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C_3-C_4 Intermediate Species in *Alternanthera* (Amaranthaceae)[1]

LEAF ANATOMY, CO_2 COMPENSATION POINT, NET CO_2 EXCHANGE AND ACTIVITIES OF PHOTOSYNTHETIC ENZYMES

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ABSTRACT

Two naturally occurring species of the genus *Alternanthera*, namely *A. ficoides* and *A. tenella*, were identified as C_3-C_4 intermediates based on leaf anatomy, photosynthetic CO_2 compensation point (Γ), O_2 response of Γ, light intensity response of Γ, and the activities of key enzymes of photosynthesis. *A. ficoides* and *A. tenella* exhibited a less distinct Kranz-like leaf anatomy with substantial accumulation of starch both in mesophyll and bundle sheath cells. Photosynthetic CO_2 compensation points of these two intermediate species at 29°C were much lower than in C_3 plants and ranged from 18 to 22 microliters per liter. Although *A. ficoides* and *A. tenella* exhibited similar intermediacy in Γ, the apparent photorespiratory component of O_2 inhibition in *A. ficoides* is lower than in *A. tenella*. The Γ progressively decreases from 35 microliters per liter at lowest light intensity to 18 microliters per liter at highest light intensity in *A. tenella*. It was, however, constant in *A. ficoides* at 20 to 25 microliters per liter between light intensities measured. The rates of net photosynthesis at 21% O_2 and 29°C by *A. ficoides* and *A. tenella* were 25 to 28 milligrams CO_2 per square decimeter per hour which are intermediate between values obtained for *Tridax procumbens* and *A. pungens*, C_3 and C_4 species, respectively. The activities of key enzymes of C_4 photosynthesis, phosphoenolpyruvate carboxylase, pyruvate Pi dikinase, NAD malic enzyme, NADP malic enzyme and phosphoenolpyruvate carboxykinase in the two intermediates, *A. ficoides* and *A. tenella* are very low or insignificant. Results indicated that the relatively low apparent photorespiratory component in these two species is presumably the basis for the C_3-C_4 intermediate photosynthesis.

The majority of the world's important crops are C_3 plants exhibiting substantial loss of photosynthetically fixed carbon through photorespiration, and increasing the efficiency of such plant species has in recent years been a goal of plant research. At present, there has been considerable interest in improving the productivity of C_3 species by screening for lines with reduced rates of photorespiration (23). Recently, one approach to this problem has been to identify the naturally occurring plant species intermediate to the C_3 and C_4 plants. The search for naturally occurring C_3-C_4 intermediate species and the study of their physiological and biochemical characteristics are important to understand the possibility of increasing photosynthetic efficiency of C_3 crops.

Among the 19 genera in 11 families of higher plants, *Alternanthera* is one already known to possess C_3 and C_4 species which indicates the possibility of occurrence of transient species having features intermediate between C_3 and C_4 plants (6, 10, 11, 25). There have been many attempts in the past to identify and characterize C_3-C_4 intermediate species. Recently, naturally occurring species with photosynthetic characteristics intermediate between C_3 and C_4 plants have been identified in the genera *Mollugo* (29), *Panicum* (4, 12, 22, 27), *Moricandia* (1, 15), *Flaveria* (2, 16, 20), and most recently *Neurachne* (10). The intermediate nature of these species includes a Kranz-like leaf anatomy, partially suppressed photorespiration as indicated by reduced Γ,[2] a reduced sensitivity of net photosynthesis to O_2, and intermediacy in biochemical process of photosynthesis.

In the present study, leaf anatomy, O_2 sensitivity of Γ, light intensity response of Γ, photosynthetic CO_2 exchange rate, and activities of some key photosynthetic enzymes in two species of *Alternanthera* (*A. tenella* and *A. ficoides*) and representative C_3 and C_4 species were investigated to report features intermediate between C_3 and C_4 plants in species hitherto not known.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Plants of *Alternanthera ficoides* L. R.Br.R. and *Alternanthera tenella* Colla. were grown from vegetative cuttings on soil supplemented with manure (three parts of soil and one part of farm yard manure) in 30 cm clay pots. Plants received full solar irradiance for most of the day in an 11 h natural photoperiod. The maximum light intensity (PAR, 400–700 nm) available at the top of the canopy was 180 to 200 nE cm^{-2} s^{-1} on a clear day. Daily maximum and minimum air temperatures had ranges 28.9 to 31.9°C and 20.8 to 21.9°C, respectively. Plants were watered every alternate d to avoid water stress throughout the growth of the plant. Other plant species used in the present study for comparison were grown under conditions similar to that of *Alternanthera* species. Young and fully expanded leaves (second or third from shoot apices) from 4 to 5 week old vigorously growing plants were used in the present study.

