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Changes of photosynthetic characteristics in relation to leaf senescence in two maize hybrids with different senescent appearance

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Abstract

A field experiment was conducted to investigate the changes in chlorophyll (Chl) and nitrogen (N) contents, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) and phospho*enol*pyruvate carboxylase (PEPC) contents and PEPC activity, and the photon-saturated net photosynthetic rate (P_{Nsat}), and their relationships with leaf senescence in two maize hybrids with different senescent appearance. One stay-green (cv. P3845) and one earlier senescent (cv. Hokkou 55) hybrid were used in this study, and we found that Chl and N contents and the P_{Nsat} in individual leaves of P3845 were greater than those in corresponding leaves of Hokkou 55 at the successive growth stages. In addition, larger contents of RuBPCO and PEPC, and a greater activity of PEPC were observed in P3845. Due to the lower rates of decrease of Chl, RuBPCO, and PEPC amounts per unit of N, and the lower net C translocation rate per unit of N in the stay-green hybrid, leaf senescence was delayed in comparison to the earlier senescent hybrid.

Additional key words: chlorophyll content; nitrogen content; phospho*enol*pyruvate carboxylase; photon-saturated photosynthetic rate; ribulose-1,5-bisphosphate carboxylase/oxygenase.

Introduction

Senescence in plants is a complex, highly regulated process that involves a decline in photosynthesis, dismantling of chloroplasts, degradation of macromolecules such as proteins, nucleic acids, and lipids, loss of chlorophyll (Chl), and mobilisation of nutrients to developing parts of the plant (Smart 1994, Buchanan-Wollaston 1997). Chl degradation is the most obvious visual change, and this is accompanied by losses of membrane lipids and proteins, eventually resulting in cell death.

During grain formation, a large amount of N is translocated from senescing leaves to the grain (Osaki *et al.* 1988a,b,c). The decomposition of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) and phospho*enol*pyruvate carboxylase (PEPC) is the most important component of the N-redistribution process, since in addition to their roles in photosynthetic CO_2 fixation, these enzymes also constitute the major reserve of N available for the developing sink (Hudspeth and Grula 1989, Rajagopalan *et al.* 1994, Osaki *et al.* 1995, Chollet *et al.* 1996, Rogers and Ellsworth 2002). However, little information is available on the changes and differences of RuBPCO and PEPC in leaves of two maize hybrids with different senescent appearance during leaf senescence.

There is a positive correlation between the photon-saturated net photosynthetic rate (P_{Nsat}) and N content during leaf senescence (Hirose and Kitajima 1986, Evans 1989, Bondada and Oosterhuis 1998, Osaki and Shinano 2001), regardless of whether the variation in leaf N was due to the microenvironment, leaf age, or N availability (Reich and Schoettle 1988). Moreover, this relationship is relatively insensitive to differences among species or growth conditions (Field and Mooney 1986), and should be considered universal when expressed per leaf mass (Reich *et al.* 1992). However, the coefficient *c*, which is calculated from the equation: $P_{Nsat} = c\,N + P_{Nsat0}$, where c and P_{Nsat0} are constants, of the P_{Nsat}-N regression in individual leaves differs among species (Evans and Seemann 1989). Therefore, the *c* within the earlier senescent and stay-green maize hybrids is probably also different.

P_{Nsat} is regulated not only by N, but also by other

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factors such as ageing and leaf longevity. A negative relationship between P_{Nsat} and leaf longevity was confirmed (Field and Mooney 1986, Koike 1988, Reich *et al.* 1992, 1995, Wright and Cannon 2001), and longevity of a leaf is intimately related to its N status (Thomas and Rogers 1990, Borrell and Hammer 2000). Mass-based P_{Nsat} and leaf N values are usually positively related to each other and negatively related to leaf thickness/density (low specific leaf area, SLA) and longevity (Reich *et al.* 1991, 1992, 1997, 1998, Haase *et al.* 2000, Craine and Reich 2001). Osaki and Shinano (2001) also found a negative correlation between P_{Nsat} and leaf longevity, and stated that the net C translocation rate (or photosynthetic rate) is closely related to the net translocation rate of N compounds. Therefore, leaf longevity decreases or increases when P_{Nsat} is high or low, respectively, because N is quickly or slowly translocated from leaves in correspondence to a high or low P_{Nsat}. Thus, a great photosynthetic rate results from a great translocation rate of C and N compounds from leaves. However, how leaf senescence is regulated by C translocation within the two different senescent types of maize hybrids remains unclear.

