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The photosynthetic characteristics of differently shaped leaves in *Populus euphratica* Olivier

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Abstract

There is a distinct leaf shape polymorphism within a single plant of *P. euphratica* Olivier. The anatomical structure, carbon isotope discrimination ($\Delta^{13}C$), and stomatal and photosynthetic behaviour were investigated in broad-ovate (BOL) and lanceolate (LL) leaves, located at the top and bottom in crown, respectively, of a mature Euphrates poplar growing in its native habitat. Both types of leaves had a non-Kranz anatomy and low $\Delta^{13}C$ values. However, $\Delta^{13}C$ of a LL was in average 3.2 ‰ larger than that of a BOL. In comparison with the LL, the BOL had a smaller stomatal conductance, causing subsequent decreases in transpiration rate and ratio of CO_2 concentrations in intercellular spaces to air. Carbon assimilation rate and water use efficiency were higher in the BOLs than in the LLs. The BOL exhibited C_4-like enzymological features, the activity of glycollate oxidase, and the ratio of activities of ribulose-1,5-bisphosphate carboxylase (RuBPC) to phosphoenolpyruvate carboxylase (PEPC) was lower in BOL than in LL throughout the whole growing season. The lowered ratio of RuBPC/PEPC in BOL was mainly associated with a marked decline in the activity of RuBPC, and only a slight increase in the activity of PEPC. These differences might contribute to microclimate adaptation in both types of leaves.

Additional key words: broad-ovate leaf; $\Delta^{13}C$; glycollate oxidase; lanceolate leaf; leaf morphology and anatomy; phosphoenolpyruvate carboxylase; polymorphism; poplar; ribulose-1,5-bisphosphate carboxylase/oxygenase.

Introduction

With differences in their origin and distribution, higher plants show diverse pathways of photosynthetic CO_2 fixation. Meanwhile, to date relatively limited studies have demonstrated the flexibility of leaf photosynthetic modes even within an individual

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plant. For instance, the C_4 pathway exists in the young leaves of tobacco at an early growth stage (Kisaki *et al.* 1973). In *Mollugo nudicaulis*, the C_3, C_3-C_4, and C_4 pathways are performed in leaves of different position (Raghavendra *et al.* 1978). The simultaneous occurrence of C_3 and C_4 photosynthesis is also observed in various C_4 species (Khanna and Sinha 1973, Crespo *et al.* 1979). Varied photosynthetic modes in the leaves of a single plant may be associated with leaf microclimate differences which result from changes in the leaf position or developmental period (Farquhar *et al.* 1989). In addition, since there is now a general agreement that the C_4 and C_3-C_4 syndromes evolved from the primitive C_3 pathway, perhaps the photosynthetic mode divergence during the leaf ontogeny will contribute to an understanding of this complex process (Troughton 1971).

Euphrates poplar, one of the oldest species of *Populus* in *Salicaceae*, is the sole species of the genus naturally growing in deserts. The tree is mainly dispersed over the middle part of Asia, and it is important in the vegetation systems of the desert regions' riversides and oases. Euphrates poplar is characterized by a great resistance to drought, salinity, high irradiance and temperature, and wind. There is a distinct leaf shape polymorphism from the lower to upper crown of the tree, so it is also described as the heterophyllous poplar. In this paper we examined the anatomical structures, carbon isotope composition, gas exchange patterns, and activities of enzymes in C_3 and C_4 photosynthesis and photorespiration in differently shaped BOL and LL types, located at the top and the bottom, respectively, of mature Euphrates poplar's crown.

Materials and methods

Plant and location: A mature tree of *P. euphratica* Olivier, naturally growing in the Academia Sinica's Desert Research Institute Area (39°31'-58'N, 100°4'-36'E; 1300 m elevation), was selected for the study. The region is a typical desert: the mean annual precipitation is 118 mm while the annual potential evaporation is 2392 mm. The air temperature has large daily fluctuations with the annual maximum temperature being 39 °C and the minimum -27 °C. The northwest wind is frequent throughout the year. The relative air humidity usually does not exceed 50 % (Wang *et al.* 1995).

