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Growth of cotton under continuous salinity stress: influence on allocation pattern, stomatal and non-stomatal components of photosynthesis and dissipation of excess light energy*

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Abstract. Cotton (*Gossypium hirsutum* L.) plants were grown in flowing-culture solutions containing 0%, 26% and 55% natural seawater under controlled and otherwise identical conditions. Leaf Na^+ content rose to 360 mM in 55% seawater, yet the K^+ content was maintained above 100 mM. The K^+/Na^+ selectivity ratio was much greater in the saline plants than in the control plants. All plants were healthy and able to complete the life cycle but relative growth rate fell by 46% in 26% seawater and by 83% in 55% seawater. Much of this reduction in growth was caused by a decreased allocation of carbon to leaf growth versus root growth. The ratio of leaf area/plant dry weight fell by 32% in 26% seawater and by 50% in 55% seawater while the rate of carbon gain per unit leaf area fell by only 20% in 26% seawater and by as much as 66% in 55% seawater. Partial stomatal closure accounted for nearly all of the fall in the photosynthesis rate in 26% seawater but in 55% seawater much of the fall also can be attributed to non-stomatal factors. As a result of the greater effect of salinity on stomatal conductance than on CO_2-uptake rate, photosynthetic water-use efficiency was markedly improved by salinity. This was also confirmed by stable-carbon-isotope analyses of leaf sugar and of leaf cellulose and starch. – Although non-stomatal photosynthetic capacity at the growth light was reduced by as much as 42% in 55% seawater, no effects were detected on the intrinsic photon yield of photosynthesis nor on the efficiency of photosystem II photochemistry, chlorophyll a/b ratio, carotenoid composition or the operation of the xanthophyll cycle. Whereas salinity caused in *increase* in mesophyll thickness and content of chloroplast pigments it caused a *decrease* in total leaf nitrogen content. The results indicate that the salinity-induced reduction in non-stomatal photosynthetic capacity was not caused by any detrimental effect on the photosynthetic apparatus but reflects a decreased allocation to enzymes of carbon fixation. – Rates of energy dissipation via CO_2 fixation and photorespiration, calculated from gas-exchange measurements, were insufficient to balance the rate of light-energy absorption at the growth light. Salinity therefore would have been expected to cause the excess excitation energy to rise, leading to an increased non-radiative dissipation in the pigment bed and resulting increases in non-photochemical fluorescence quenching and zeaxanthin formation. However, no such changes could be detected, implying that salinity may have increased energy dissipation via a yet unidentified energy-consuming process. This lack of a response to salinity stress is in contrast to the responses elicited by short-term water stress which caused strong non-photochemical quenching and massive zeaxanthin formation.

Key words: Carbon isotope discrimination – Energy dissipation – *Gossypium* – Growth (under salinity) – Photosynthesis (under salinity) – Salinity stress – Stomatal closure – Xanthophyll cycle

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Abbreviations and symbols: A = net rate of CO_2 uptake; A_c = calculated rate of CO_2 uptake at constant p_i; Chl = chlorophyll; E = rate of transpiration; EPS = epoxidation state of xanthophyll cycle components; F, F_m = fluorescence emission at the actual, full reduction of PSII reaction centers; F_v = variable fluorescence; g_s = stomatal conductance to water vapor; g_w = conductance to CO_2 transfer from intercellular spaces to chloroplasts; NPQ = non-photochemical fluorescence quenching; p_a, p_i, p_c = atmospheric, intercellular and chloroplastic partial pressures of CO_2; PCRO = photosynthetic carbon reduction and oxygenation cycle; P(ET) = sum of the rate of carboxylation and oxygenation; PFD = photon flux density; PSII = photosystem II; V + A + Z = pool size of xanthophyll cycle components; $\delta^{13}C$ = carbon-isotope composition; φ_{PSII} = photon yield of PSII photochemistry at the actual reduction state in the light