Therefore, the objective of this study was to determine the changes in Chl, N, RuBPCO, PEPC, and P_{Nsat}, and their relationships with leaf senescence in two different senescence types of maize. This should reveal the physiological mechanisms of high yield in the stay-green maize hybrid.

Materials and methods

Plants: Seeds of two maize (*Zea mays* L.) hybrids, the representative stay-green (cv. P3845) and the representative earlier senescent (cv. Hokkou 55), were soaked with distilled water for germination. Three days later, the germinated seeds were sown in the field of the Graduate School of Agriculture, Hokkaido University, Sapporo, Japan (43°03'N, 141°20'E). The earlier senescent and stay-green hybrids are morphologically similar and do not differ in phenology. These two hybrid types have been useful in studying physiological processes contributing to yield increases (He *et al.* 2001, 2002). The plots (54 m^2) were arranged in a randomised complete block design with three replications and a plant density of 66 667 plants per ha, *i.e.* about 6.7 plants per m^2 (space: 0.6×0.25 m). Nitrogen [300 kg(N) ha^{-1} = 30 g(N) m^{-2}, *i.e.* 50 kg N supplied by a rapid release fertiliser, and the other 250 kg N supplied as a slow release fertiliser], phosphorus [200 kg(P_2O_5) ha^{-1}, 20 g m^{-2}], and potassium [200 kg(K_2O) ha^{-1}, 20 g m^{-2}] were applied as basal applications with $(NH_4)_2SO_4$ or urea, superphosphate, and potassium sulfate, respectively.

Individual leaves were sampled at two-week intervals from leafing to senescence, which corresponded to 47, 62, 75, 89, 106, 117, and 132 d after sowing (DAS). For the dry mass and N content determinations, a portion of the leaf samples were dried at 80 °C in an air-forced oven for 72 h, weighed, and ground. The other portion was immediately frozen with liquid N and stored at −80 °C until analysis for RuBPCO and PEPC contents. P_{Nsat} was measured 54, 77, 96, 121, and 133 DAS in the field, and corresponding leaves were taken simultaneously for the measurement of N content.

N and Chl contents: N content was determined using the Kjeldahl method (Hind 1993). For the determination of the Chl content, 0.25 g of fresh leaves were placed in a 100 cm^3 test tube, then 10-15 cm^3 pure methanol was added, and homogenised with a polytron. The homogenate was then filtered and made up to 100 cm^3 with pure methanol. The Chl concentration in the supernatant was spectrophotometrically determined by measuring the absorbances at 652.0 and 665.2 nm for Chl *a* and Chl *b*, respectively, and calculated according to Porra *et al.* (1989).

P_{Nsat}: To determine leaf position, individual leaves were marked from the ground level up. P_{Nsat} was measured in the field at photon saturation by placing the intact leaf in various sized transparent plastic chambers connected to an infrared gas analyser (model *ADC-3*, *Shimadzu*, Kyoto, Japan). Irradiance of about 1 500 μmol m^{-2} s^{-1} was supplied by a halogen lamp (*KTS-100R*, *Kenko Co.*, Tokyo, Japan). The air temperature, relative humidity, and CO_2 concentration in the chamber were 20 to 25 °C, 40-50 %, and 350-370 g m^{-3}, respectively.

RuBPCO and PEPC contents: RuBPCO and PEPC were extracted according to Osaki *et al.* (1993). 200 mg of the fully expanded maize leaves were ground in a chilled mortar with a pestle and acid washed quartz sand under ice-cold conditions and homogenised in 1 cm^3 of the following solution: 100 mM Tris-HCl (pH 7.5) buffer, containing 5 % (v/v) glycerol, 5 % (v/v) ethylene glycerol, 5 mM Na-phosphate, 50 mM NaF, 1 mM $MgCl_2$, 10 mM EDTA, 1 mM iodoacetic acid sodium, 14 mM 2-mercaptoethanol, 2 mM benzamidine-HCl, 1 mM phenylmethylsulfonyl fluoride, 10 μM leupeptin, 10 μg cm^{-3} chymostatin, 1 μM microcystin-LR, and 5 % (m/v) polyvinyl-polypyrrolidone. The homogenate was centrifuged at 15 000×g at 4 °C for 10 min, and then the supernatants were collected for RuBPCO and PEPC content determination. The soluble protein content in the supernatants was determined using the method of Bradford (1976).

