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Flaveria pringlei (C_3) and *Flaveria trinervia* (C_4) under NaCl stress

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Abstract

The C_4 species *Flaveria trinervia* is obviously better adapted to saline environments than the C_3 species *F. pringlei*. Treatment with 100 mM NaCl diminished crop growth rate in *F. pringlei* by 38 % but not in *F. trinervia*. Under saline conditions, more assimilates were invested in leaf growth in *F. trinervia* but not in *F. pringlei*. Electrolyte concentration in *F. trinervia* in control and salt treated plants is lower than in *F. pringlei*. Fluorescence data do not indicate a damage of PS 2 charge separation in both species. Whether the C_4 photosynthetic pathway in *F. trinervia* is responsible for the improved salt tolerance compared to *F. pringlei* remains an open question.

Key words: assimilates, chlorophyll, electrolytes, fluorescence, growth analysis, isotope discrimination, photosystem 2, stomata

Introduction

The saline environments could have been an ecological niche which favoured genotypes exhibiting C_4 photosynthesis. Distribution of C_4 *Atriplex* species and their obvious preference of saline habitats support this assumption (Osmond *et al.* 1980). Powell (1978) reported in his monography of the genus *Flaveria* (*Asteraceae*) the salinity of natural habitats of *F. australasica* (C_4), *F. campestris* (C_4), *F. trinervia* (C_4), *F. brownii* (C_4-like), *F. chloraefolia* (C_3-C_4), *F. floridana* (C_3-C_4) and *F. oppositifolia* (C_3-C_4).

Although adaptation to salinity is a complex phenomenon (for review see *e.g.* Poljakoff-Mayber and Gale 1975, Greenway and Munns 1980, Wainwright 1980, Staples and Toenniessen 1984) and many C_3 species are well adapted to saline conditions too, it seems worthwhile to compare closely related species, exhibiting C_3 and C_4 photosynthesis, with regard to their salt tolerance.

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Materials and methods

Plants of *Flaveria pringlei* Gandoger (C_3) and *F. trinervia* (Spreng.) C. Mohr (C_4) were grown from seeds in a growth chamber [irradiance 450 μmol(PAR) m^{-2} s^{-1}, photoperiod 16/8 h, relative humidity of 70 % and day/night temperature of 21/16 °C]. Plants with two pairs of fully developed leaves were planted in plastic pots (600 cm^3) in a mixture of compost and sand. Soil water in the NaCl treated group was exchanged by daily watering with 100 mM NaCl solution during the first 4 d after planting. Thereafter they were normally watered as the controls. Soil water content was held constant at 70 % water holding capacity by weighing. Each treatment was run with 10 replicates.

Growth analysis data were calculated from dry matter and leaf area from the first and second harvest (15 and 22 d after planting) using the formulae given by Květ *et al.* (1971). Leaf area was determined by a leaf area meter (*LI 3000, LICOR,* Lincoln USA).

Chlorophyll *a+b* concentrations were measured by the method described by Porra *et al.* (1989). Chlorophyll *a/b* ratios were determined fluorometrically according to Meister (1992).

Fluorescence measurements: Prior to chlorophyll fluorescence measurements, plants were dark-adapted for 8 h. Leaf fluorescence was measured with a *PAM 100 fluorometer* (*H.Walz GmbH*, Effeltrich, Germany). Initial fluorescence, F_0, was determined with low excitation irradiance of 1.6 kHz modulation frequency. Maximum fluorescence, F_m, was obtained during saturating pulses of 0.7 s length and irradiance of 1400 μmol m^{-2} s^{-1}, delivered from a *Schott KL 1500* light source (*Schott*, Wiesbaden, Germany). Variable fluorescence, F_v, was calculated as $F_m - F_0$.

Electrolyte determination: Pulverized plant dry matter was boiled with tridistilled water and gently shaken for 24 h. Electrolyte content of the supernatant was measured with a conductivity meter (*LF 2000*, electrode *LTA 1*, *WTW*, Weilheim, Germany), calibrated with NaCl solutions.

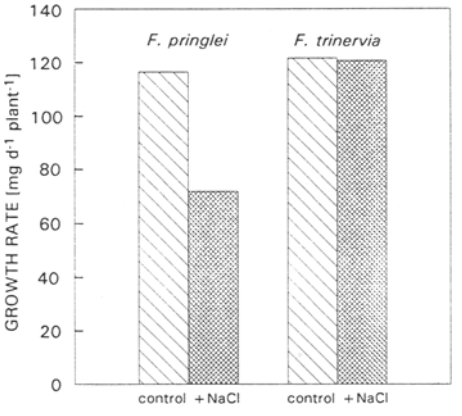
Isotope discrimination: $\delta^{13}C$ values were determined from pulverized leaf dry matter by mass spectrometry using PDB as a reference. Standard error of determination was 0.15 ‰.