Introduction

Crop yield often is strongly reduced by soil salinization. Although species differ in their tolerance to salinity, plant

growth is ultimately affected (for a review, see Munns and Termaat 1986). The underlying causes are complex and can range from changes in allocation patterns such as an increased allocation to root at the expense of leaf growth (Delane et al. 1982); to partial stomatal closure caused by an accompanying water stress which results in a decreased rate of photosynthesis per unit leaf area (Robinson et al. 1983; West et al. 1986; Flanagan an Jefferies 1989; Brugnoli and Lauteri 1991); to detrimental effects on the photosynthetic system, caused by excessive salt build-up and-or an ionic imbalance in the leaves (Yeo et al. 1985). The K^+ content may be especially critical in maintaining the functional integrity of the photosynthetic system (Chow et al. 1990). The relative contribution of each of these groups of factors to the salt-induced decrease in growth rate is unclear. It is probable that it may depend on the species and on the manner in which the salinity stress is imposed. However, assessments are difficult since studies have focussed either on overall growth rate and allocation patterns, or growth and ion uptake, or leaf photosynthesis and stomatal conductance, while comparisons of the impact on growth of the relative effects of salinity of each of these factors in the same organism and under similar conditions are lacking.

A consequence of salt-induced decreases in photosynthetic rate, not previously addressed, is the fate of the accompanying increases in excess excitation energy, i.e., that portion of the light energy absorbed by the light-harvesting system not consumed by carbon fixation and photorespiration. Such excess energy is potentially harmful to the photosynthetic system (photoinhibition). It is now a widely held view that much of this energy is normally dissipated via non-radiative energy dissipation in the pigment bed, reflected in non-photochemical quenching of chlorophyll fluorescence (for reviews, see Björkman 1987; Demmig-Adams 1990; Krause and Weis 1991). Development of excessive energy is also accompanied by de-epoxidation of violaxanthin to zeaxanthin and there is increasing evidence that zeaxanthin is involved in mediating non-radiative energy dissipation although the mechanism is not known (Demmig et al. 1987; Bilger et al. 1989; Bilger and Björkman 1990, 1991; Noctor et al. 1991; for a review, see Demmig-Adams 1990).

Table 1. Water potential, Na^+/K^+ ratio and concentration of macronutrients in the flowing-culture solutions

	Seawater, %		
	0	26	55
Water potential, bar	−0.4	−5.8	−15.6
Na^+/K^+, mol · mol^{-1}	0.33	15.2	29.8
Na^+, mM	1.0	131	277
K^+, mM	3.0	8.6	9.3
Mg^{2+}, mM	1.0	14.6	29.7
Ca^{2+}, mM	1.3	3.9	6.8
NH_4^+, mM	0.5	0.5	0.5
Cl^-, mM	1.5	155	324
SO_4^{2-}, mM	1	8	16
NO_3^-, mM	2.5	2.5	2.5
HPO_4^{2-}, mM	1.5	1.5	1.5

The objective of the present study was to provide a comprehensive basis for assessing the effects of salinity on growth, resource allocation, photosynthesis, water-use efficiency and energy dissipation in cotton, a crop considered to be moderately salt tolerant. To achieve this objective different approaches were combined, ranging from classical growth analysis, through determinations of photosynthetic gas-exchange characteristics, carbon-isotope discrimination and chloroplast pigment composition, to measurements of chlorophyll fluorescence, light-induced absorbance changes and zeaxanthin formation. Salinity stress was imposed by addition of seawater to flowing culture solutions as this seems more realistic than addition of NaCl alone; it is noteworthy that seawater contains relatively high concentrations of Ca^{2+} and K^+ which may be beneficial at high NaCl concentrations (Cramer et al. 1986; Chow et al. 1990).

Material and methods

Plant material and growing conditions. Seeds of upland cotton (*Gossypium hirsutum* L., cv. Acala SJC–1), obtained from California Planting Cotton Seed Distributor (Bakersfield, USA), were germinated in a perlite-vermiculite mixture (1/1, v/v) watered with nutrient solutions at different seawater concentrations. Perlite and vermiculite (#2 Coarse) were obtained from Therm-O-Rock Industries (Chandler, Ariz., USA). After emergence of cotyledons, young seedlings were transferred to two identical flowing nutrient-culture systems as described by Schäfer and Björkman (1989). Briefly, each of the systems consisted of a network of 2–1 cylinders through which a culture solution from a 200-l tank was passed at a rate of 1.5–2 l · min^{-1} per cylinder. Culture solutions were prepared either from deionized water (control) or from seawater obtained freshly from the Steinhart Aquarium, California Academy of Science, San Francisco. Different seawater concentrations were obtained by mixing appropriate proportions of 0% and 100% seawater solutions. The macronutrient compositions and water potentials of the culture solutions are given in Table 1. K^+ and Na^+ concentrations were determined as described below and solution water potentials were measured by a thermocouple psychrometer system (Model SC–10A; Decagon Devices, Pullman, Wash., USA). Other macronutrient compositions were calculated from the known amounts of added nutrients and the mean macronutrient concentration of seawater provided by the supplier. The culture solutions also contained standard amounts of micronutrients and pH was 6.5–6.9. Total ionic strengths of the flowing solutions were checked daily by measuring the solution conductivity with a calibrated Model 1481–60 conductivity meter and model 1481–64 platinum cell (Cole-Parmer Instruments Co., Chicago, III., USA). When the conductivity deviated from standard values by 3%, fresh culture solution at the appropriate seawater concentration was added to the system so that the standard conductivity was restored. Because of the large volume (200 l) of each culture solution it is likely that nutrient concentration remained essentially unchanged during the interval between the additions of fresh culture solutions.