Polypeptides in the extracted sample were further separated by SDS-PAGE according to Laemmli (1970). The gel image was then scanned (*EPSON 7000G*) with purified maize RuBPCO and PEPC (*Sigma*) as a standard, and the RuBPCO and PEPC contents were determined with a densitometric method using *NIH ImageJ*

1.24, 2001 software (National Institute of Health, USA).

PEPC activity: 200 mm^3 of supernatant was centrifuged at 15 000×*g* at 4 °C for 5 min, then 25 mm^3 of the supernatant was reacted in a 1 cm^3 mixture containing: 2 units of NAD-malate dehydrogenase, 10 mM Hepes-NaOH (pH 7.5), 10 mM $MgCl_2$, 1 mM $NaHCO_3$, 0.2 mM NADH, 200 mM glucose-6-phosphate, and 100 mM PEP. The PEPC activity was measured spectrophotometrically at 30 °C by coupling the reaction to NADH oxidation mediated by exogenous NAD-malate dehydrogenase at 340 nm in a dual beam UV spectrophotometer (*UV-1600, Shimadzu*, Japan) equipped with a kinetics software package.

Statistical analysis: Standard analyses of variance techniques were used to assess the significance of treatment means and the differences between treatment means were compared using the least significant difference (LSD). The regression was subjected to statistical analysis by *SPSS 10.0* (*SPSS for Windows 1999*).

Results

Chl content in individual leaves: Chl content in the sixth leaf of Hokkou 55 declined from the leafing stage to maturity, while that in P3845 increased after leaf emergence, reaching maximum values 62 DAS, and, thereafter, declined dramatically (Fig. 1). For both hybrids, the Chl content in the 9th and 12th leaves declined with growth. However, the Chl content in the 15th leaf increased after emergence, reaching maximum values 89 DAS and, thereafter, declined gradually with leaf senescence. The Chl content in individual leaves in the stay-green hybrid P3845 were markedly greater than those in corresponding leaves in the earlier senescing hybrid Hokkou 55. At the last measurement, Chl contents in the 6th, 9th, 12th, and 15th leaves in P3845 were 1.29, 2.64, 1.56, and 1.56 times greater than those in Hokkou 55, indicating that Hokkou 55 senesced faster, especially at harvest time.

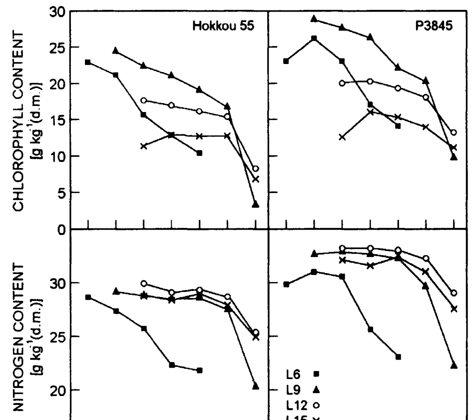

Fig. 1. Changes of chlorophyll and nitrogen contents at successive growth stages. *The L numbers* in the figure indicate leaf position counted from the bottom.

N content in individual leaves: The trend of N content in the 6th leaves of both hybrids was similar to that of the Chl contents, while those for the 9th, 12th, and 15th leaves of both hybrids declined gradually after emergence. At the last measurement, N content in the 6th, 9th, 12th, and 15th leaves of P3845 were 1.07, 1.09, 1.15, and 1.10 times greater than in Hokkou 55. This shows that the differences of N content between the two hybrids were not as large as that of the Chl content (Fig. 1).