Gas exchange: During the growing season of 1994, diurnal courses (06:00-20:00) of the net photosynthetic rate (P_N), air and intercellular CO_2 concentrations (c_a, c_i), stomatal conductance (g_s), leaf temperature (T_L), and transpiration rate (E) of the youngest fully expanded BOL and LL (Fig. 1), located at the top and the bottom of this tree's crown, respectively (vertical distance roughly 4.5 m), were individually measured at an interval of 2 h on 20 May, 25 July, and 8 September with a *LI-6000* Portable Photosynthesis System (*Li-Cor*, Lincoln, USA). Environmental parameters, namely the air temperature (T_A), relative air humidity (RH), photosynthetically active radiation (PAR), at the top and the bottom of the tree's crown were simultaneously obtained by the used instrument. The air vapour pressure difference (VPD) and water use efficiency (WUE) were calculated from the values of T_A, RH, P_N, and E.

Leaf anatomy: Leaf samples for the anatomical study were cut into small sections and fixed in 5 % (v/v) glutaraldehyde/50 mM sodium phosphate buffer, pH 7.3. The fixed leaves were dehydrated through a graded ethanol series, embedded in paraffin, and sectioned with glass knives on a rotary ultramicrotome. The sections were stained with safranin and fast green, then viewed and photographed with an *Olympus BH-2* microscope (*Olympus Optical*, Japan).

Fig. 1. Morphology of broad-ovate (*left*) and lanceolate (*right*) leaves in Euphrates poplar.

Carbon isotope composition: 40 leaves from each sample were oven-dried at 80 °C and then ground to a fine powder (cf. O'Leary 1981). Subsamples were combusted, and relative abundances of ^{13}C and ^{12}C in the produced CO_2 were determined by an *MAT-251* mass spectrometer (*Finnigan MAT*, Bremen, Germany) in the Academia Sinica's State Key Laboratory of Gas Geochemistry. The results are reported in terms of $^{13}C/^{12}C$ ratios ($\delta^{13}C$) relative to the PDB standard (Craig 1957). Random duplicate measurements were also performed as checks, indicating an accuracy within 0.16 ‰. Assuming the $\delta^{13}C$ of air source was -8.0 ‰ (Farquhar *et al.* 1989), isotope discrimination ($\Delta^{13}C$) was calculated as

$$\Delta^{13}C = \frac{\delta_a - \delta_p}{1 + \delta_p}$$

with δ_a and δ_p being the isotopic composition of air and plant material, respectively.

Enzyme activities: Enzymes were extracted from the leaf tissue frozen in liquid nitrogen by grinding in a chilled mortar and pestle. The grinding medium contained 100 mM Hepes-KOH (pH 7.5), 5 mM $MgCl_2$, 2 mM $MnCl_2$, 1 mM EDTA, 3 mM dithioerythritol, 0.05 % *Triton X-100,* and 25 % (m/m) polyvinylpolypyrrolidone. The resulting homogenate was filtered through 3 layers of cheesecloth, and centrifuged at 15 000×*g* for 5 min. The supernatant was used for enzyme assays. The protein content was measured by the method of Bradford (1976), using bovine serum albumin as standard.