Leaf Anatomy. Free hand sections of leaves fixed in formalin-acetic acid-ethanol mixture were taken and observed under a light microscope. Starch accumulation in fresh leaf sections were observed after staining with I_2-KI solution. Fresh leaf segments (0.25 cm^2) were placed in boiling 80% (v/v) ethanol until Chl had been extracted and cleared with 10% (w/v) aqueous NaOH solution. Cleared leaf segments were rinsed repeatedly with distilled H_2O and stained with I_2-KI solution. A paradermal view

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[2] Abbrevations: Γ, photosynthetic CO_2 compensation concentration; RuBP, ribulose 1,5-bisphosphate; PEP, phosphoenolpyruvate; OAA, oxaloacetate; pO_2, partial pressure of oxygen; IRGA, infrared gas analyzer.

of stained leaf tissue was observed under the light microscope.

CO_2 Compensation Point (Γ). The CO_2 compensation point in a closed gas circuit system was determined using IRGA (model 225-MK 3 from Analytical Development Company Ltd., England) calibrated for CO_2 on an absolute mode. A plexiglass cuvette (4 × 4 × 0.5 cm) was used as the photosynthetic chamber enclosing the lower and upper surfaces of an attached leaf (usually second or third from the top of plants). Air entering into the photosynthetic chamber was humidified and maintained at constant temperature (29 ± 1°C) by bubbling through distilled H_2O at constant temperature. The air flow through the system was adjusted to 0.4 L/min. The leaves were illuminated at 145 nE cm^{-2} s^{-1} (PAR, 400–700 nm) by a slide projector with a 150 W halogen lamp (Photophone, model Slidomatic) held perpendicular to the adaxial leaf surface. After preillumination for 30 to 40 min, the CO_2 depletion by the intact leaf in a closed system at 21% O_2 was monitored until equilibrium was reached. Nitrogen was flushed through the system to achieve zero O_2 concentration and the response for Γ was determined. The equilibrium value of CO_2 was recorded as the CO_2 compensation point. The Γ measurements for a leaf were repeated two to three times and then averaged. Similar measurements were also repeated on two to three different days and on different individual plants of the same species and the variation is not significant. The intensity of the light within the leaf cuvette was adjusted to the required level by manipulating the distance between the light source and the photosynthetic chamber. Light intensities (PAR, 400–700 nm) were measured with a quantum sensor (model LI 190S) connected to LI-COR model LI 170 Quantum/Radiometer/Photometer.

CO_2 Exchange Rate Measurements. Net CO_2 exchange of intact individual leaves was measured according to Monson *et al.* (21) with an IRGA (model 225-MK3, ADC, England) using a differential mode and open gas circuit system. Air entering into the photosynthetic chamber was humidified and maintained at constant temperature (29 ± 1°C) by bubbling through water at constant temperature. Air flow containing 340 μl/L CO_2 and 21% O_2 was adjusted to 0.4 L/min. After preillumination for 30 to 40 min, CO_2 depletion in the atmospheric air by intact leaves was monitored until a steady state was recorded. CO_2 exchange rate measurements for individual leaves (usually second or third from the top of plants) were repeated two to three times on different d and on different individual plants and then averaged.

Preparation of Leaf Tissue Extract for Enzyme Assay. Leaf samples (1.0–1.5 g) were rapidly homogenized at 4°C using a prechilled mortar and pestle with a pinch of acid washed sand and 1.5 ml of ice cold (0°C) extraction medium. After homogenization, 1.0 ml of the medium was added and the homogenate was filtered through two layers of muslin cloth. An aliquot of the filtered homogenate (0.1 ml) was taken before centrifugation and determined for Chl based on the method of Arnon (3). The homogenate was centrifuged at 10,000*g* for 10 min in a refrigerated centrifuge and the supernatant was assayed for enzyme activities. The extraction medium for RuBP carboxylase contained 50 mM Hepes-NaOH (pH 7.8), 2 mM EDTA, 5mM $MgCl_2$, and 5 mM DTT. For PEP carboxylase, the extraction medium included 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 5mM $MgCl_2$, 5 mM DTT, and 2% (w/v) PVP-40. For the assay of pyruvate, Pi dikinase, a buffer solution containing 50 mM Hepes-KOH (pH 7.5), 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA, 5 mM cysteine, 1.5 mM sodium pyruvate, and 2.5% (w/v) PVP-40 was used for enzyme extraction (19). The extraction procedure for the assay of NAD- and NADP-malic enzyme was similar to that of Edwards *et al.* (7). The extraction medium contained 50 mM Hepes- KOH (pH 7.2), 2 mM $MnCl_2$, 5 mM $MgCl_2$, 10 mM DTT, and 1% (w/v) BSA. Triton X-100 was added to the filtered homogenate to give a final concentration of 0.5% (v/v). The extraction medium for PEP carboxykinase was essentially similar as described by Hatch and Mau (8) which included 50 mM Hepes-KOH (pH 8.0), 1 mM $MgCl_2$, 5 mM DTT, and 1% (w/v) PVP-40.