Plants were grown in an environmentally controlled growth room (Schäfer and Björkman 1989). Light was provided by a bank of 10 multivapor lamps (Metalarc 1000 W, phosphor coated; GTE, Sylvania, Winchester, Ky., USA). The daily light period was 14 h and the photon flux density (PFD) at the height of leaves used for experiments was 670–800 μmol · m^{-2} · s^{-1} (34–40 mol photon · m^{-2} · d^{-1}). Air temperature was 30 ± 1° C in the light and 24 ± 1° C in the dark periods. Culture-solution temperature varied from 34 ± 1° C in the light to 24 ± 1° C in the dark. Air relative

humidity ranged between 35% and 42%. Plants were spaced so that mutual shading among neighboring plants was avoided.

In each experiment, two salinity treatment were compared, taking advantage of the two separate flowing nutrient-culture systems. In Exp. I, cotton plants grown in 26% seawater were compared with those grown in 0% seawater. Plants were germinated directly in the final seawater concentration (0% and 26%) as described above and then transferred to the flowing systems. In Exp. II, plants grown in 26% were compared with those grown in 55% seawater solutions. Because of the very slow germination rate in 55% seawater, seeds were germinated in 26% seawater, then after root emergence, the concentration was increased to 40%, and finally the seedlings (still at the cotyledon stage) were transferred to the 55% flowing-seawater culture. In order to obtain plants of more similar size and developmental stage, germination of seeds in the flower salinity treatment was started 7–10 d later than in the higher-salinity treatment. Fully expanded and exposed leaves were always used. Where no significant differences were observed between the two 26% seawater batches, results from Exp. I and II were pooled.

The plants used for the water-stress experiments shown in Fig. 9 were grown in 2.5-l pots containing a perlite-vermiculite mixture (1/1, v/v) and watered twice daily with standard 0% seawater solution. Plants were stressed by withholding the water supply to the roots. For comparison, acute salinity stress was imposed on hydroponically grown plants by sudden transfer from 0% to 26% seawater culture solutions. Both the water-stress and the acute salinity-stress treatments were conducted in the growth room, in order to minimize perturbation of plants by other factors. The PFD incident on each leaf was measured using a quantum meter (Li–185B and quantum sensor Li–192SB; Li Cor, Lincoln, Neb., USA). Water or salinity stress was imposed after collecting samples for pigment analysis and gas-exchange measurements. Special care was taken to keep plants at the same PFD before and after each transfer. In both the experiments leaves were restrained in a horizontal position with thin nylon threads. The time courses of gas-exchange characteristics and changes in the epoxidation state of the xanthophyll-cycle components were followed for at least 5 h (acute salinity stress) or 4 d (water stress).

Analysis of K^+, Na^+ and N. For measurements of leaf ion contents, discs (10 cm^2) were punched from leaves of different plant treatments and stored at −70° C before extracting with water. The extract was spun at 12000 · g for 2 min. Aliquots of the supernatant or of the culture solution were diluted with 1% Sterox solution (Perkin-Elmer, Instrument Division, Oak Brook, Ill., USA) and K^+ and Na^+ contents determined by flame emission spectrophotometry (Model Coleman S1–Ca; Perkin-Elmer, Oak Brook, Ill., USA). Leaf organic nitrogen content was determined on leaf discs (4.92 cm^2) according to Isaac and Johnson (1976) using an automated micro-Kjeldahl system (Alpkem, Clackmas, Ore., USA).