All enzymes were assayed at 30 °C. Ribulose 1,5-bisphosphate (RuBP) carboxylase (RuBPC, EC 4.1.1.39) was assayed following the substrate dependent $H^{14}CO_3$ incorporation into acid stable products (Rajendrudu *et al.* 1986). The reaction medium contained 50 mM Hepes-NaOH (pH 7.8), 5 mM dithiothreitol, 10 mM $MgCl_2$, 20 mM $NaH^{14}CO_3$, and 1 mM RuBP. The reaction was stopped by adding 20 % (m/v) trichloracetic acid. Phosphoenolpyruvate (PEP) carboxylase (PEPC, EC 4.1.1.31) was spectrophotometrically assayed (Devi *et al.* 1993), using the following reaction medium: 20 mM Tricine-KOH (pH 7.8), 5 mM $MgCl_2$, 10 mM $NaHCO_3$, 2 units of malate dehydrogenase, 0.2 mM NADH, and 2.5 mM PEP in a total volume of 1 cm^3. Glycollate oxidase (GO, EC 1.1.3.1) was assayed spectrophotometrically according to Moore *et al.* (1988). The reaction medium of 1 cm^3 contained 50 mM Tricine-KOH (pH 7.5), 2.5 mM $MgCl_2$, 3 mM phenylhydrazine, and 5 mM glycollate.

Results

Differences in environmental parameters: In the crown of the sampled tree, the average hourly T_A was about 1.9 °C higher at the top than at the bottom (Fig. 2).

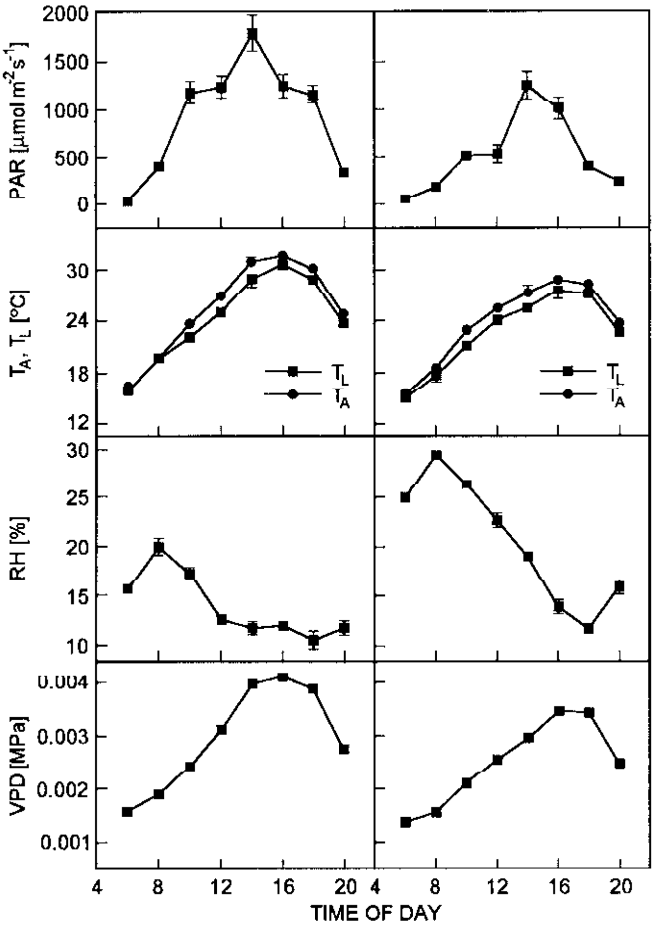

Fig. 2. Diurnal time courses of photosynthetically active radiation (PAR), air and leaf temperature (T_A, T_L), relative air humidity (RH), and air vapour pressure difference (VPD) at the top (*left*) and the bottom (*right*) of mature Euphrates poplar's crown in which broad-ovate and lanceolate leaves are located, respectively. Values of PAR, T_A, T_L, and RH are the means of 6 measurements performed on 25 July, 1994. Bars indicate ± SE where these exceed the sizes of symbols. VPD was calculated from the means of T_A and RH.

The maximum difference of T_A between the two positions in the tree's crown was 3.9 °C at midday. In addition, the RH at the top was lower than at the bottom. The effects of T_A and RH both resulted in steadily higher VPD at the top of the tree's crown, implying an increased evaporative demand for the BOL. The microclimate of the BOL at the top were characterized by higher irradiance and temperature values compared to the LL at the bottom; the maximum differences in T_L and PAR were 3.8 °C and 522 μmol m^{-2} s^{-1}, respectively.

