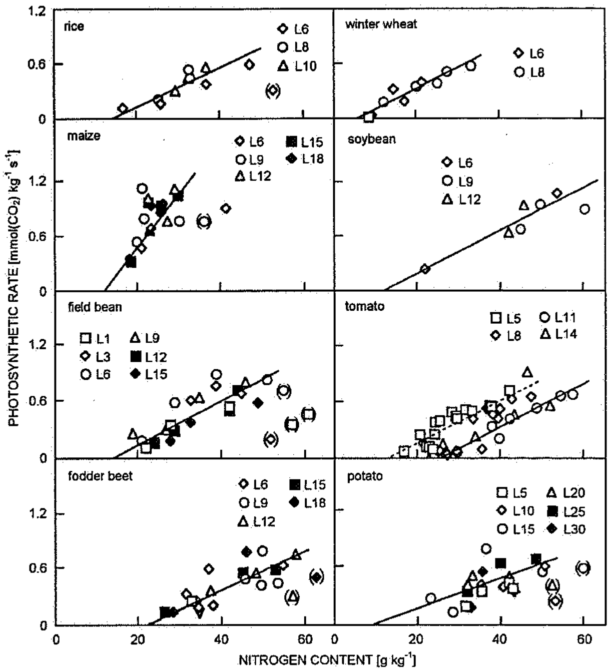

Fig. 3. Relationship between photosynthetic rate and nitrogen content. Values in parentheses (corresponding to the N content at very early leafing) were omitted for estimating the regression between the photosynthetic rate and nitrogen content for the reasons mentioned in the text. The L numbers in the figure indicate leaf position counted from the bottom. Regression coefficients are in Table 2.

The P_{sat}-Nc relation remained constant regardless of leaf position except for tomato (Fig. 3). Presumably because we expressed P_{sat} and Nc on a dry mass basis and not on a leaf area basis. If P_{sat} and Nc were expressed on a leaf area basis, the Cf value would differ considerably among the leaf positions (values not shown). Thus the effect of LMA on the Cf value was cancelled, because the P_{sat}-LMA relationship varied among the leaf position or with leaf ageing (Fig. 4). Therefore, we estimated Cf of each crop from all the data including possible leaf positions. The Cf variation among leaf positions was negligible in spite of the difference in growth condition as indicated by correspondingly low SE values (Table 2). The Cf value was very low in tomato, potato, and fodder beet, intermediate in rice, winter wheat, soybean, and field bean, and very high in maize. Since Cf was different among the crops, inter-specific differences in P_{sat} could not be explained solely by the function of Nc. Other factors such as sugar and/or starch contents in leaves were not related to P_{sat} (Fig. 5).

Relationship between P_{sat} and concentration of N in RuBPCO: Although P_{sat} was related to RuBPCO-N except in maize (Fig. 6), the regression coefficient was remarkably different among the C_3 crops. The regression

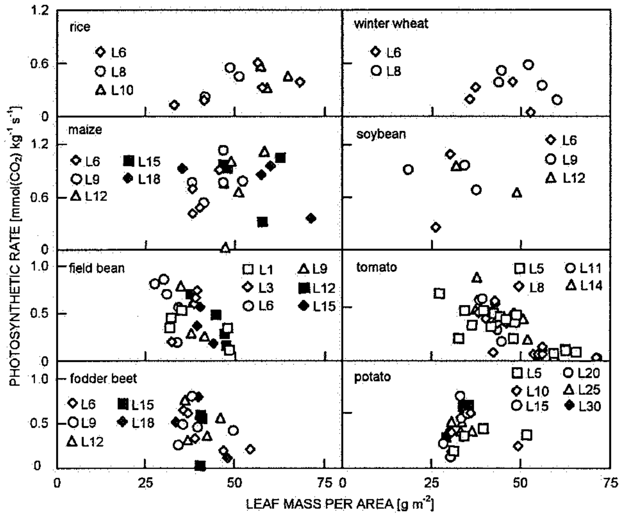

Fig. 4. Relationship between photosynthetic rate and leaf mass per area. The L numbers in the figure indicate leaf position counted from the bottom.