Growth analysis. Four plants per treatment were periodically harvested. Fresh weight and dry weight were determined for leaves, shoots and roots, and leaf area was measured with an area meter (Delta-T Devices, Cambridge, UK). Relative growth rate (RGR), net assimilation rate (NAR), also called unit leaf rate (ULR), and leaf area ratio (LAR) were calculated by classical growth analysis methods (Evans 1972, chpts. 13–19).

Gas exchange. Exchange of CO_2 and water vapor was measured on attached, fully expanded leaves, using a Li-Cor model 6200 portable photosynthesis system, incorporating a model 6250 infra-red gas analyzer and 1-l chamber (Li Cor). Each leaf was fully exposed and its natural orientiation was near normal to the light. This was also true for leaves used for measurements of carbon-isotope composition ($\delta^{13}C$), chlorophyll (Chl) fluorescence, absorbance changes and pigment, ion, and nitrogen contents, described below. The boundary-layer conductance to water-vapor transfer was 2.0 mol · m^{-2} · s^{-1} · bar^{-1}. The system was equilibrated in open mode and when no change was detected in the air-vapor pressure inside the chamber, the system was closed and measurements were taken. In the water-stress experiment stomatal conductance was measured using a steady-state porometer model Li–1600 (Li Cor). The stomatal conductances given here represent the sum of the conductances for the abaxial and the adaxial leaf surfaces.

Calculation of gas-exchange parameters were made according to von Caemmerer and Farquhar (1981). The sum of the rate of carboxylation and oxygenation (P(ET)) was calculated as

$$P(ET) = (A + R_d)(p_i + 2\Gamma^*)/(p_i - \Gamma^*),$$

where, A is the net CO_2 uptake, R_d is the dark-respiration rate, p_i the intercellular CO_2 partial pressure, and Γ^* the compensation point in absence of dark respiration. Γ^* was calculated from the leaf temperature according to Brooks and Farquhar (1985). The rate of linear electron transport through the carbon-reduction and carbon-oxidation cycles (PCRO) may be obtained by multiplying P(ET) by a factor of 4–4.5 (von Caemmerer and Farquhar 1981).

Rates of photosynthetic O_2 evolution were measured using a leaf-disc oxygen electrode as described in detail by Björkman and Demmig (1987). The CO_2 concentration inside the leaf-disc chamber was kept at 5% by flowing a mixture of 5% CO_2 in air before closing the chamber and recording the rate of O_2 evolution.

Carbon-isotope analysis. Leaves were collected in the evening of the days when gas exchange had been measured. Soluble sugars were extracted as described by Brugnoli et al. (1988). Whole leaves were extracted in boiling water for 30 min and then spun at 12000 · g for 10 min. The supernatant was passed through Dowex-50 (H^+ form) and then through Dowex-1 (Cl^- form) resins (Sigma Chemical Co., St. Louis, Mo., USA) so that amino acids and organic acids were eliminated from the extract. After filtration, the eluate was freeze-dried and packed in dry ice. The insoluble fraction was oven-dried at 75° C. The samples were then air-shipped to the Department of Botany at Duke University, Durham, N.C., USA for carbon-isotope analysis ($\delta^{13}C$). This was as previously described (Brugnoli and Lauteri 1991). Internal precision for individual measurements of $\delta^{13}C$ was always greater than $0.01 \cdot 10^{-3}$.

Fluorescence and absorbance-change measurements. Fluorescence measurements were made on fully expanded cotton leaves using a PAM chlorophyll fluorometer (Walz, Effeltrich, FRG), as described by Schäfer and Björkman (1989) with slight modifications. The leaf temperature was controlled by circulating water in a heat exchanger from an external temperature-controlled water bath, as described in detail by Bilger and Björkman (1991). Non-photochemical fluorescence quenching was calculated according to the Stern-Volmer equation (see Bilger and Björkman 1990) as $NPQ = F_m/F'_m - 1$, where F_m and F_m' are the fluorescence yields when all PSII reaction centers are temporarily closed following a saturating pulse of light before and after exposure to actinic light, respectively. Photochemical quenching was calculated according to Bilger and Schreiber (1986), and the actual photon yield of PSII in the light (φ_{PSII}) from the equation $\varphi_{PSII} = (F'_m - F)/F'_m$ where F is the fluorescence at the actual reduction state of PSII reaction centers in the light (Genty et al. 1989; Bilger and Björkman 1990).