Leaf anatomy: There was no marked difference between the vascular bundle sheath and mesophyll cells of the two type leaves, so both the BOL and LL did not have the Kranz leaf anatomy (Fig. 3). Both differently shaped leaves exhibited xeromorphic

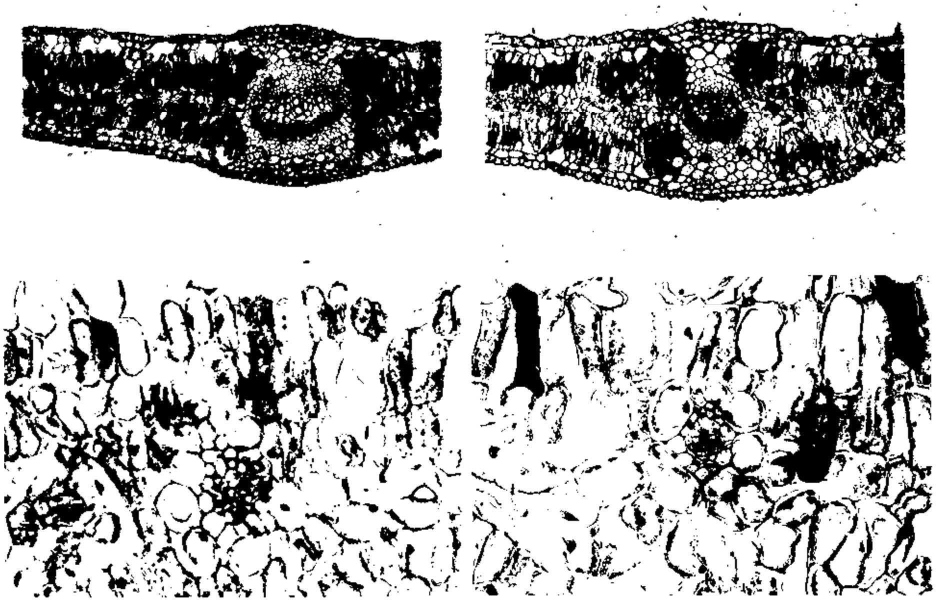

Fig. 3. Transectional anatomy of broad-ovate (*left*) and lanceolate (*right*) leaves in Euphrates poplar. bs, bundle sheath cell; pp, palisade parenchyma cell; sp, spongy parenchyma cell; bar = 300 μm.

features such as well-developed cuticle, hypodermis, and stoma with substomatal chamber. According to the measurements on 8 transections of each leaf type, nevertheless, the mean diameter and wall thickness of the main vein vessel were 16.4 and 1.9 μm in the BOL, and 15.3 and 1.1 μm in the LL, respectively. In contrast to the discontinuous spongy tissue in the BOL, the spongy parenchyma was well-developed and continuous in the LL. The thickness of palisade tissue was 60.9 % of the total leaf thickness in the BOL, and 25.2 % in the LL. The BOL had a more strengthened palisade tissue and a weak spongy one compared to the LL. Obviously, the BOL possessed a more developed xeromorphic structure than the LL.

Variation in stable carbon isotope composition: The $\delta^{13}C$ values of the BOL and LL were close to those of the extreme C_3 plants (Table 1). The $\Delta^{13}C$ variation of leaf material was less pronounced in each of the two leaf shapes throughout the growing season, whereas the $\Delta^{13}C$ of the LL was on average 3.2 ‰ larger than that of the BOL.

Table 1. Carbon isotope composition ($\delta^{13}C$) in differently shaped leaves of Euphrates poplar. Values in parentheses refer to carbon isotopic discrimination [Δ, ‰].