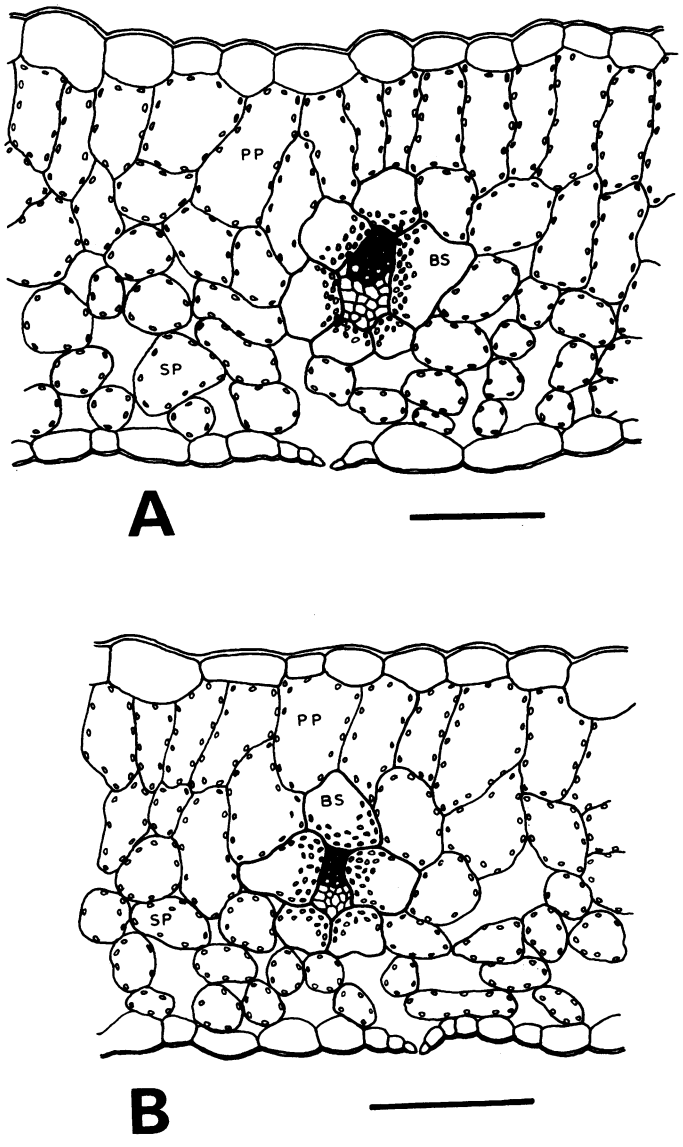

FIG. 1. Traces of leaf transections of two species of *Alternanthera*, *A. ficoides* (A) and *A. tenella* (B). BS, bundle sheath cells; PP, palisade parenchyma cells; SP, spongy parenchyma cells. Bar = 100 μm.

Assay of Enzyme Activities. RuBP carboxylase was assayed following the substrate dependent $H^{14}CO_3$ incorporation into acid stable products. The assay medium contained 50 mM Hepes-NaOH (pH 7.8), 5 mM DTT, 10 mM $MgCl_2$, and 20 mM Na-$H^{14}CO_3$ (0.2 mCi/mmol) plus enzyme extract in a total volume of 0.5 ml. After 4 min of preincubation at 30°C RuBP was added to make it a final concentration of 1 mM and stopped after 40 to 60 s by adding 0.5 ml 20% (w/v) TCA. Radioactivity into acid stable products was determined using LKB model 1217 liquid scintillation counting system. PEP carboxylase was assayed spectrophotometrically by coupling oxaloacetate formation with malate dehydrogenase. The assay mixture (3.0 ml) contained 100 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 0.14 mM NADH, 10 mM $NaHCO_3$, 4.5 units of malate dehydrogenase, and an aliquot of enzyme extract. The reaction was initiated at 30°C by addition of PEP to give a final concentration of 2.0 mM and the rate of decrease in extinction at 340 nm was measured using Hitachi