Results

Growth analysis: Growth in *F. pringlei* was severely inhibited by 100 mM NaCl but not in *F. trinervia* (Fig. 1). Inhibition in *F. pringlei* was caused by reduced net assimilation rate (NAR) and relative growth rate (RGR) whereas growth of leaf area was not affected (Table 1). In *F. trinervia,* NAR and RGR were only slightly

influenced by salt treatment but relative leaf growth rate was drastically enhanced. Specific leaf mass (SLM) in both species was slightly decreased by NaCl.

Shoot-root ratio in salt treated plants was generally lower as compared with controls but only in *F. trinervia* the differences were significant ($P < 0.05$). Generally in *F. trinervia* the ratios were lower than in *F. pringlei* (Fig. 2).



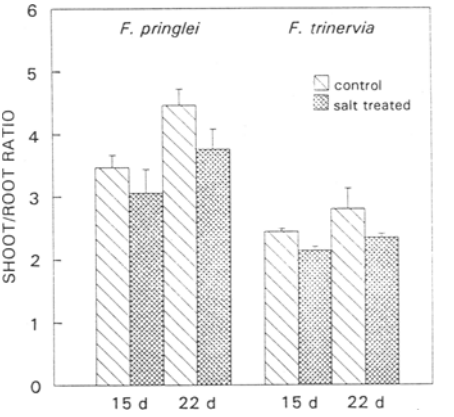


Fig. 1 (*left*). Crop growth rate in *F. trinervia* and *F. pringlei*. Time interval: 15th to 22nd day after salt treatment (100 mM NaCl).

Fig. 2 (*right*). Shoot/root ratio in *F. trinervia* and *F. pringlei* 15 and 22 d after salt treatment.

Stomata: Leaves of *Flaveria* species are amphistomatous. The absolute values depend on growth conditions. The differences between *F. trinervia* and *F. pringlei* were significant ($P < 0.05$) and there was a small but significant decrease in stomatal number in both species by salt treatment (Table 1).

Chlorophyll (*a*+*b*) content in both species increased between 15 and 22 d after treatment in both species as is expected during leaf development. The differences between NaCl treated and control plants were insignificant (Table 1). It is remarkable that salinity stress decreased chl *a*/*b* ratio in *F. pringlei* whereas it was increased in *F. trinervia*. Since bulk part of chl *b* is organized in the LHC 2 complex and core complexes of PS 1 and PS 2 are chl *b*-free (Siefermann-Harms 1985) a decrease of chl *a*/*b* ratio may indicate a decrease in core complexes relative to LHC 2.

Fluorescence: The ratio of variable to maximum fluorescence (F_v/F_m), a measure of the intrinsic efficiency of energy conversion by PS 2, was higher in the C_3 species compared to the C_4 species (Table 1). We have observed such pattern for other species of *Flaveria* (data not shown) and suggest that the lower F_v/F_m ratio in C_4 species is a result of increased contribution of PS 1 emission to room temperature fluorescence (*cf.* Genty *et al.* 1990). No significant effect of salt treatment on F_v/F_m ratio was observed indicating that salinity did not inhibit PS 2 energy conversion. Similar observations were made for cotton (C_3) by Brugnoli and Björkman (1992).

Electrolyte concentration (EC): EC in both species is increased by salt treatment (Fig. 3). In *F. pringlei* in both control and salt treated plants EC was higher than in *F. trinervia*. In both species EC 22 d after treatment was lower than 15 d after treatment. Perhaps this is a „dilution effect" due to the growth of the plants, which were irrigated only with water during the first and second harvest.

Table 1. Net assimilation rate (NAR), relative growth rate (RGR), relative leaf growth rate (RLGR), specific leaf mass (SLM), stomata frequency and distribution, chlorophyll (*a+b*) content and chl *a/b* ratio, variable to maximal fluorescence ratio (F_v/F_m) and carbon isotope discrimination ($\delta^{13}C$) in *Flaveria priglei* (C_3) and *F. trinervia* (C_4) under salt stress (100 mM NaCl).