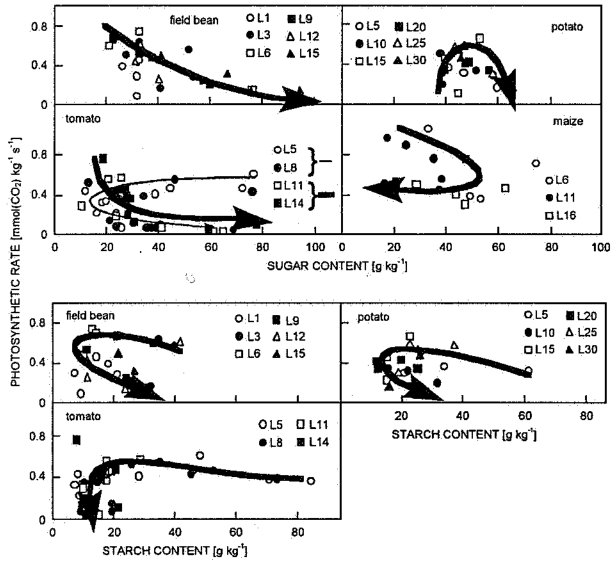

Fig. 5. Relationship between photosynthetic rate and sugar, starch, and nitrogen contents. *Arrows* indicate the progression of growth from leafing to senescence of individual leaves.

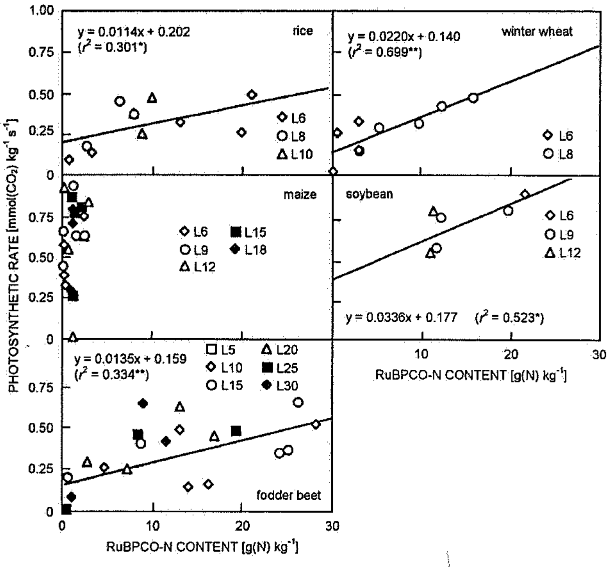

Fig. 6. Relationship between photosynthetic rate and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) based on nitrogen content. **significant at 1 % level, *significant at 5 % level.

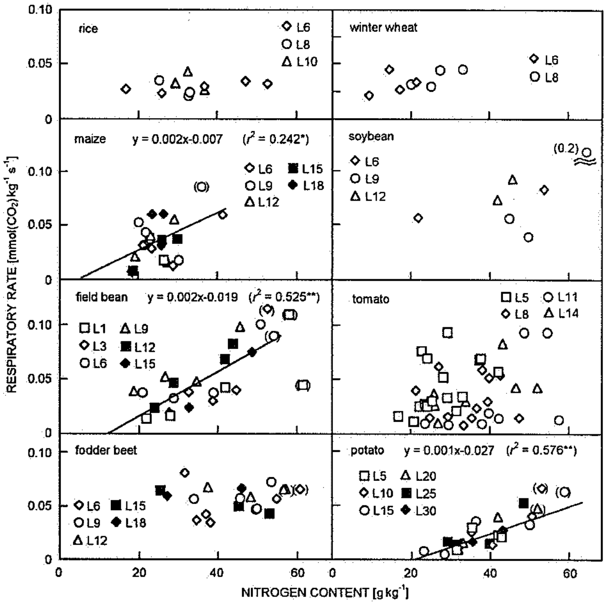

Fig. 7. Relationship between respiratory rate and nitrogen content (values corresponding to at very early leafing). **significant at 1 % level, *significant at 5 % level.

coefficient was high in soybean, medium in winter wheat, and low in rice and fodder beet. Thus the difference in the P_{sat}-Nc relationship among the crops was not associated with RuBPCO-N.

Relationship between R_{D} and Nc or saccharide content: R_{D} decreased significantly with the decrease of Nc in maize, field bean, and potato (Fig. 7). In tomato, R_{D} decreased with the decrease of Nc to approximately 30 g(N) kg^{-1}, then increased with the decrease of Nc. In fodder beet, R_{D} increased slightly with the decrease of Nc. Thus, R_{D} among crops did not show a consistent relationship with Nc.