	$\delta^{13}C$ [‰] 20 May	25 July	8 September
Broad-ovate leaf	-26.4 (18.9)	-26.7 (19.2)	-26.5 (19.0)
Lanceolate leaf	-29.3 (21.9)	-29.7 (22.4)	-29.7 (22.4)

Gas exchange: Both the BOL and LL showed a similar tendency for reduced g_s after stomatal opening in the morning (values not shown), but the average g_s in the BOL was lower than in the LL during the whole growing season (Table 2). In accordance with the difference of g_s, the BOL had a lower ratio of c_i/c_a and a lower E than the LL. Although a significant decrease of P_N at midday was observed only in the BOL (results not shown), the LL, with its higher c_i, did not perform predominant P_N in the course of the season. Subsequently, the average hourly WUE, expressed as P_N/E, was lower in the LL than in the BOL.

Table 2. Diurnal means of net photosynthetic rate (P_N), transpiration rate (E), water use efficiency (WUE), stomatal conductance (g_s), and the ratio of intercellular to air concentration of CO_2 (c_i/c_a) in differently shaped leaves of Euphrates poplar. Values are means of 6 leaves ± SE. * and ** indicate the differences of pair means are significant at the 0.05 and 0.01 probability levels based on ANOVA, respectively.

	Broad-ovate leaf			Lanceolate leaf		
	20 May	25 July	8 September	20 May	25 July	8 September
P_N [mg m^{-2} s^{-1}]	0.30±0.05	0.43±0.04	0.24±0.04	0.27±0.03	0.42±0.03	0.23±0.07
E [mg m^{-2} s^{-1}]	53.1±6.4	56.0±6.9	41.0±7.5	74.5±6.8*	80.4±12.0	65.7±16.4
WUE [g kg^{-1}]	5.8±0.9*	7.9±0.4**	6.2±0.7	3.6±0.3	5.6±0.6	3.7±1.3
g_s [cm s^{-1}]	0.26±0.04	0.34±0.04	0.30±0.04	0.32±0.03	0.44±0.06	0.33±0.05
c_i/c_a	0.55±0.04	0.58±0.05	0.64±0.05	1.01±0.10**	0.78±0.05**	0.91±0.10*

Activities of photosynthetic and photorespiratory enzymes: Differently shaped leaves exhibited a parallel increase in the activity of RuBPC and GO in the course of the season; the reverse was true for PEPC (Table 3). The BOL showed a slight increase in the activity of PEPC compared to the LL while the activity of RuBPC was 1.6- to 2.4-fold higher in the LL than in the BOL during the whole growing season, resulting in a lower ratio of RuBP/PEPC in the BOL. The activity of GO in the BOL was 46 to 80 % less than in the LL.

Table 3. Activities of selected photosynthetic and photorespiratory enzymes [mmol kg^{-1}(protein) s^{-1}] in differently shaped leaves of Euphrates poplar. Values are the means of 4 independent extractions ± SE. *, **, and *** indicate the differences of pair means were significant at the 0.05, 0.01, and 0.001 probability levels based on ANOVA, respectively.

	Broad-ovate leaf			Lanceolate leaf		
	20 May	25 July	8 September	20 May	25 July	8 September
RuBPC	0.81±0.09	0.89±0.08	1.13±0.11	1.91±0.25**	1.60±0.16**	1.80±0.18*
PEPC	0.43±0.04	0.35±0.03	0.34±0.03	0.36±0.03	0.29±0.03	0.29±0.02
GO	11.58±0.62	12.27±0.52	14.53±1.50	21.7±1.33***	45.53±3.73***	72.51±6.68***
RuBPC/PEPC	1.87	2.52	3.29	5.26	5.44	6.23

