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Suaeda monoica, a C_4 Plant without Typical Bundle Sheaths

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ABSTRACT

***Suaeda monoica* Forssk. ex J. F. Gmel was found to possess the C_4 pathway of photosynthesis. The succulent leaves of *Suaeda* lack a green bundle sheath formation but have a layer of chlorenchyma, containing large and centripetally arranged chloroplasts, which surrounds the water tissue. We suggest that the proximity of a chlorenchymatous cell layer to the vascular bundles is not necessary for the operation of the C_4 pathway.**

Since the discovery of the C_4 carbon fixation pathway (7, 12), an increasing number of plants possessing this pathway have been recognized. It was found that in addition to the primary fixation of CO_2 into 4-carbon acids, C_4 plants possess certain other characteristics, *i.e.* a low CO_2 compensation point (5), low discrimination against ^{13}C (3, 17), and a specific anatomical leaf structure (9, 13, 14, 20). The latter, frequently called the Kranz anatomy, consists of a layer of large cells which usually envelope the vascular bundles and which are densely packed with chloroplasts. This bundle sheath formation is surrounded by chlorenchymatous mesophyll cells. The existence of such differentiated chlorenchyma, with the inner layer attached to the vascular bundles, is commonly believed to be necessary for the operation of the C_4 carbon fixation pathway.

The characterization of C_4 plants by the above mentioned features has been generally recognized. Since it was shown that the balance between C_3 and C_4 carboxylation could be altered by factors such as salinization, ontogeny, and leaf age (10, 11, 16), it seemed that the correlation between the appearance of a chlorenchymatous bundle sheath layer and the C_4 carbon fixation pathway was not of a basic nature (4). The possibility of finding a C_4 plant which lacks these typical structural features was therefore very likely.

MATERIALS AND METHODS

Suaeda monoica Forssk. ex J. F. Gmel plants were grown on aqueous solutions in a growth chamber (16 or 9 hr light period;