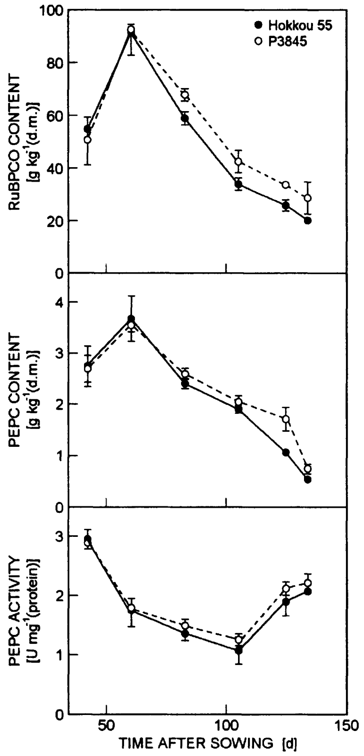

Fig. 2. RuBPCO and PEPC contents and PEPC activity in total leaves of the two maize hybrids.

Contents of RuBPCO and PEPC in whole leaves followed similar trends: both increased rapidly after leaf emergence, reached a maximum around full expansion (61 DAS), and then declined gradually with growth. Larger RuBPCO and PEPC contents were observed in P3845 during the entire growth stage compared with Hokkou 55 (Fig. 2), which benefited photosynthetic CO_2 assimilation. A similar tendency was observed for the contents of RuBPCO and PEPC based on leaf N (values not shown).

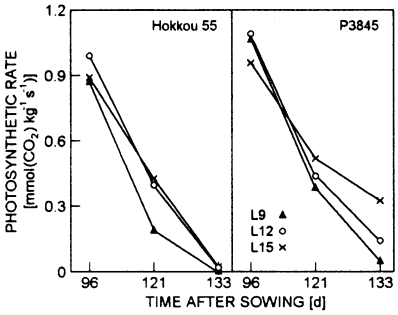

Fig. 3. Changes of P_{Nsat} in individual leaves of the two maize hybrids. The *L numbers* in the figure indicate leaf position, counted from the bottom.

PEPC activity in whole leaves was higher in P3845 than in Hokkou 55 during growth, indicating that the stay-green hybrid could retain a greater photosynthetic capacity, which benefited dry matter accumulation (Fig. 2).

P_{Nsat} in individual leaves (on leaf dry mass basis) in individual leaves in P3845 was greater than in Hokkou 55 during the entire growth stage (Fig. 3). At harvest, P_{Nsat} in the 9th, 12th, and 15th leaves of Hokkou 55 dropped nearly to zero, while those in the 12th and 15th leaves of P3845 remained relatively greater (Fig. 3). A similar trend was observed for P_{Nsat}-based leaf area (values not shown), demonstrating that stay-green hybrids could maintain a larger photosynthetic capacity, especially at harvest time.

Discussion

Senescence is the final stage of development in plants. The loss of Chl is normally apparent to the eye as leaf yellowing, although a series of other biochemical and physiological changes are also involved (Nooden 1988, Matile 1992, Feller and Fischer 1994). Many senescence-related mutants have been found in crop plants that maintain leaf greenness after the grain-ripening stage and are referred to as stay-green or non-yellowing (Thomas and Smart 1993). In this study, the Chl content in P3845 was greater than in Hokkou 55 in the individual leaves during the whole growth stage, showing that the process of leaf senescence in stay-green hybrids was delayed compared with the earlier senescent ones. Since both Chl and N contents decreased with growth, we derived a linear and positive correlation between them within both hybrids (Fig. 4). The higher coefficient of Chl-N in Hokkou 55 indicated that the decreased Chl content per unit of N was greater in the earlier senescing hybrid, which accelerated leaf senescence of Hokkou 55 in comparison to P3845.

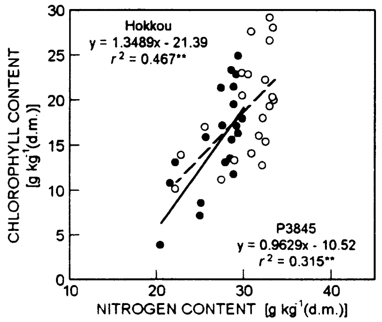

Fig. 4. Correlations between chlorophyll and N contents in canopy leaves of Hokkou 55 (●) and P3845 (○). ** denotes significance at the 0.01 probability level.