R_{D} was not explained by a pattern of simple regression in the case of sugar or starch contents (Fig. 8). Since the starch concentration increased with the decrease of Nc and P_{sat}, starch probably accumulated due to the low rate of saccharide translocation, especially during the late growth stage of leaves (starch accumulation was estimated from Figs. 1, 2, and 5). Since the decrease of

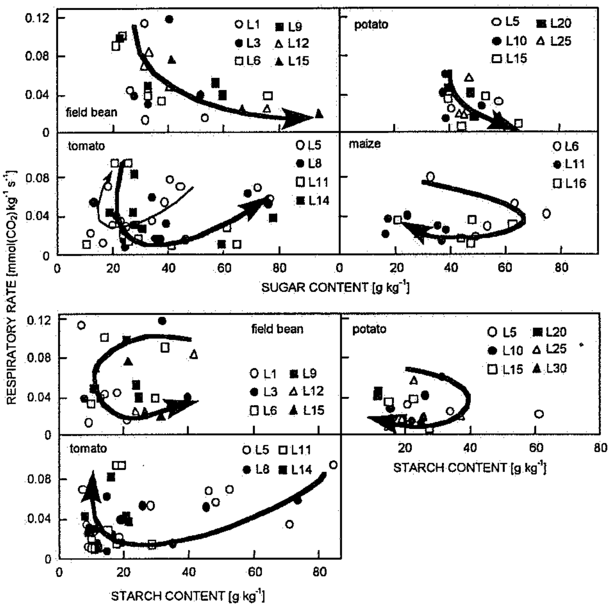

Fig. 8. Relationship between respiratory rate and sugar or starch contents. *Arrows* indicate progression of growth from leafing to senescence of individual leaves.

Nc was caused by the degradation of protein from leaves, it indicates that respiration, which utilises storage substances, was promoted by the decrease of Nc in some crops.

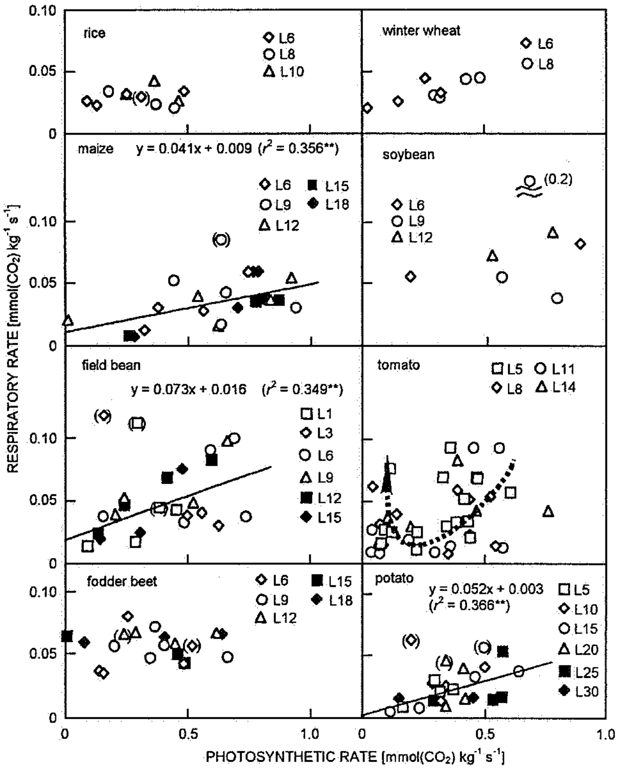

Fig. 9. Relationship between photosynthetic rate and respiratory rate (values corresponding to at very early leafing). **significant at 1 % level, *significant at 5 % level.

Relationship between R_D and P_{sat}: If values at very early leafing stage were eliminated as shown in Fig. 3, R_D was positively correlated with Nc in maize, field bean, and potato (Fig. 9). In rice, winter wheat, soybean, and fodder beet, R_D remained constant regardless of P_{sat}. In tomato, at the early stage, R_D decreased with the decrease of P_{sat}, then remained constant, and finally increased with the decrease of P_{sat}.