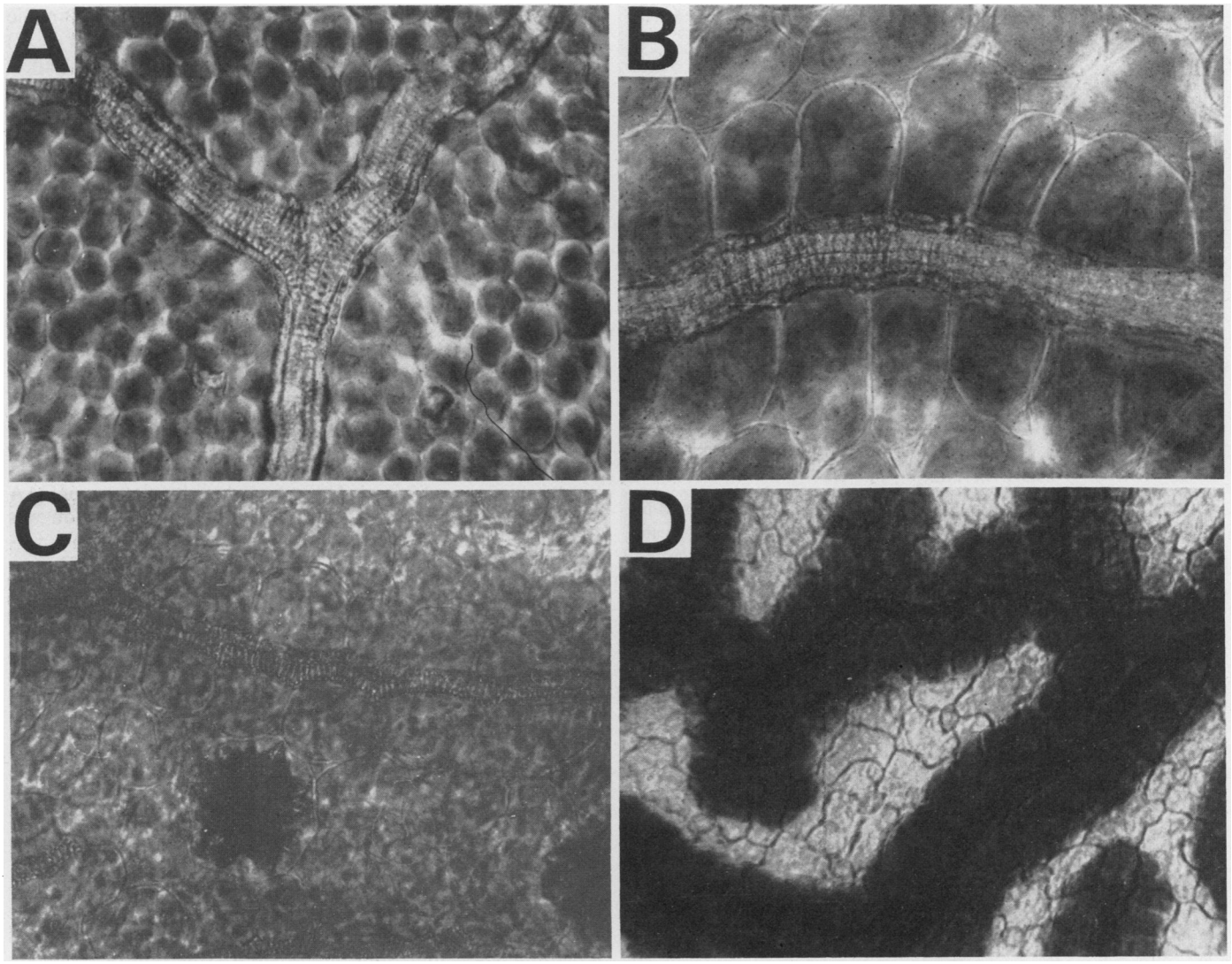

FIG. 2. Paradermal view of a portion of leaf in *Alternanthera sessilis* (A), *A. ficoides* (B), *A. tenella* (C), and *Amaranthus viridis* (D). Bundle sheath cells are indistinct and the mesophyll cells are stained for starch in *A. sessilis*, a C_3 plant. The starch is found in both mesophyll and bundle sheath cells of intermediate species, *A. ficoides* and *A. tenella* while it is exclusively found in bundle sheath cells of *Amaranthus viridis*, a C_4 plant (× 200).

