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Ribulose-1,5-bisphosphate Carboxylase Activity, Chlorophyll and Protein Concentrations in Different *Populus* Clones

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Abstract. Ribulose-1,5-bisphosphate carboxylase activity (RuBPC), chlorophyll (chl) and protein (prot) concentrations and chlorophyll/protein (chl/prot) ratios were determined in five different *Populus* clones together with their maximal net CO_2 uptake rates (P_{max}). A classic reference clone (*Populus* × *euramericana* "Robusta" (DODE) GUINIER) was compared with four recently selected euramerican and interamerican crossings. Chl/prot ratio and RuBPC activity varied among the different clones, while chl *a*/chl *b* ratio showed only a very low coefficient of variation (1.7%) for the five clones. Poplar clone "Robusta" could be distinguished from the recent faster growing clones based on the different biochemical characteristics.

A significant correlation was found between both total chl concentration and chl/prot ratio with P_{max} for the five clones.

Additional index words: chlorophyll *a* / chlorophyll *b* ratio; *Populus trichocarpa* × *Populus deltoides*; net CO_2 exchange rate.

Ribulose-1,5-bisphosphate carboxylase (RuBPC: EC 4.1.1.39) as the primary acceptor in the photosynthetic CO_2 fixation in C_3 plants has been used in many photosynthesis studies (TROUGHTON 1975). While enzyme kinetics and biochemical aspects of the RuBPC carboxylation reaction were studied by COOPER *et al.* (1969) and by LORIMER *et al.* (1977), COOPER *et al.* (1969) — using ^{14}C incorporation technique — proved that CO_2 is indeed the active species of the RuBPC enzyme. Several authors (WAREING *et al.* 1968, DICKMANN 1971, DICKMANN and GORDON 1975, REYSS and PRIOUL 1975) showed a significant correlation between maximum net CO_2 exchange rate (P_{max}) and the specific activity of the RuBPC enzyme.

Different authors (DICKMANN 1971, PIETERS 1974, COOPER 1975, MALKINA 1976, LEWANDOWSKA and JARVIS 1977) also included the total amount of chlorophylls (chl), the chl *a*/chl *b* ratio and the chl/prot ratio in their studies on photosynthetic efficiency, since chlorophylls are the most important photon absorbing and transforming plant pigments involved in the photo-

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synthetic process. A significant correlation between the total chl concentration and P_{max} – mainly at low photon flux densities – has been shown already by GABRIELSEN 1948, RABINOWITCH 1951, LOACH 1967, BHAGSARI 1974. Differences in chl concentrations can be caused as a result of genotypic differences, by decrease in nutrient components, by diseases or by greening of etiolated leaf material (BHAGSARI 1974, COOPER 1975).

It was the main purpose of this study to quantify differences and variations in some biochemical characteristics – as RuBPC activity, chl and prot concentrations – among five different poplar (*Populus*) clones and to check which of these characteristics showed the greatest variation in relation to their P_{max} rates.

MATERIAL AND METHODS

Five one-year-old potted cuttings from five different *Populus* clones (*Populus deltoides* S. 9-2 × *P. nigra* GHOY, clone "Ghoy"; *Populus trichocarpa* V. 235 × *P. deltoides* S. 1.173, clones "Unal" (male) and "Beaupré" (female); *P. trichocarpa* V. 235 × V. 24, clone "Trichobel" and *P.* × *euramericana* (DODE) GUINIER, clone "Robusta") were rooted in a greenhouse. Complete description of the clones with main characteristics was given earlier (CEULEMANS and IMPENS 1980). After one month the plants in 7.5 dm^3 pots were transferred to a controlled environment growth chamber (WEISS phytotron, Germany), with day/night air temperature 22.5/19.0 °C; day/night atmospheric humidity 15. 6/12. 4 $g\,m^{-3}$; photon flux density 310 $\mu mol\ m^{-2}s^{-1}$ and photoperiod 12 h. Photon flux density in the phytotron was provided by 40 white fluorescent PHILIPS lamps and 9 incandescent OSRAM lamps giving a total irradiance of 1580 $W\ m^{-2}$. Plants were watered daily with tap water and twice weekly with a modified Hoagland nutrient solution (GEENEN, personal communication).