Discussion

Regulating factors of leaf P_N: At the early leafing stage, Nc was high, while P_{sat} was very low due to low contents of chlorophyll and RuBPCO (Osaki *et al.* 1993, 1995a). In the current study, P_{sat} increased with the decrease of Nc during the leafing stage (values in parentheses of Fig. 3), but after the leafing stage they decreased linearly with the decrease of Nc. At the early leafing stage, the leaf mass was too small to evaluate P_{sat}; values at this stage can therefore be omitted from discussion on productivity. Thus, based on the ontogenetic changes of P_{sat} and Nc, a general model of the P_{sat}-Nc relationship was derived (Eq. 2) (Fig. 3).

LMA, a parameter related to the amount of air space of leaf (Koike 1988), regulates the diffusion rate of CO_2 (Nobel 1977, Björkman 1981). In previous studies; leaf mass per area (LMA) increased with the progression of growth, indicating a positive relationship between LMA and P_{sat} (Jurik 1986, Oren *et al.* 1986). However, this relationship was not applicable when data on various tree species were compiled (Koike 1988). In the current report, P_{sat} was not related to LMA (Fig. 4), indicating that at least in field crops, the difference of P_{sat} per unit of Nc (estimated from Cf) cannot be explained by the leaf structure, particularly by its thickness. On the other hand, the low distribution ratio of total N to RuBPCO-N tended to give a low regression coefficient of P_{sat}-Nc (*e.g.*, Evans and Seemann 1989), and that the distribution ratio of total N to RuBPCO-N may account for the fact that the P_{sat}-Nc regression varied among species. However, in our observations the coefficient of regression between P_{sat} and RuBPCO-N was markedly different among the species (Fig. 6). This means that Cf differences among crops were not ascribed to the N distribution to functional proteins and that chlorophyll content (which forms the light-harvesting-pigment-protein complex) did not explain the Cf difference among crops. Other factors such as sugar and/or starch contents in leaves are probably related to P_{sat} (Krapp and Stitt 1995), while P_{sat} in the current report was not related at all to the sugar or starch contents (Fig. 5). Although it was difficult to determine the physiological factors responsible for the difference in Cf among species, we assumed that Cf is primarily regulated by the efflux rate of C and N compounds from leaves, as discussed by Osaki and Shinano (2001).

Since Nc in individual leaves decreased with the progress of growth, it was supposed, based on ontogenetic factors (Tanaka 1961), that N in old leaves is re-translocated to newly growing organs. The Nc change after leaf expansion is described in Eq. 1. These facts indicate that Nc is strictly regulated by inherited ontogenetic programs in leaves. On the other hand, since leaf senescence is accelerated by shading or mutual shading (Hirose *et al.* 1988, 1989), it is assumed that N re-translocation is also affected by environmental factors. In the current report, Nf was very similar among leaf positions, except for legume crops (soybean and field bean). This suggests that Nf is regulated more by ontogenetic factors than by environment, based on the following: (*1*) SE value of Nf among the leaf positions was low (Table 2). (*2*) Irradiation of each leaf changed considerably according to canopy growth. Our previous results (Osaki *et al.* 1995b) also support this assumption, because even if the sink (ears) of cereal crops is cut, N continues to be translocated from leaves to stem. The accumulation of N in the stem indicates that N translocation from leaves of cereal crops is regulated mainly by an autonomous mechanism in leaves.

Regulating factors of R_D: R_D is related to P_{sat} or Nc (*e.g.*, Amthor 1989). Moreover, it is often suggested that saccharide content is related to R_D (*e.g.*, Azcón-Bieto and Osmond 1983, Azcón-Bieto *et al.* 1983, Hrubec *et al.* 1985, Baysdorfer *et al.* 1987, Williams and Farrar 1990, Farrar and Williams 1991), but several reports indicated no clear relationship between the saccharide content and R_D (Challa 1976, Farrar 1980, Journet *et al.* 1986, Brouquisse *et al.* 1991, Douce *et al.* 1991, Noguchi *et al.* 1996). We found that saccharide (sugars and/or starch) content was not the determinant factor (Fig. 8). According to McCree (1974), $R_D = k P_G + cM$, where P_G is gross photosynthetic rate, k and c are constants, and M is dry matter. P_G/M is expressed as $P_{sat} + 0.5 R_D$, assuming that R_D during a light period (half day) is the same as that during a dark period. Therefore, the above equation can be rewritten as $R_D = k/(1 - 0.5 k) P_{sat} + c/(1 - 0.5 k)$, which was discussed by Hesketh *et al.* (1971). If this equation can be applied to ontogenetic data, R_D must be related linearly to P_{sat}. However, a significant relationship of R_D and P_{sat} was found in maize, field bean, and potato, while there was no significant relationship in other crops (Fig. 9).