Light-induced spectral absorbance change was measured using a photodiode array spectroradiometer (Model SE–590; Spectron Engineering, Denver, Colo., USA). The system was operated using the same leaf holder and optical system as was used for fluorescence measurements. An additional fiber-optic was placed vertically at about 2 mm from the lower leaf surface, in order to collect and guide the light transmitted through the leaf to the measuring head of the spectroradiometer. A data acquisition and control system (3421A; Hewlett Packard, Palo Alto, Cal., USA) was used to collect the digital data from the spectroradiometer and to trigger the shutter opening and closure. Measuring light was provided by the same light source as was used for fluorescence measurements. To reduce the intensity in the green region, where leaves have high transmittance, a magenta CC 40M filter (Eastman Kodak, Rochester, N.Y., USA) was inserted in the measuring beam. In both fluorescence and absorbance-change measurements, a red dichroic filter (OCLI, Santa Rosa, Cal., USA) was used in the actinic light

beam to minimize the interference of blue-light-induced chloroplast movements, which can be considerable, particularly in the absorbance-change measurement (Brugnoli and Björkman 1992). The acquisition of each spectrum involved the following computer-controlled sequence: the actinic red light was turned off and concurrently the measuring light was turned on by opening the electronic shutter (Uniblitz 2141; Vincent Associates, Rochester, N.Y., USA), the spectrum was taken by the spectroradiometer, the shutter was closed, and the actinic light was again turned on. The entire sequence took 0.8–1.2 s, depending on flash intensity and leaf transmittance. The spectrum was taken at 2.4-nm intervals from 415 to 852 nm. The absorbance change caused by de-epoxidation of violaxanthin to zeaxanthin peaked at 508–512 nm. The net ΔA_{540}, attributed to a ΔpH-dependent increase in light scattering, was calculated by subtracting the direct contribution of zeaxanthin formation at this wavelength. This contribution was obtained by linear regression between the gross ΔA_{540} and ΔA_{510} during recovery in the dark (when the 540-nm component had completely recovered and only the contribution of the tail of the 510-nm absorbance change was present). ΔA as used here is the sum of changes in absorption and reflectance.

Pigment analysis. Leaf discs (0.82–1.64 cm^2) were punched from leaves, frozen in liquid nitrogen, and stored at −70° C. Extraction and high performance liquid chromatography (HPLC) analysis of pigment composition was as described by Thayer and Björkman (1990). Epoxidation state (EPS) of xanthophyll-cycle components was calculated as EPS = (V + 0.5A)/(V + A + Z), where V, A and Z are the contents of violaxanthin, antheraxanthin and zeaxanthin. Samples for pigments composition of undisturbed cotton leaves were always collected at noon.

Light response of epoxidation state of the xanthophyll-cycle components. Intact fully expanded leaves of plants grown at different salinity concentrations were selected so that the incident PFD was in the range 680–720 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Plants were transferred to a cylinder containing a continuously aerated nutrient solution at the appropriate seawater concentration. The solution temperature was kept at 28° C. A leaf was inserted into the leaf chamber of an open gas-exchange system as previously described (Schäfer and Björkman 1989). Before starting experiment, the EPS of the leaf was measured by HPLC. Leaf temperature was maintained at 30° C. The CO_2 and O_2 partial pressures were kept at 340 μbar and 210 mbar, respectively. Measurements of light-response curves were started at the lower PFD and the PFD was then increased step-by-step by changing the neutral-density-filter combination between the light source and leaf chamber. The leaf was kept at each light level for 35 min before punching a leaf disc (0.82 cm^2) for HPLC analysis, and for an additional 5-min period before increasing the PFD. Each disc represented approx. 1% of the total area of each leaf. Tests showed that this punching of discs did not affect the EPS of the remaining leaf.

Results

Although the growth of the cotton plants was retarded by salinity, especially in 55% seawater, all plants appeared healthy at all developmental stages. In spite of their small size, plants grown in 55% seawater, were able to flower and to produce cotton bolls and seeds (not shown).