Photosynthetic Measurements

P_{max} was measured on single attached leaves of optimal leaf age under saturating photon flux densities using a ventilated gas exchange chamber and an infrared gas analyser (Unor, MAIHAK, Germany) as described earlier (CEULEMANS and IMPENS 1980).

Biochemical Assays

For the biochemical replications leaf material from eight leaves at optimal leaf age (CEULEMANS and IMPENS 1979) were used. The fresh mass and leaf area were measured prior to the enzymatic assays, by means of an analytical balance (accuracy 0.001 g) and an area meter (LAMBDA, type LiCor 185, USA) respectively.

Carboxylase Activity

Crude extracts were prepared by a rapid grinding of the leaf (*ca.* 2 g) in a mortar with 20 ml of a hypotonic buffer (tris-HCl 0.1 M, pH 8.2), containing 1 mM EDTA and 5 mM dithiothreitol. The slurry was filtered through 8 layers of cheese cloth, and the filtrate obtained was used for estimating enzyme activity. All preparative procedures and conservations were carried

TABLE 1
Biochemical leaf characteristics for five different poplar clones grown under controlled environment conditions. RuBPC activity per unit leaf area and per unit weight of protein, chlorophyll and protein concentrations per unit leaf area, chlorophyll/protein and chl *a*/chl *b* ratios. Max. net CO_2 exchange rates are also presented. Mean values of eight replications per clone are shown, as well as standard deviations (in brackets)

Clones	RuBPC [mU cm^{-2}]	RuBPC mU[mg prot]$^{-1}$	chl (a + b) [μg cm^{-2}]	prot [μg cm^{-2}]	chl/prot [$\times 10^{-3}$]	chl a/chl b —	P_{max} [mg CO_2 m^{-2} s^{-1}]
Ghoy	53.5 (8.0)	90.7 (21.1)	26.50 (3.77)	612.3 (152.2)	43.3 (12.4)	2.96 (0.36)	0.49
Unal	34.1 (14.1)	71.4 (21.1)	26.15 (8.31)	468.6 (87.4)	55.8 (20.6)	2.93 (0.31)	0.51
Beaupré	49.2 (7.5)	79.5 (12.6)	27.36 (4.15)	631.7 (102.1)	43.3 (9.6)	3.03 (0.29)	0.52
Trichobel	53.0 (10.5)	93.6 (13.1)	21.02 (3.57)	567.3 (90.5)	37.1 (8.6)	3.00 (0.23)	0.48
Robusta	31.7 (8.7)	48.3 (11.0)	22.01 (5.92)	658.5 (110.4)	33.4 (10.6)	3.06 (0.21)	0.41
Mean	44.3	76.7	24.61	587.7	42.6	3.00	0.48
Stand. deviat.	10.6	18.2	2.88	74.4	8.5	0.05	0.04
Coeff. variat. [%]	23.9	23.7	11.7	12.7	20.0	1.7	9.0

out at 0 °C. RuBPC activity was measured according to LILLEY and WALKER (1974). Absorption changes at 340 nm were measured on a VARIAN U.V. Spectrophotometer (type DB 635).

Chlorophyll and Protein Analyses

100 µl leaf extract were solved in 4.9 ml acetone 80% (v/v) for chl analyses and 500 µl in 9.5 ml acetone 85% (v/v) for prot analyses. The homogenate was centrifuged for 10 min in the dark at 0 °C and at 1000 *g*. Chl analyses were further performed according to MC KINNEY (1941). The method of LOWRY *et al.* (1951) was utilised for prot analysis.

RESULTS AND DISCUSSION

RuBPC activities were expressed per unit of leaf area as well as per unit weight of prot (Table 1). Specific RuBPC activity (*i.e.* enzyme activity expressed per unit of weight of prot) of clone "Robusta" was significantly different (at $P = 0.05$) from these of the four other — inter-American and Euro-American — crossings. Since most of the literature data on RuBPC activities were expressed in specific activities of the ^{14}C, it was rather difficult to compare our data with those from literature. Nevertheless, the specific activities reported here for poplar were of the same order of magnitude as those obtained by VAN ASSCHE *et al.* (1979) for *Phaseolus*.