Discussion

Varied shape leaves, such as LL, long-narrow, rhombus, reniform, nephroid, and BOL ones, occur with a change in the height of Euphrates poplar's crown. It is very rare that the same organ within an individual tree exhibits so marked morphodifferentiation in desert regions. In the present study, we found a difference of average 3.2 ‰ in $\Delta^{13}C$ value between the BOL and LL of the tree species, assuming a value for δ_a of -8.0 ‰. The reduction of δ_p value in leaves at lower crown layers is partly ascribed to a lighter source of CO_2 resulting from soil respiration under closed canopy, but much of the decrease in δ_p value seems to be correlated with physiological effects (Farquhar *et al.* 1989). The natural forest of Euphrates poplar is sparse and, moreover, strong turbulent conditions prevail in its native habitats, suggesting that there are no marked differences of source CO_2 composition from the lower to upper crown (Francey *et al.* 1985, Schleser and Jayasekera 1985). Thus, rather the physiological state than the source air CO_2 mainly contributes to the discrepancy of δ_p value between the BOL and LL types.

A minor difference of δ_p value usually reflects large changes in physiological behaviour (Hattersley 1982, Hattersley and Roksandic 1983). According to gas exchange measurements during the growing season, the decrease of g_s in BOL was pronounced with respect to the LL. Concomitantly with the depression of g_s, E and c_i/c_a were lower in the BOL than in the LL. Earlier studies demonstrate that the $\Delta^{13}C$ is associated with c_i/c_a (Farquhar *et al.* 1989). A positive correlation between the $\Delta^{13}C$ value and leaf conductance has been established by Ehleringer (1990). Therefore, the decline of g_s, causing a subsequent decrease in c_i/c_a, could be responsible for the lower $\Delta^{13}C$ value in the BOL than in the LL.

The g_s and photosynthetic capacity generally change in parallel under a variety of environmental conditions (Wong *et al.* 1979, 1985), but their ratio may change with leaf age (Sobrado 1996). Compared to the LL, however, the decrease of g_s did not induce a decline of P_N in the BOL. This could be related to the difference between the top and the bottom of the Euphrates poplar's crown in microenvironmental factors such as irradiance and temperature, which affect photosynthetic competence. Furthermore, the corresponding biochemical properties in the mesophyll should also be involved to maintain higher P_N in the BOL (Farquhar and Sharkey 1982, Briggs *et al.* 1986).

The decrease in the ratio of RuBPC/PEPC was pronounced in the BOL than in the LL. The lower ratio in the BOL was mainly associated with a marked decline in the activity of RuBPC, because of only a slight increase in the activity of PEPC. The lowered GO activity was also observed in the BOL throughout the whole growing seasons. These enzymological traits showed that the BOL of Euphrates poplar tended to assimilate CO_2 in a C_4-like mode.

It is difficult to ascertain the key microenvironmental factor contributing to the different physiological states of the two different shape leaves in the field. The stomatal and photosynthetic performance in the BOL could be correlated with the whole microclimate, characterized by higher irradiance and temperature, and lower air humidity compared to the LL. The discrepancy in the development of xeromorphic features between the two leaf types seemed also to support the opinion. However, the present study could not elucidate specific grounds for leaf shape polymorphism in the Euphrates poplar. The narrow leaves at low crown layers benefit from decreasing evaporation area and utilizing limited incident irradiance due to reduction of shelter of each other, but the young tree of Euphrates poplar exposed to full of irradiance also possesses only LL or long-narrow leaves. The ecological significance of morphodifferentiation in the leaves of Euphrates poplar remains to be studied.

References

Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Briggs, G.M., Jurik, T.W., Gates, D.M.: Non-stomatal limitation of CO_2 assimilation in three tree species during natural drought conditions. - Physiol. Plant. **66**: 521-526, 1986.

Craig, H.: Isotopic standards for carbon and oxygen and correction factors for mass spectrometric analysis of carbon dioxide. - Geochim. cosmochim. Acta **12**: 133-149, 1957.

Crespo, H.M., Frean, M., Cresswell, C.F., Tew, J.: The occurrence of both C_3 and C_4 photosynthetic characteristics in a single *Zea mays* plant. - Planta **147**: 257-263, 1979.

Devi, M.T., Raghavendra, A.S.: Partial reduction in activities of photorespiratory enzymes in C_3-C_4 intermediates of *Alternanthera* and *Parthenium*. - J. exp. Bot. **44**: 779-784, 1993.