Leaf K^+ and Na^+, and K^+/Na^+ selectivity ratio. As shown in Table 2, leaf Na^+ concentration in the bulk mesophyll water strongly increased with increasing external salinity level, from 13.6 mM in control leaves to 362 mM in leaves of plants grown in 55% seawater. The increase of leaf Na^+ concentration was proportional to the external Na^+ concentration in the 26% seawater treatment while it was more than proportional in the 55% seawater treatment. Because of increased leaf succulence, the relative increase in Na^+ content on a dry-weight basis was greater than the increase in the Na^+ concentration in the bulk leaf water. In leaves from plants grown at 55% seawater salinity. NaCl accounted for as much as 15% of total dry matter.

On the basis of dry weight, K^+ content remained constant in the 0% and 26% seawater treatments and actually rose in the 55% seawater plants. In all treatments the K^+ concentration in the bulk leaf solution exceeded 100 mM although it was highest in the control plants. As expected, the Na^+/K^+ ratio in leaves was much higher in salt-stressed than in control leaves (Table 2). The selectivity ratio, expressed as the ratio of K^+/Na^+ in leaves divided by the K^+/Na^+ in the culture solution, was much higher in the saline treatments than in control plants.

Growth analysis. In Fig. 1 are shown the time courses of dry-weight accumulation for plants grown at 0%, 26% and 55% seawater. Salinity reduced the growth rate during the entire growth period. The initial lag phase of dry-weight accumulation was prolonged in salinity-stressed plants, and was particularly pronounced in 55% seawater. Beyond this lag phase the growth rate was reduced to approximately one-half in 26% seawater and to one-fifth in 55% seawater.

Salinity also had strong influence on dry-weight allocation patterns. The proportion of dry weight allocated to roots compared with that allocated to leaves strongly increased with increasing salinity, as shown by the salt-induced decline of the slope of leaf-to-root dry weight (Fig. 2). The leaf-to-root dry-weight ratio varied from 3.1 in control plants to 1.5 and 1.1 in 26% and 55% seawater plants, respectively. In control plants, the relationship between leaf and root dry weight was curvilinear, such that with increasing plant dry weight, an increasing

Table 2. Leaf Na^+ and K^+ contents, and selectivity ratios, of cotton plants grown at different seawater concentrations. Each value is the mean of three independent determinations

Seawater (%)	Na^+ (mM)	Na^+ (mmol · (g DW)$^{-1}$)	NaCl (% of DW)	K^+ (mM)	K^+ (mmol · (g DW)$^{-1}$)	KCl (% of DW)	Na^+/K^+ (mol · mol^{-1})	Selectivity ratio	Water content. (% of FW)
0	14± 1	0.05±0.01	0.3±0.06	163± 7	0.59±0.04	4.3±0.32	0.09	3.9	78
26	127± 9	0.77±0.10	4.5±0.58	104±11	0.58±0.07	4.3±0.52	1.22	12.5	84
55	362±14	2.57±0.29	15.0±0.89	109±26	0.73±0.12	5.4±0.89	3.32	9.0	87

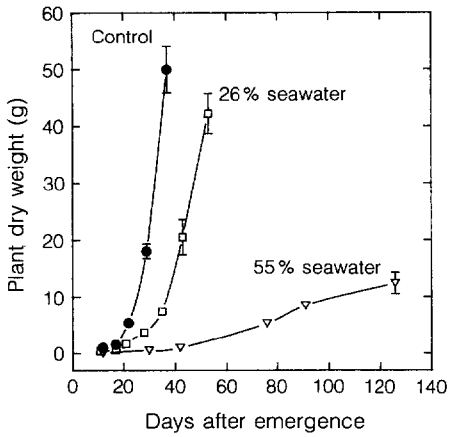

Fig. 1. Effect of salinity on plant dry-weight accumulation in cotton. Each value is the mean of four measurements on different individual plants. Standard errors, when larger than symbols, are shown as vertical bars. Symbols: ●, control; □, 26% seawater; ▽, 55% seawater