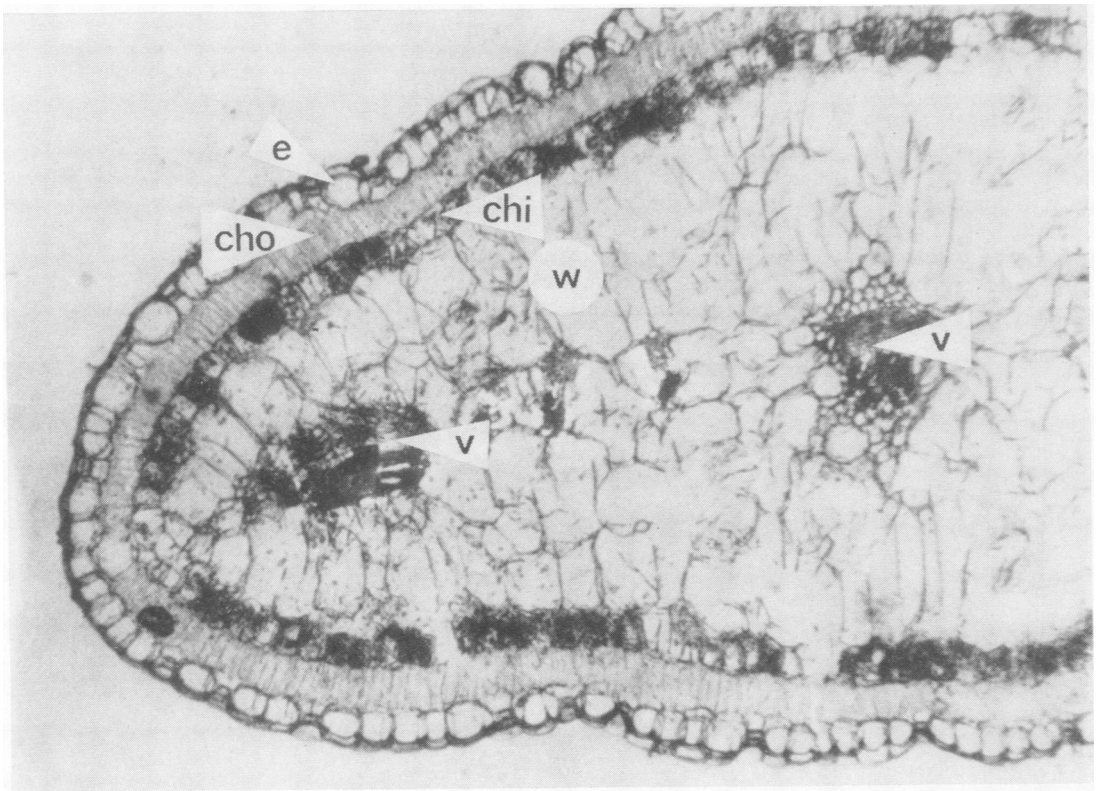

FIG. 1. Cross section of *Suaeda monoica* leaf (190 ×). e: epidermis; cho: outer layer of chlorenchyma; chi: inner layer of chlorenchyma; w: water tissue; v: vascular bundle.