Ehleringer, J.R.: Correlations between carbon isotope discrimination and leaf conductance to water vapor in common beans. - Plant Physiol. **93**: 1422-1425, 1990.

Farquhar, G.D., Ehleringer, J.R., Hubick, K.T.: Carbon isotope discrimination and photosynthesis. - Annu. Rev. Plant Physiol. Plant mol. Biol. **40**: 503-537, 1989.

Farquhar, G.D., Sharkey, T.D.: Stomatal conductance and photosynthesis. - Annu. Rev. Plant Physiol. **33**: 317-345, 1982.

Francey, R.J., Gifford, R.M., Sharkey, T.D., Weir, B.: Physiological influences on carbon isotope discrimination in huon pine (*Lagarostrobos franklinii*). - Oecologia **66**: 211-218, 1985.

Hattersley, P.W.: $\delta^{13}C$ values of C_4 types in grasses. - Aust. J. Plant Physiol. **9**: 139-154, 1982.

Hattersley, P.W., Roksandic, Z.: $\delta^{13}C$ values of C_3 and C_4 species of Australian *Neurachne* and its allies (*Poaceae*). - Aust. J. Bot. **31**: 317-321, 1983.

Khanna, R., Sinha, S.K.: Change in the predominance from C_4 to C_3 pathway following anthesis in *Sorghum*. - Biochem. biophys. Res. Commun. **52**: 121-124, 1973.

Kisaki, H., Hirabayashi, S., Yano, N.: Effect of the age of tobacco leaves on photosynthesis and photorespiration. - Plant Cell Physiol. **14**: 505-514, 1973.

Moore, B.d., Monson, R.K., Ku, M.S.B., Edwards, G.E.: Activities of principal photosynthetic and photorespiratory enzymes in leaf mesophyll and bundle sheath protoplasts from the C_3-C_4 intermediate *Flaveria ramosissima*. - Plant Cell Physiol. **29**: 999-1006, 1988.

O'Leary, M.H.: Carbon isotope fractionation in plants. - Phytochemistry **20**: 553-567, 1981.

Raghavendra, A.S., Rajendrudu, G., Das, V.S.R.: Simultaneous occurrence of C_3 and C_4 photosynthesis in relation to leaf position in *Mollugo nudicaulis*. - Nature **273**: 143-144, 1978.

Rajendrudu, G., Prasad, J.S.R., Rama Das, V.S.: C_3-C_4 intermediate species in *Alternanthera* (*Amaranthaceae*). - Plant Physiol. **80**: 409-414, 1986.

Schleser, G.H., Jayasekera, R.: δ^{13}C-variations of leaves in forests as an indication of reassimilated CO_2 from the soil. - Oecologia **65**: 536-542, 1985.

Sobrado, M.A.: Leaf photosynthesis and water loss as influenced by leaf age and seasonal drought in an evergreen tree. - Photosynthetica **32**: 563-568, 1996.

Troughton, J.H.: Aspects of the evolution of the photosynthetic carboxylation reaction in plants. - In: Hatch, M.D., Osmond, C.B., Slatyer, R.O. (ed.): Photosynthesis and Photorespiration. Pp. 124-129. Wiley-Interscience, New York - London - Sydney - Toronto 1971.

Wang, H.L., Zhang, C.L., Liang, H.G.: Seasonal changes of polyamines in habitat adaptation of different ecotypes of reed plants. - Oecologia **101**: 119-123, 1995.

Wong, S.C., Cowan, I.R., Farquhar, G.D.: Stomatal conductance correlates with photosynthetic capacity. - Nature **282**: 424-426, 1979.

Wong, S.-C., Cowan, I.R., Farquhar, G.D.: Leaf conductance in relation to rate of CO_2 assimilation. III. Influences of water stress and photoinhibition. - Plant Physiol. **78**: 830-834, 1985.