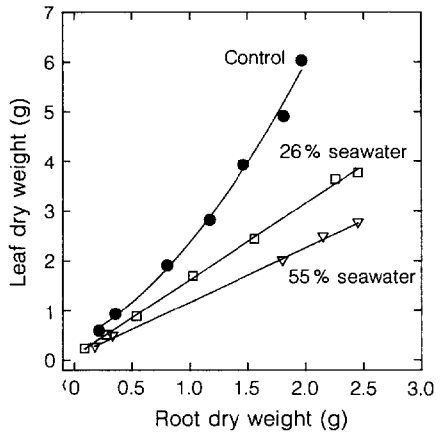

Fig. 2. Effect of salinity on the relationship between leaf dry weight and root dry weight in cotton plants grown at different salinity concentrations. Curves represent the best-fit relationship obtained by regression analysis. The relationships for the 25% and 50% seawater treatments are linear, that for the 0% seawater is a second degree regression. Each datum point is the mean of four measurements on different individual plants. Symbols: ●, control; □, 26% seawater; ▽, 55% seawater. $r^2 = 0.997$ for the control and 0.999 for the two salt treatments

proportion of the dry-weight gain was invested preferentially in leaves rather than in roots (Fig. 2). Such a progressive change in allocation pattern was not evident in the two salinity treatments; here the relationship between leaf and root dry weight was linear over the entire course of growth. It is noteworthy that the average ratio of root diameter to root length increased with increased salinity (data not shown).

The effects of salinity on relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR; leaf area per total plant DW) and specific leaf area (SLA; leaf area per leaf DW) are compared in Fig. 3. The RGR declined almost linearly with increasing salinity level, decreasing by 46% at the intermediate salinity and by 83% at the high salinity. The NAR decreased by only

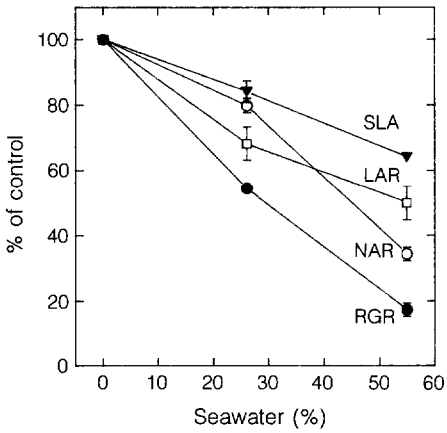

Fig. 3. Effect of salinity on net assimilation rate (NAR; g DW · m^{-2} · d^{-1}), relative growth rate (RGR; g DW · g^{-1} · d^{-1}), leaf area ratio (LAR; m^2 · (g plant DW)$^{-1}$ and specific leaf area (SLA; m^2 · g^{-1} leaf DW) of cotton plants grown at different salinity concentrations. Values are percent of control. The absolute values for control plants were: NAR = 18.3 ± 0.3, RGR = 0.215 ± 0.006, LAR = 0.01175 ± 0.0008, and SLA = 0.0271 ± 0.0009. Each datum point is the mean of three independent determinations made when the plants from the different treatment were of similar size (6–20 g DW per plant). Standard errors, when larger than symbols, are shown as vertical bars

20% at the intermediate salinity and by as much as 66% at the high salinity. The reduction in RGR at the intermediate salinity was caused by a 32% reduction in leaf area development (LAR) together with only a 20% reduction in NAR. At the high salinity, the relative contributions of NAR and LAR to the reduction in RGR were reversed: LAR decreased by 50% and NAR by as much as 66%. The SLA decreased by 16% and 38% at the intermediate and the high salinity, respectively (Fig. 3). The ratio of leaf-to-plant dry weight decreased about 20% in both salinity treatments, compared to control. A portion of the decrease in SLA was caused by an increased contribution of NaCl to the total leaf dry weight (Table 2). The SLA, adjusted for the increased salt contents, decreased by about 15% at the intermediate salinity and by about 31% at the high salinity.

Photosynthetic-rate and gas-exchange characteristics. To investigate the source of the salinity-induced reduction in NAR, measurements of net CO_2 uptake were made on individual leaves in situ (Table 3). Values for transpiration rate, stomatal conductance and other derived parameters are also included. There were no significant differences between means in most of the gas-exchange parameters of 26% seawater plants from Expts. I and II. However, since there was a slight, yet significant, difference in the p_i/p_a ratios in the 26% seawater treatment between Expts. I and II the data were not pooled in Table 3.