Table I. *Leaf Anatomy, Photosynthetic CO_2 Compensation Point (at zero and 21% O_2) and Photosynthetic Rate at 21% O_2, 340 µl/L CO_2 and 29°C in Plants Exhibiting C_3, C_3-C_4 Intermediate and C_4 Type of Photosynthesis and Photorespiration*

Values are the average of two to three determinations on different individual plants.

Plant Species	Leaf Anatomy	CO_2 Compensation Point at 29°C		Photosynthetic Rate
		21% O_2	Zero O_2	
		µl/L		mg CO_2 dm^{-2} h^{-1}
Achyranthes aspera	C_3	62.0	6.5	18.0
Alternanthera ficoides	C_3-C_4	22.0	17.0	25.0
Alternanthera pungens	C_4	3.0	2.5	46.5
Alternanthera tenella	C_3-C_4	18.0	5.0	27.6
Amaranthus viridis	C_4	3.0	3.0	46.0
Cleome gynandra	C_4	3.5	2.5	43.5
Tridax procumbens	C_3	55.0	6.0	21.5

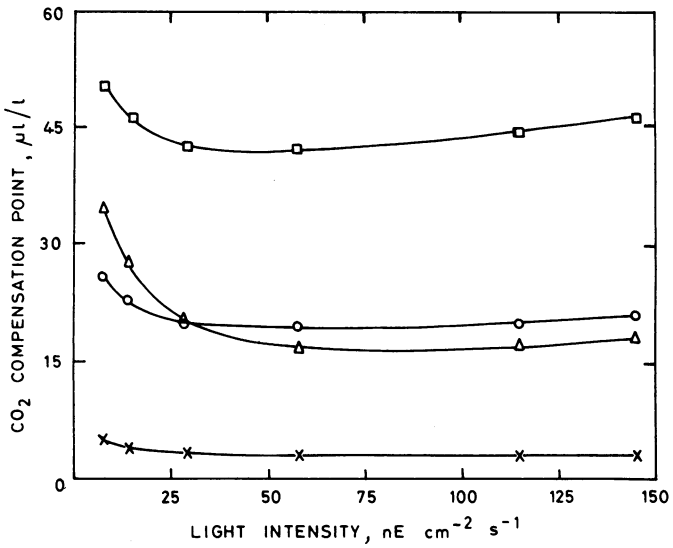

FIG. 3. Response of photosynthetic CO_2 compensation points to increasing light intensities in two intermediate species, *A. ficoides* (○) and *A. tenella* (△) and a representative C_3, *Tridax procumbens* (□) and C_4, *Cleome gynandra* (×) species.

