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 ($P_{\text{sat}}$) and dark respiration ($R_0$) of individual leaves were examined in relation to nitrogen content (Nc) in rice, winter wheat, maize, soybean, field bean, tomato, potato, and beet. $P_{\text{sat}}$ was positively correlated with Nc as follows: $P_{\text{sat}} = C_f \cdot Nc + P_{\text{sat}0}$, where $C_f$ and $P_{\text{sat}0}$ are coefficients. The value of $C_f$ 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-bisphosphate carboxylase/oxygenase (RuBPCO) content. $R_0$ was explained by $P_{\text{sat}}$ and/or Nc, however, two models must be applied according to plant species. $R_0$ related linearly with $P_{\text{sat}}$ and Nc in maize, field bean, and potato as follows: $R_0 = a \cdot P_{\text{sat}} + b$, or $R_0 = a' \cdot Nc + b'$, where $a$, $a'$, $b$ and $b'$ are coefficients. In other species, the $R_0/P_{\text{sat}}$ ratio increased exponentially with the decrease of Nc as follows: $R_0/P_{\text{sat}} = \text{a exp}(b \cdot Nc)$, where $a$ and $b$ are coefficients. Therefore, $R_0$ in these crops was expressed as follows: $\ln(R_0) = \ln(a \cdot P_{\text{sat}}) + b' \cdot Nc$, indicating that $R_0$ in these crops was regulated by both $P_{\text{sat}}$ 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 ($P_{\text{sat}}$) has often been reported (Gulmon and Chu 1981, Field and Mooney 1986, Hirose and Kitajima 1986, Evans 1989). Moreover, $P_{\text{sat}}$ 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 $P_{\text{sat}}$ 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 $P_{\text{sat}}$ and leaf Nc remains constant. However, the $P_{\text{sat}}$-Nc relation varies with growth stages (Murata 1961, Hayami 1982) and species (Evans and Seemann 1989). Thus $P_{\text{sat}}$ 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 $P_{\text{sat}}$ is negatively correlated with leaf longevity (Chabot and Hicks 1982, Koike 1988).

Leaf dark respiration rate ($R_0$) is also an important factor in the regulation of the C balance in leaves. Plant $R_0$ 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. $R_0$ can be related to Nc of leaf tissue (Connor et al. 1993), but Byrd et al. (1992) showed that $R_0$ is not correlated with Nc. $P_{\text{sat}}$ is generally in a positive correlation with $R_0$ (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 $P_{\text{sat}}$. According to McCree (1974), $R_0$ of whole plants can be...
divided into two components—growth respiration and maintenance respiration—as indicated in the equation: 
\[ R_D = k P_O + c \], where \( P_O \) 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 \(^{14}\text{C}[-\text{U}]-\text{sucrose} \) was introduced at the tip of the leaf. At 24 h after the introduction of the \(^{14}\text{C}[-\text{U}]\)-compounds, \(^{14}\text{C} \) distribution to organic acids, free amino acids, proteins, sugars, polysaccharides, and respiration was analysed. If the above hypothesis is correct, the \(^{14}\text{C} \)-compounds introduced were distributed mainly to the fraction of organic acids and respiration under maintenance status. However, when \(^{14}\text{C} \)-sucrose was introduced to rice leaf, the \(^{14}\text{C} \) 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 \(^{14}\text{C} \)-distribution ratio was similar to that in the upper leaves (new growing). Based on the above results, we suggest that since the \(^{14}\text{C} \)-distribution ratio into each chemical component did not change regardless of irradiation or leaf age, it was impossible to distinguish the components of growth and maintenance 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 \( N_C \) 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 \( P_{sat} \), \( R_D \), \( N_C \), 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. \( \text{CO}_2 \) 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 Seisakusho, Tokyo, Japan, for the remaining crops). \( P_{sat} \) was measured at photon saturation: namely, at \([\mu \text{mol} \text{ m}^{-2} \text{ s}^{-1}] \) 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 \( \mu \text{mol} \text{ m}^{-2} \text{ s}^{-1} \), due to the limit of the facility, \( P_{sat} \) was estimated at around 1 500 \( \mu \text{mol} \text{ m}^{-2} \text{ s}^{-1} \), 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 humidity 40-50 %, and \( \text{CO}_2 \) concentration 350-370 g m\(^{-3} \). The air flow rate was 16.7 cm\(^2\) s\(^{-1} \) for LIA-2 and 8.3 cm\(^2\) 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 monooiodoacetate, 10 \( \mu \text{M} \) leupeptin, and 12 500 cm\(^{-3} \) glycine, 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 3 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.