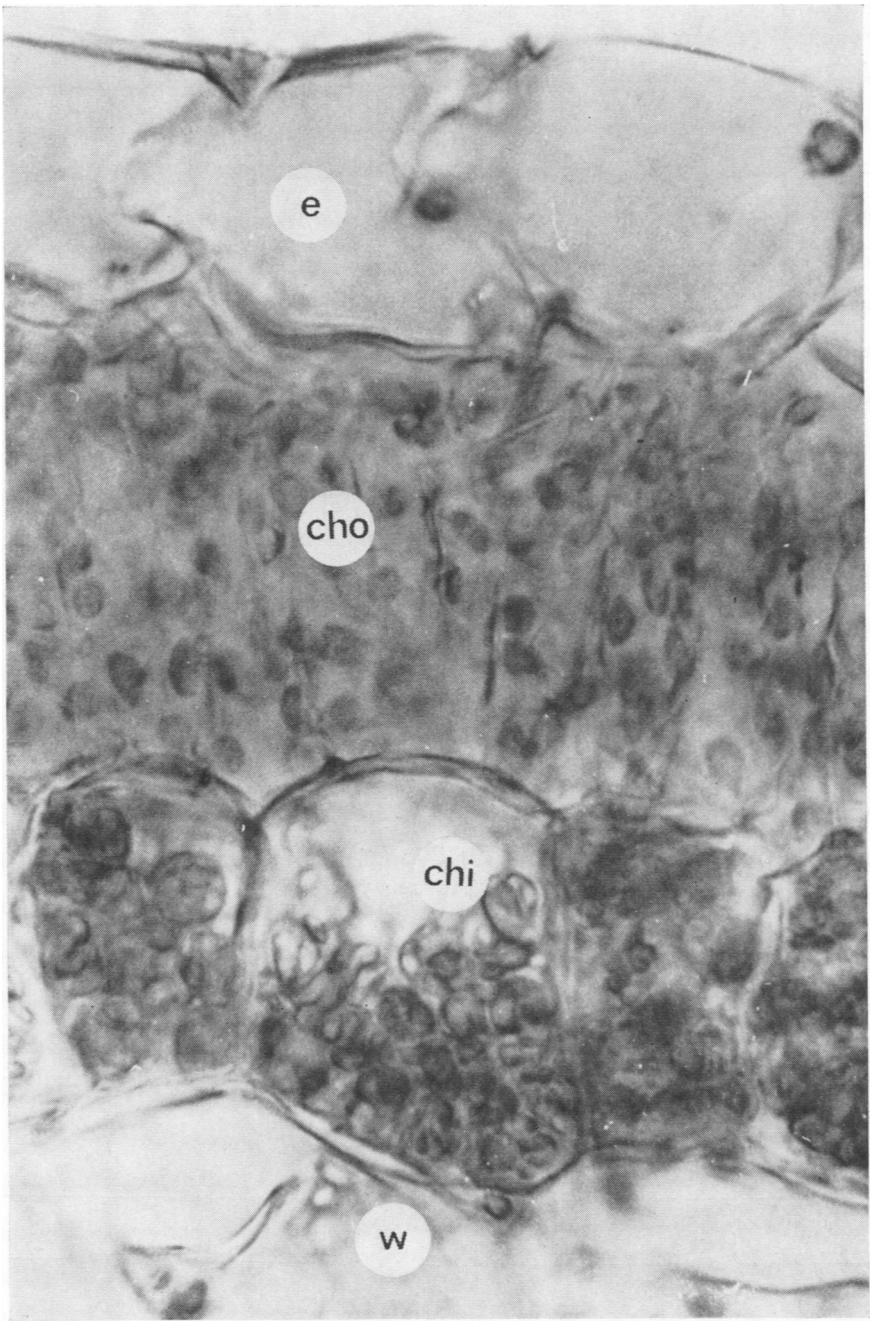

FIG. 2. Magnified section of the leaf shown in Fig. 1 (740 ×). e: epidermis; cho: outer layer of chlorenchyma; chi: inner layer of chlorenchyma with centripetally arranged chloroplasts; w: water tissue.

1.8×10^4 ergs $\times$ $cm^{-2} \times sec^{-1}$ light intensity, Sylvania white and cool white VHO lamps supported by incandescent light; 25 C day and 18 C night temperature; 70% relative humidity). Two types of nutrient solutions were used: (*a*) full strength Hoagland solution (8) and (*b*) the same solution to which NaCl was added to a final concentration of 100 mM. The solutions were constantly aerated. Three- to four-month-old plants were used in the following analysis.

Phosphoenolpyruvate Carboxylase Activity. PEP-case[1] from 5 g leaf material was extracted and assayed as previously described (2). The assay mixtures were free of sodium chloride.

Carbon Isotope Ratios. The $^{13}C/^{12}C$ ratios ($\delta^{13}C$) were measured using a mass spectrometer. Values were calculated relative to a PDB limestone standard.

Malate Content. Malate was extracted by crushing and boiling 5 g of leaf material in 10 ml of H_2O for 20 min; 1.5 ml of the extract was assayed in 0.47 M glycine, 0.20 M hydrazine sulfate, 0.23 mM EDTA, 0.03 M NAD, and 50 IU malate dehydrogenase (Sigma). The reaction mixture was kept at pH 9.5. The final reaction volume was 3.2 ml. The reduction of NAD was measured spectrophotometrically at 340 nm.

CO_2 Compensation Point. Compensation points were measured using an IRGA (Beckman Model 865) at saturating light intensities.

Short Time Light Fixation. Branches of *Suaeda monoica* were

[1] Abbreviations: PEP-case: phosphoenolpyruvate carboxylase; IRGA: infrared gas analyzer; CAM: Crassulacean acid metabolism.

Table I. *Some Characteristics of Carbon Metabolism of Suaeda monoica and Chloris gayana*

Data are means, in some cases ± standard deviation.

Characteristics	*Suaeda monoica*		*Chloris gayana*
	High salt	Low salt	
PEP-case activity (μmoles $CO_2 \times$ mg protein$^{-1} \times$ min^{-1})	0.49	0.34	0.51
CO_2 compensation concentration (μl/l)	<5	5	<5
$\delta^{13}C$ (0/00)	−15.89	−17.02	−14.84 to −17.62
Malate content (μmoles × g dry weight^{-1})			
Light (6 hr)	125 ± 10	440 ± 75	
Dark (6 hr)	120 ± 12	360 ± 60	

exposed to $^{14}CO_2$ for 5 sec. The plants were then flushed with $^{12}CO_2$ for different time periods. Leaves were killed with liquid nitrogen. Labeled compounds were extracted in 80% ethanol at pH 4 and then in H_2O. Extract constituents were separated by TLC according to Feige *et al.* (6). The chromatographic plates were then autoradiographed. The specific spots were removed, and ^{14}C activity was determined by liquid scintillation spectrophotometry.