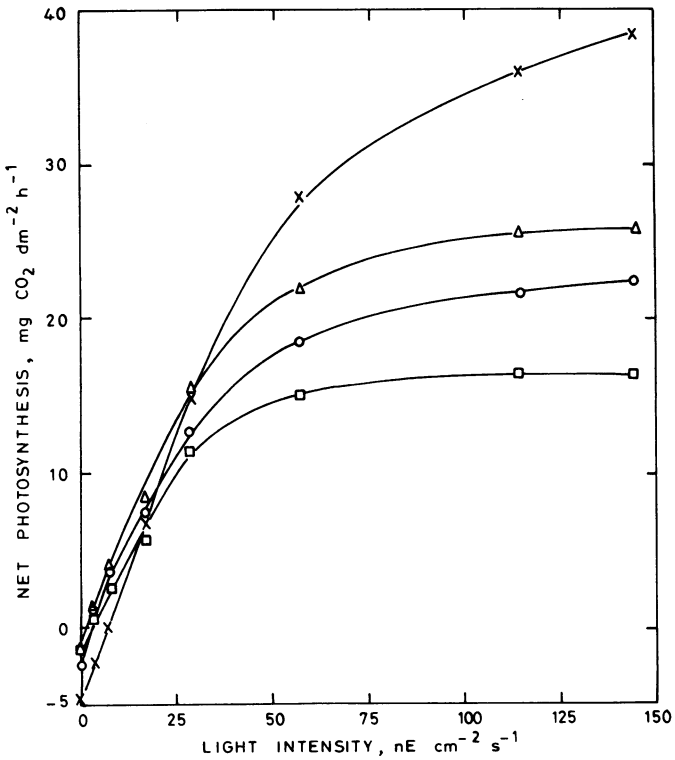

FIG. 4. Rate of net CO_2 exchange as a function of incident light intensity for young and fully expanded intact leaves of *A. ficoides* (○), *A. tenella* (△), *Tridax procumbens* (□), and *Cleome gynandra* (×). Measurements were made at 29°C in the atmospheric air containing 340 µl CO_2 L^{-1} and 21% O_2.

model 557 spectrophotometer. The spectrophotometric assay of NAD-malic enzyme developed by Hatch *et al.* (9) was used to eliminate possible sources of error in the determination of enzyme activity particularly in plants with low activity of this enzyme. In addition to enzyme extract, the assay medium for NAD-malic enzyme included 25 mM Hepes-KOH (pH 7.2), 5mM malate, 2 mM NAD, 4 mM $MnCl_2$, 0.15 mM CoA, 0.2 mM EDTA, 5 mM DTT, 25 µM NADH, and 1 unit of malate dehydrogenase in a volume of 3.0 ml. The assay mixture for NADP-malic enzyme was essentially similar as described by Edwards *et al.* (7) which contained in addition to enzyme extract 50 mM Hepes-KOH (pH 8.0), 1 mM EDTA, 0.25 mM NADP, 2 mM malate, 25 µM NADPH, and 5 mM $MgCl_2$. The reaction was initiated by addition of $MgCl_2$. Pyruvate, Pi dikinase was assayed spectrophotometrically in a manner similar to that of Ku *et al.* (19). The reaction mixture contained in addition to enzyme, 100 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 5 mM DTT, 0.1 mM EDTA, 1.5 mM sodium pyruvate, 2.5 mM K_2HPO_4, 10 mM NH_4Cl, 25 mM $NaHCO_3$, 1.25 mM ATP, 0.2 mM NADH, 1.5 units of malate dehydrogenase (Sigma), and 1.5 units of PEP carboxylase (Corn, Sigma) in a total volume of 3.0 ml. Reaction was started by the addition of ATP and the rate of decrease in extinction at 340 nm was measured at 30°C using Hitachi 557 Spectrophotometer. The PEP carboxykinase decarboxylation reaction was assayed spectrophotometrically by the method of Hatch and Mau (8). The reaction mixture contained 50 mM Hepes-KOH (pH 8.0), 2 mM $MnCl_2$, 3 units of pyruvate kinase (type 1, from rabbit muscle, Sigma), 0.6 mM OAA, 0.4 mM ATP, and enzyme extract in a total volume of 3.0 ml. The nonenzymic breakdown of OAA was initially recorded for 5 min and the enzyme reaction was started with the addition of ATP.

RESULTS AND DISCUSSION

Light microscopic observation of leaf tissue of *A. ficoides* and of *A. tenella* showed a distinct layer of large parenchymatous bundle sheath cells with substantial number of chloroplasts around the vascular tissue (Fig. 1). However, the parenchyma sheath chloroplasts appeared similar in size to those of mesophyll chloroplasts and were arranged centripetally in bundle sheath cells. Mesophyll cells surrounding and in contact with the parenchyma sheath cells were not radially arranged but had two types of mesophyll cells, spongy and palisade as in a non-Kranz species. Starch was found both in the mesophyll and bundle sheath cells as indicated by positive reaction for I_2-KI solution. The paradermal view of leaves of two *Alternanthera* species and the representative C_3 and C_4 plants are shown in Figure 2. The two species of *Alternanthera*, *A. ficoides* and *A. tenella*, exhibited a definite parenchymatous bundle sheath, the cells of which are relatively larger than the other mesophyll cells. Starch was accumulated both in mesophyll and bundle sheath cells of *A. ficoides* and *A. tenella*. However, *Amaranthus viridis*, a C_4 plant showed a distinct layer of bundle sheath cells exclusively stained for starch around the vascular tissue. In contrast, *A. sessilis*, a C_3 plant, exhibited no distinct bundle sheath and dense starch accumulation in mesophyll cells. From the results it is found that the leaf anatomy in *A. ficoides* and *A. tenella* is less Kranz-like and resembles other known C_3-C_4 intermediate species, *Panicum milioides* (4, 18, 22), *Moricandia arvensis* (14, 30), and *Flaveria species* (20). Although the electron microscopic study for the structural details of leaves in *Alternanthera* has not been presented, the light microscopic studies of the leaf anatomy and the paradermal view of leaves in two species, *A. ficoides* and *A. tenella*, are distinct enough to indicate the C_3-C_4 intermediate characteristics.