Parameter	Treatment [d]	*F. pringlei* control	NaCl	*F. trinervia* control	NaCl
NAR [mg cm^{-2} d^{-1}]	15 - 22	1.215	1.030	0.967	1.035
RGR [mg mg^{-1} d^{-1}]	15 - 22	0.130	0.114	0.095	0.102
RLGR [cm^2 cm^{-2} d^{-1}]	15 - 22	0.091	0.092	0.046	0.065
SLM [mg cm^{-2}]	22	6.88	6.29	4.94	4.59
Stomata number [mm^{-2}]	22	189.8	172.0	210.6	202.4
Abaxial/adaxial ratio	22	0.74	0.89	1.13	0.94
Chlorophyll *a+b*	15	0.465±0.0008	0.456±0.0001	0.546±0.0007	0.650±0.0004
[mmol m^{-2}]	22	0.667±0.0004	0.712±0.0002	0.819±0.0012	0.769±0.0006
Chl *a/b*	15	4.03 ±0.03	3.85 ±0.02	4.35 ±0.04	4.45 ±0.05
	22	4.29 ±0.02	4.11 ±0.07	4.23 ±0.04	4.54 ±0.03
F_v/F_m	22	0.842±0.019	0.826±0.009	0.767±0.007	0.790±0.006
$\delta^{13}C$ [‰]	15	-29.20	-29.15	-15.15	-15.60
	22	-29.15	-29.65	-15.60	-15.90

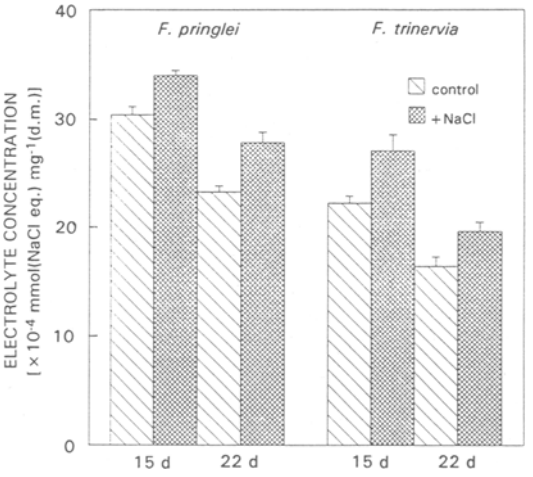


Fig. 3. Electrolyte concentration in *Flaveria priglei* and *F. trinervia* 15 and 22 d after salt treatment (100 mM NaCl).

Isotope discrimination: Contrary to the effects of salinity known from other C_3 species (*e.g.* Brugnoli and Lauteri 1991, Brugnoli and Björkman 1992, for other references see Farquhar *et al.* 1989) in *F. pringlei* no change (15d treatment) or even a small decrease (22 d) in the $\delta^{13}C$ value of leaf dry matter was found. In accordanc with the known small variability of carbon isotope discrimination in C_4 plants (Peisker and Henderson 1992) in *F. trinervia* the $\delta^{13}C$ values of control and salt treated plants differed only slightly (Table 1).

Discussion

Obviously, salt treatment had inhibitory effects on *F. pringlei*. Salt was taken up at a higher concentration as in the control plants. Growth was inhibited. Gas exchange measurements (data not shown) indicate a partial stomatal closure (perhaps osmotically induced), by which carbon gain was reduced. However, considering the measured $\delta^{13}C$ values any effect of salinity on the stomatal apparatus must be compensated or even exceeded by a concomitant effect on photosynthetic metabolism (Farquhar *et al.* 1982). Fluorescence data indicate that charge separation in PS 2 was not damaged. In *F. trinervia*, salt treatment with the concentration used in this experiment was not experienced as stress at all. Overall growth was not reduced; net assimilation rate and relative growth rate were slightly increased. The drastically enhanced rate of leaf area growth rate indicates a shift in allocation pattern in favour of development of the photosynthetic potential of the whole plant. Clearly, the electrolyte concentration within the plants was increased by salt treatment, but the level was generally lower than in *F. pringlei*. Therefore, *F. trinervia* cannot be evaluated as an ideal "salt excluder", but perhaps a type which can at least partially prevent salt uptake and besides this can tolerate a higher ion concentration without metabolic damage. With regard to the evolution of the C_4 photosynthetic pathway it must be assessed that in *F. trinervia* the special features of C_4 photosynthesis are not primarily responsible for the better salt tolerance as compared to the C_3 species *F. pringlei*. The results do not contradict the hypothesis that the C_4 pathway of photosynthesis is of adaptive value under saline conditions but the mechanism at the metabolic level remains unclear.

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