RESULTS

Leaf Anatomy. *Suaeda monoica* plants have succulent and semicylindrical leaves. Two different types of chlorenchyma were recognized underneath the epidermis: an outer layer with relatively small chloroplasts and an inner chlorenchymatous layer which contains many large chloroplasts in a centripetal arrangement (Figs. 1 and 2). The center of the leaf was comprised of several layers of a parenchymatous water tissue embedding the vascular bundles. No bundle sheath formation was observed. The cells of the water tissue contained few scattered chloroplasts which were smaller than those of the inner layer of the chlorenchyma, but resembled those of the outer chlorenchymatous layer.

No structural dimorphism between chloroplasts of the different green layers could be seen. Electron micrographs showed that both types of chloroplasts contained grana and accumulated starch.

Phosphoenolpyruvate Carboxylase. The initial carbon fixation reaction in C_4 plants is catalyzed by PEP-case. As it was shown that the pH optimum for this enzyme varied in halophytes under different salt treatments (18, 19), it was necessary to determine the pH optimum in *Suaeda* before measuring enzyme activity. Optimal activities of PEP-case extracted from salt-treated plants were obtained at pH 7.9, whereas values obtained for salt-depleted plants were lower (pH 7.5).

By comparing the specific activity of PEP-case extracted from salt-treated *Suaeda monoica* leaves with that extracted from *Chloris gayana*, a known C_4 plant, Andrews *et al.* (1) showed that the values were approximately equal. The activity in salt-depleted *Suaeda* plants was about 15% lower (Table I).

Carbon Isotope Ratios. Examination of the $^{13}C/^{12}C$ ratios in *Suaeda monoica* leaves following different salt treatments and at different leaf ages yielded values between −15 and −17 $\delta^{13}C$ ‰. Similar values were obtained for *Chloris* plants grown under the same conditions.

Malate Content. The possibility of *Suaeda monoica* being a CAM plant was investigated by analyzing the variation in malate content of the leaves during consequent periods of light and dark. The results presented in Table I show that no excess malate had accumulated during the dark period. This pattern was unchanged in plants grown under long (16 hr) or short (9 hr) photoperiods.

CO_2 Compensation Point. Measurements showed that salt-depleted *Suaeda monoica* plants had a CO_2 compensation point of 5 μl/l. The compensation point of salt-treated plants was even lower (Table I).

Short Time Light $^{14}CO_2$ Fixation. The best proof for the existence of the C_4 pathway is the determination of the primary products of photosynthesis. Results of short time light fixation experiments in salt-treated *Suaeda* plants show that after 5-sec exposure to $^{14}CO_2$ a typical C_4 pattern of labeling was found: 85% of the label was found in aspartate, 8% in malate, and 3% in alanine and serine (Fig. 3). Different chase periods in $^{12}CO_2$ following the 5-sec pulse of $^{14}CO_2$ resulted in a rapid decrease in the