In this study, no significant linear correlation (at $P = 0.01$) was found between P_{max} at optimal leaf age and RuBPC activity expressed per unit of leaf area. In contrast, a weak significant linear correlation (at $P = 0.01$) was found between P_{max} and the specific enzyme activity, as described by others in the past.

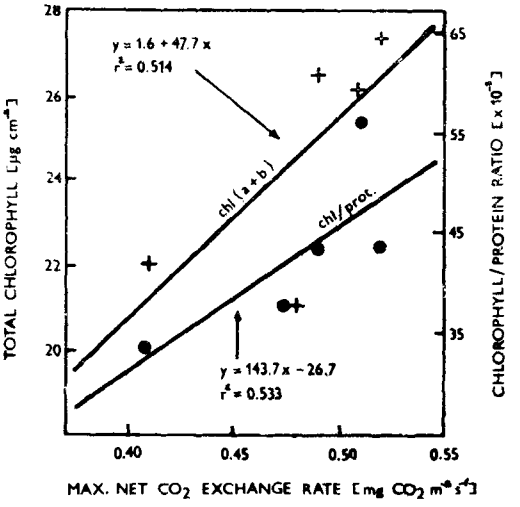

Fig. 1. Total chlorophyll (*a* + *b*) concentration (+) and chlorophyll/protein ratio (●) *versus* maximal net CO_2 exchange rate for five different poplar clones. Mean values of eight replications per clone are presented. Chlorophyll concentrations were expressed per unit leaf area. Linear relationship is significant at the 0.1 and 0.2 level, respectively.

In contradiction to REYSS and PRIOUL (1975), who found a very significant correlation between RuBPC and carboxylation resistances to CO_2 for plants under different light regimes, these two parameters were not correlated for the five poplar clones studied here. Most probably this is due to the fact that the carboxylation resistance has a broader meaning than assumed and is quite difficult to calculate. With a clonal mean value of 24.6 $\mu g\ cm^{-2}$, chl concentrations for the five poplar clones were in the same order of magnitude as the values for many other species cited by ŠESTÁK (1977) and as the data for cottonwood by DICKMANN (1971) and HERNANDEZ-GIL and SCHAEDLE (1972). LOACH (1967) obtained for *Populus tremuloides* slightly higher values (*ca.* 40 $\mu g\ cm^{-2}$). Prot concentrations reported here were also in good agreement with those of DICKMANN amd GORDON (1975) and DICKMANN *et al.* (1975) for poplar and those of VAN ASSCHE *et al.* (1979) for beans. No significant correlation was found between P_{max} and prot concentration for the different clones.

The chl *a*/chl *b* ratio had nearly the same value as this reported by DICKMANN (1971).

A significant correlation (at $P = 0.01$) was found between total chl concentration and P_{max} as well as between chl/prot ratio and P_{max} (Fig. 1). A direct relationship between chl content and photosynthesis in developing leaves of cottonwood has been shown by DICKMANN (1971), while HOLMGREN *et al.* (1965) stated that the chl/prot ratio gives an indication of the shade adaptation of the plants. The internal resistance for carbon dioxide at photon flux density saturation of the different poplar clones was not significantly correlated either with the total chl concentration or with the chl *a*/chl *b* ratio.

No correlations were found between chl/prot ratio and either internal mesophyll resistances. A very tight correlation existed between P_{max} and the internal resistances, but this relationship is obvious and inherent to the method of resistance calculation.

For all the biochemical characteristics studied, poplar clone "Robusta" showed the most extreme values compared to the other, more recently selected Euro-American and inter-American clones. Indeed, clone "Robusta" showed the lowest RuBPC activity, the lowest chl/prot ratio, but far the highest total prot concentration and chl *a*/chl *b* ratio. Resulting P_{max} was also lowest for this clone, which could be correlated with lower growth potential.

In conclusion, in the five clones studied RuBPC activities and chl/prot ratios showed the highest variation, while chl *a*/chl *b* ratio and P_{max} differed only slightly between the five clones.

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