RuBPCO and PEPC contents were negatively correlated with N at the very early stage, because they were very low while N content was very high. After leaf expansion, both were positively correlated with N. Coefficients of RuBPCO-N and PEPC-N from the linear equations for Hokkou 55 were greater than those for P3845 (Fig. 5). This indicated that the decreases of both RuBPCO and PEPC contents per unit of N, or N translocation rate per unit of N content, were larger for the earlier senescent hybrid. This could speed up the progress of leaf senescence and not benefit the production of photosynthates during the late growth period.

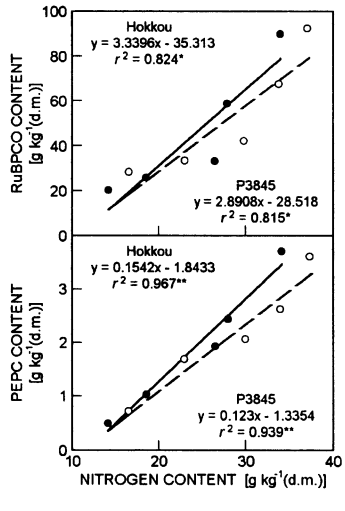

Fig. 5. Correlations of RuBPCO-N and PEPC-N in whole leaves of Hokkou 55 (●) and P3845 (○). * and ** denote significance at the 0.05 and 0.01 probability levels, respectively.

During the early leaf expanding stage, the N content in individual leaves was high, while P_{Nsat} was very low due to low contents of Chl (Fig. 1) and RuBPCO (Fig. 2). Therefore, P_{Nsat} in individual leaves at the early stage was not linearly related to N for both hybrids. After the leafing stage, P_{Nsat} decreased linearly with the decrease of N content, and a linear relationship between P_{Nsat} and N was developed as $P_{Nsat} = c\,N + P_{Nsat0}$ (Fig. 6*A*), where *c* is the coefficient of net carbon translocation rate in the leaf, and P_{Nsat0} is a constant. Fig. 6*A* indicates that *c* in the individual leaves of Hokkou 55 was higher than that of P3845, showing that the decreased P_{Nsat} per unit N in individual leaves was higher in Hokkou 55. Hence the decrease in C translocation rate from lower leaves was larger in the earlier senescent hybrid, which inevitably promoted leaf senescence. Similar results on the coefficients of P_{Nsat}-Chl within the two maize hybrids were observed (Fig. 6*B*), demonstrating that leaf senescence was sped up when the C translocation rate per unit of both N and Chl was high.

In conclusion, higher Chl and N contents and P_{Nsat} were maintained in individual leaves. There was also a higher content of RuBPCO and PEPC, and a higher activity of PEPC was observed in canopy leaves in P3845. This demonstrated that the stay-green hybrids stayed green longer because their leaves contained more N (high contents of Chl, N, RuBPCO, and PEPC) and maintained a greater photosynthetic capacity (high PEPC activity and high P_{Nsat} in leaves). A further investigation found that the lower decreases of Chl, RuBPCO, and PEPC, and the lower C translocation rate per unit of N were also retained, which was related to delayed leaf senescence. Therefore, lower leaf activity contributed to late senescence, which caused greater LAD. However, in the earlier senescent hybrid, the lower Chl and N contents and P_{Nsat}, combined with the larger decrease rates of Chl, RuBPCO, and PEPC, and the greater C translocation rate from the lower leaves to upper leaves resulted in earlier leaf senescence. Hence a novel method using C translocation and leaf activity can be applied to explain the differences in leaf senescence mechanisms between the earlier senescent and stay-green maize hybrids.

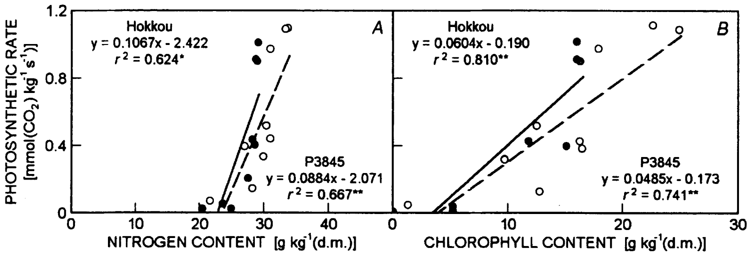

Fig. 6. Correlations of P_{Nsat}-N and P_{Nsat}-Chl in individual leaves of Hokkou 55 (●) and P3845 (○). * and ** denote significance at the 0.05 and 0.01 probability levels, respectively.

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