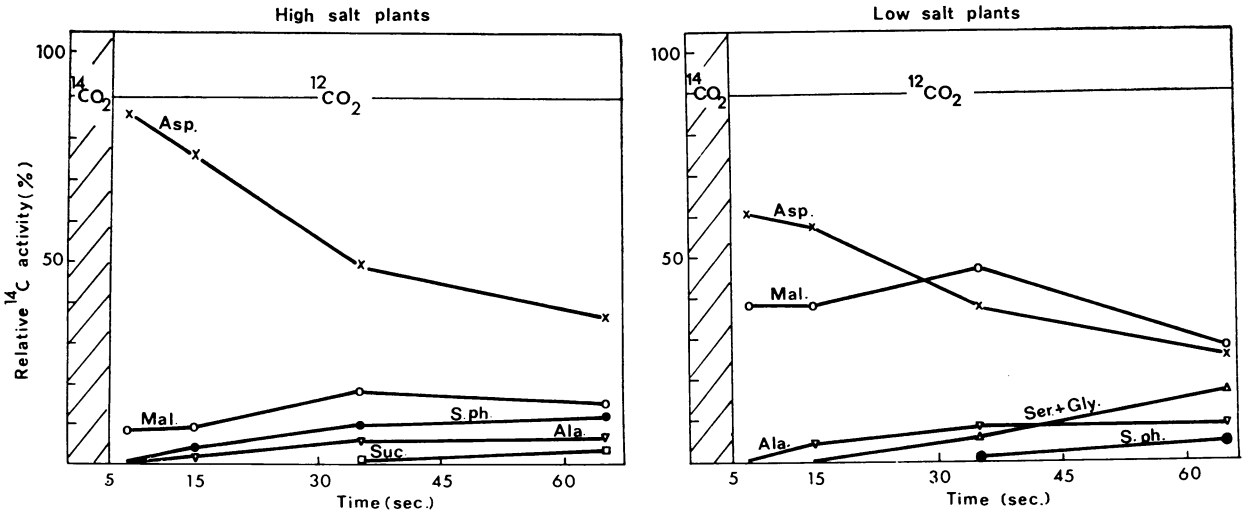

FIG. 3. Time course of ^{14}C labeling pattern. Approximately 20,000 cpm were applied to each plate. Data presented in fractions of the total recovered ^{14}C. Asp: aspartate; Mal: malate; S.ph.: phosphorylated metabolites; Ala: alanine; Suc: sucrose; Ser + Gly: serine + glycine.

content of labeled aspartate and in the appearance of the label in phosphorylated metabolites and sucrose.

DISCUSSION

The various characteristics which were investigated are generally believed to be important criteria for proving that a plant is of the C_4 type. The low discrimination values against ^{13}C, obviously resulting from the high activity of PEP-case, show that *Suaeda monoica* could have been either a C_4 or a CAM plant. If this species could perform Crassulacean acid metabolism, a considerable accumulation of organic acids, mainly malate, in the dark would be expected. The lack of such accumulation in the dark evidently shows that *Suaeda monoica* is not a CAM plant. Furthermore, the low CO_2 compensation point in light found in *Suaeda monoica* is another indication that this is a C_4 rather than a CAM plant.

The labeling pattern which follows short time light fixation is again typical for C_4 plants. In this case the flow of carbon seems to go more via aspartate than via malate.

A higher content of malate was found in the salt-depleted as compared with the salt-treated plants (Table I). Relatively more malate appeared in the salt-depleted plants also as the first product of light fixation. Thus, NaCl seems to affect the balance between the malate and aspartate formation in *Suaeda monoica*.

It is not known whether the special structural differentiation of the chlorenchyma in *Suaeda* is what enables the C_4 metabolism of this plant. If it does, it is evident that the location of a chlorenchymatous cell layer near the vascular bundles is not essential. C_4 metabolism can exist therefore even in plants with such chlorenchyma located at some distance from the vascular bundles.

A similar case was reported for the C_4 plant *Salsola kali* (15). The appearance of small vascular bundles in the periphery of the water tissue indicates that, unlike in *Suaeda*, the inner chlorenchymatous layer of *Salsola* leaves is a true bundle sheath formation.

We conclude that two types of chlorenchymatic cells may be necessary for a successful operation of the C_4 carbon fixation pathway. However, there is no absolute requirement for the Kranz cells (4) to be associated with the vascular bundles. The commonly accepted structural description of C_4 plants should therefore be somewhat modified.

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