The sensitivity of Γ to changes in O_2 concentration and the photosynthetic CO_2 exchange at 21% O_2 in two species of *Alternanthera* compared with representative C_3 and C_4 species are shown in Table I. Invariably, *A. ficoides* and *A. tenella* exhibited intermediate values of Γ ranging from 18 to 22 µl/L at 21% O_2 suggesting lower rates of apparent photorespiration than C_3 species, *Tridax procumbens* and *Achyranthes aspera* and definitely higher rates than in C_4 plants, *Alternanthera pungens* and *Cleome gynandra*. Increasing O_2 concentration from zero to 21% had greatly increased Γ from 6.0 and 6.5 to 55.0 and 62.0 µl/L in C_3 species, *T. procumbens* and *A. aspera*, respectively, but had

Table II. *Activities of Key Enzymes of C_4 Photosynthesis in Leaf Extracts of the Two Species of Alternanthera and the Representative C_3 and C_4 Species*

PEPC, PEP carboxylase; RuBPC, RuBP carboxylase; PPDK, pyruvate Pi dikinase; NAD-ME, NAD malic enzyme; NADP-ME, NADP malic enzyme; PEP-CK, PEP carboxykinase. Each value is the average of at least two independent determinations.

Plant Species	Photosynthetic Type	Enzyme activity					
		RuBPC	PEPC	PPDK	NAD-ME	NADP-ME	PEP-CK
		μmol mg^{-1} Chl h^{-1}					
Alternanthera ficoides	C_3-C_4	306	41.5	19.2	2.1	1.0	ND[a]
Alternanthera tenella	C_3-C_4	251	17.3	12.2	ND	ND	ND
Cleome gynandra	C_4 (NAD-ME)	193	624	243	360	3.5	ND
Chloris barbata	C_4 (PEP-CK)	—[b]	—	—	—	—	295
Sorghum vulgare	C_4 (NADP-ME)	—	—	—	16.8	323	ND
Tridax procumbens	C_3	307	13.3	ND	ND	ND	ND

[a] Enzyme activity not detectable. [b] Not determined.

little effect on C_4 species, *A. pungens* and *C. gynandra*. However, the two species of *Alternanthera*, *A. ficoides* and *A. tenella*, differ with respect to the sensitivity of Γ to changes in O_2 concentration. Decreasing O_2 from 21% to zero had markedly decreased the Γ from intermediate value (18 μl/L) to a minimum (6μl/L) in *A. tenella* as in other C_3 species, *T. procumbens* and *A. aspera*. In *A. ficoides*, decreasing O_2 concentration from 21% to zero had slightly decreased Γ from 22 to 17 μl/L. This response was much less than that of Γ for *A. tenella* or other C_3 species, which suggests the possibility of O_2 inhibition of apparent photorespiratory component in *A. ficoides* is lower than in other known C_3 species. Although *A. tenella* exhibited C_3-C_4 intermediate Γ values at 21% O_2, the decrease in Γ to a minimum value at zero O_2 concentration strongly suggests the occurrence of O_2 sensitive photorespiration in this species.

The response of Γ to increasing light intensities at 21% O_2 in different plant species is shown in Figure 3. The two species of *Alternanthera* exhibited intermediate values for Γ relative to other C_3 and C_4 species at all the light intensities measured. The Γ of the C_4 species, *C. gynandra*, was constant at 3 to 4 μl/L between light intensities, 7.5 and 145 nE cm^{-2} s^{-1} while *T. procumbens*, a C_3 species, exhibited a little increase at lower light intensities. In contrast, the Γ in *A. tenella* was progressively decreased from 35 μl/L at lowest light intensity to 17 to 18 μl/L at 60 nE cm^{-2} s^{-1} light intensity and subsequently remained constant as the light intensity increased further. In *A. ficoides*, the Γ was not much affected as the light intensity varied and was relatively constant at 20 to 25 μl/L between light intensities measured.