Thus, in rice, winter wheat, soybean, tomato, and fodder beet, R_D was not explained by P_{sat} or Nc because R_D remained constant or increased with the decrease of P_{sat} or Nc (Figs. 7 and 9). R_D/P_{sat} will increase with the decrease of Nc because of contribution of storage substance or reconstruction of structural compounds at late growth leaf stage. Consequently, it is assumed that R_D in leaves is not regulated solely by photosynthesis (substrate supply), and cannot therefore be represented by the equation of McCree (1974) for all species. Also, R_D had significant relationship with Nc only in maize, field bean, and potato (Fig. 7), which relationship was similar with the relationship between R_D and P_{sat}. Therefore, we tested various models on R_D/P_{sat} and Nc parameters. Among these models we found that the R_D/P_{sat} ratio of various crops increased exponentially with the decrease of Nc, the R_D/P_{sat} ratio could be described by the following equation:

$$R_D/P_{sat} = a \exp(b\ Nc) \qquad (3)$$

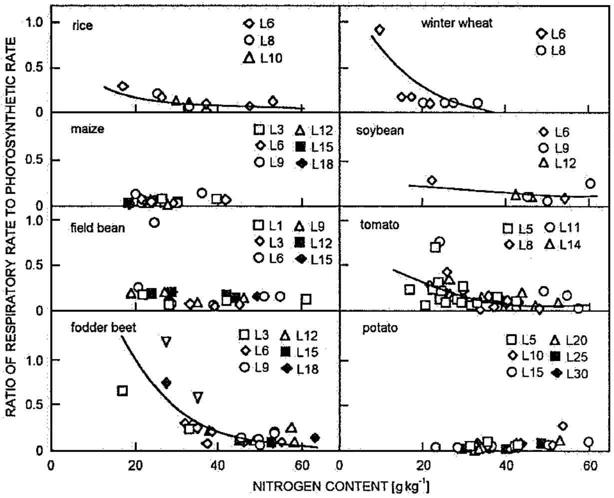

Fig. 10. Relationship between nitrogen content and ratio of respiratory rate to photosynthetic rate. Results of statistic analysis are shown in Table 3.

Table 3. Relationship between R_D/P_{sat} ratio and nitrogen content (Nc). Constants a and b were estimated from the equation R_D/P_N = a exp(b Nc). *significant at 5 % level, **significant at 1 % level.

		a	b	r^2
Species	Rice	0.2703	-0.0271	0.353*
	Winter wheat	2.3724	-0.1217	0.663**
	Maize	0.0557	-0.0280	0.001
	Soybean	0.2763	-0.0173	0.122
	Field bean	0.1507	-0.0068	0.026
	Tomato	0.6281	-0.0452	0.258**
	Potato	0.2100	-0.0112	0.326*
	Fodder beet	1.7837	-0.0479	0.449**
Families	C_3-*Gramineae*	0.6489	-0.0536	0.449**
	C_4-*Gramineae*	0.0557	-0.0280	0.001
	Leguminosae	0.1595	-0.0074	0.032
	Solanaceae	0.3250	-0.0287	0.266*
	Chenopodiaceae	1.7837	-0.0479	0.449**

where a and b are coefficients (Fig. 10). R_D, then, could be estimated from the following equation:

$$\ln(R_D) = \ln(a\,P_{sat}) + b\,Nc \qquad (3').$$

Thus, the regulation of R_D by P_{sat} and Nc is essential in rice, winter wheat, soybean, tomato, and fodder beet. Coefficients a and b were estimated among crops (Table 3). The regression coefficient b was markedly different among the species. However, in maize, field bean, and potato, the regression was not significant in this equation (Table 3). At the late stage of leaf development, owing to the low rate of saccharide translocation, the starch content may increase with the decrease of Nc, and protein is degraded due to re-translocation from leaves. This suggests that the usage of storage substances will be enhanced by the decrease of Nc. Therefore, R_D/P_{sat} increased with the decrease of Nc in many crops except for maize, field bean, and potato.

Consequently, P_{sat} of each species was explained by Nc function. R_D was explained by Nc (or P_{sat}) function in maize, field bean, and potato, and by P_{sat} and Nc function in rice, winter wheat, soybean, tomato, and fodder beet, because storage substance contribution was presumably different among crops.

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