Although the response of Γ to increasing light intensities at different pO_2 has recently provided evidence for the efficient CO_2 recycling mechanism for reduced photorespiration in C_3-C_4 intermediate *Panicum* species (5), the data presented in this paper is inadequate to speculate similar mechanism in *A. ficoides* and *A. tenella*. However, the results on Γ response to increasing light intensities in *A. ficoides* and *A. tenella* further confirm the intermediate feature of Γ between C_3 and C_4 species.

Photosynthetic CO_2 uptake at 21% O_2 in *A. ficoides* and *A. tenella* showed intermediate values in relation to representative C_3 and C_4 species (Table I). However, these values are much lower than in related C_4 species, *A. pungens* and slightly higher than in C_3 species, *T. procumbens* and *A. aspera*. Figure 4 shows the data for the response of net CO_2 exchange per unit leaf area as a function of incident light intensity for *A. ficoides* and *A. tenella* with a representative C_3 and C_4 species. The net photosynthetic CO_2 exchange in C_4 species, *C. gynandra*, was nearly 2.5 times of that for *T. procumbens*, a C_3 plant, and was not light saturated at the highest light intensity used (145 nE cm^{-2} s^{-1}). *A. ficoides* and *A. tenella* were intermediate between these extremes with regard to the rate of net photosynthesis as well as to the shape of the response curve at higher light intensities (Fig. 4). However, at low light intensities there was no significant difference in the rates of net photosynthesis between all four species.

Activities of some key enzymes of photosynthesis in *A. ficoides* and *A. tenella* compared with those of representative C_3 and C_4 species are shown in Table II. RuBP carboxylase activity in whole leaf extracts of *A. ficoides* was higher than that of *C. gynandra*, a C_4 species and *A. tenella*, a low photorespiring plant but similar to those obtained for *T. procumbens*, a C_3 plant. Although *A. ficoides* and *A. tenella* exhibited intermediacy with respect to Γ, activities of C_4 photosynthetic key enzymes were very low or not detectable in these two species. The activity of PEP carboxylase in *A. ficoides* was three times higher than in *T. procumbens* (a C_3 species) but still much lower than in C_4 species, *C. gynandra*. This activity is only about 6 to 7% of the activity recorded for the C_4 species. In *A. tenella*, the activity of PEP carboxylase was nearly the same as in C_3 species, *T. procumbens*. The activity of pyruvate, Pi dikinase in *A. ficoides* and *A. tenella*, ranged between 12.2 to 19.2 μmol mg^{-1} Chl h^{-1}, which was much less than in *C. gynandra*, a C_4 species. As shown in Table II, the decarboxylase activities, NAD malic enzyme, NADP malic enzyme and PEP carboxykinase in *A. ficoides* and *A. tenella* were low or not detectable compared with that of representative C_4 species. From the results it is assumed that the higher levels of PEP carboxylase observed in *A. ficoides*, perhaps plays a role in recycling of photorespired CO_2 but without a concentrating mechanism found in C_4 plants to decrease Γ. Further, it is suggested that *A. ficoides* and *A. tenella* may not be biochemically true C_3-C_4 photosynthetic intermediates but other mechanisms must be considered as the cause of the reduced level of the Γ and its sensitivity to O_2 in these species.

Recent studies have shown that a C_4-like CO_2 concentrating mechanism is not responsible for reduced apparent photorespiration in *Panicum milioides* and *Moricandia arvensis* but have indicated the efficient interval recycling of photorespiratory CO_2 via ribulose bisphosphate carboxylase/oxygenase in either of these species (7, 13, 14, 17, 26). On the contrary, photorespiration is thought to be reduced through a CO_2 concentrating mechanism by limited C_4-type of photosynthesis in the C_3-C_4 intermediate *Flaveria* species (20, 24, 28). The evidences obtained in the present study indicate that *A. ficoides* (same as *A. ficoidea* ?), is neither a typical C_4 species exhibiting Kranz leaf anatomy and low Γ (11) nor a typical C_3 species as reported by Pathan and Nimbalkar (25) based on the leaf anatomy, initial product of CO_2 fixation and certain C_4 photosynthetic enzymes. However, the present results based on the intermediate nature of leaf anatomy, Γ, O_2 sensitivity of Γ, and activities of photosynthetic enzymes suggest that apparent photorespiration is reduced in the

two species of *Alternanthera* studied here. Further studies are needed to understand the mechanism of reduced apparent photorespiration in these species. The present study is also believed to extend our knowledge of the natural existence of plants with intermediate characteristics between C_3 and C_4 pathways.

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