Highly significant differences in all gas-exchange parameters were found among all salinity treatments (Table 3). The rate of CO_2 uptake (A) was markedly reduced in 26% seawater and dramatically reduced in 55% seawater. The reductions of A by 20% in 26% seawater and by 60%

Table 3. Gas-exchange characteristics in situ of leaves of cotton plants grown at different salinity concentrations. Mean values and SE from Expt. I (0 vs. 26% seawater) and Expt. II (26 vs. 55% seawater). Differences between different treatments are always significant ($P \leq 0.005$). In the two 26% seawater treatments only the difference in p_i p_a ratio is significant ($P \leq 0.01$).

Parameter	Expt. I		Expt. II	
	0% Seawater ($n=38$)	26% Seawater ($n=38$)	26% Seawater ($n=37$)	55% Seawater ($n=42$)
A ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	14.1 ±0.4	11.3 ±0.3	11.3 ±0.2	5.7 ±0.2
E ($mmol \cdot m^{-2} \cdot s^{-1}$)	11.0 ±0.3	7.4 ±0.2	7.9 ±0.2	3.5 ±0.2
A/E ($mmol \cdot mol^{-1}$)	1.28	1.53	1.43	1.63
g_s ($mol \cdot m^{-2} \cdot s^{-1} \cdot bar^{-1}$)	0.443 ±0.018	0.224 ±0.007	0.213 ±0.007	0.084 ±0.004
p_i (μbar)	277 ±2	242 ±2	239 ±2	212 ±3
p_i/p_a	0.79 ±0.01	0.70 ±0.01	0.68 ±0.004	0.60 ±0.01

in 55% seawater (Table 3) are in good agreement with the observed salt-induced decrease of NAR obtained by growth analysis. On the assumption that respiratory losses by non-photosynthetic organs at day and by the whole-plants at night were approx. 4.3 g $DW \cdot m^{-2} \cdot d^{-1}$, the sum of NAR and respiratory losses calculated in terms of μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ would be 13.8 in the control, 11.2 in the 26% seawater and 6.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the 55% seawater; these values are close to the net CO_2 uptake (A) measured by gas exchange (Table 3).

Stomatal conductance to water-vapor exchange was strongly reduced by salinity, from 0.443 $mol \cdot m^{-2} \cdot s^{-1} \cdot bar^{-1}$ in the control, to 0.219 and to 0.084 $mol \cdot m^{-2} \cdot s^{-1} \cdot bar^{-1}$ in the 26% and in the 55% seawater, respectively (Table 3). The relative reduction of the stomatal conductance to water vapour (g_s) always exceeded that of A. As shown in Fig. 4 the relationship between A and g_s is curvilinear such that the salinity-induced decline in conductance was greater than that in A. Thus the photosynthetic water-use efficiency was improved by salinity (compare Fig. 4 and A/E in Table 3). Consistent with this observation, the mean p_i/p_a value decreased from 0.79 in control leaves to 0.69 in 26% and to 0.60 in 55% seawater (Table 3).

That increased salinity indeed resulted in an improved plant water-use efficiency was confirmed by the results from carbon-isotope analysis of the soluble sugars and non-soluble constituents (mostly cellulose and starch) in the leaf. To minimize any influence on the $\delta^{13}C$ by the presence of organic acids which may be derived from β-carboxylation of phosphoenolpyruvate (Farquhar

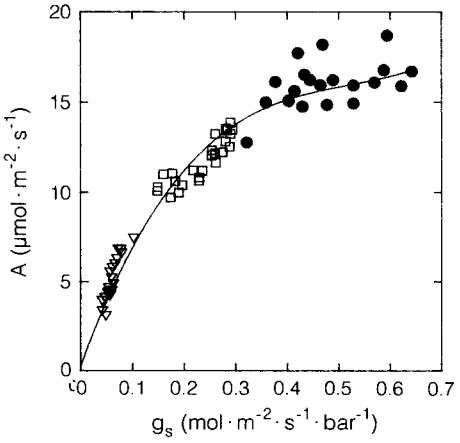

Fig. 4. Effect of salinity on the relationship between assimilation rate (A; $\mu mol \cdot m^{-2} \cdot s^{-1}$) and stomatal conductance (g_s; $mol \cdot m^{-2} \cdot s^{-1} \cdot bar^{-1}$) in cotton plants grown at different salinity concentrations. Measurements were taken directly inside the growth room using a portable gas-exchange system. Air temperature was 30° C, relative humidity was between 35 and 40%; PFD incident on leaves during measurements ranged from 670 to 710 $\mu mol\, m^{-2} \cdot s^{-1}$. Symbols: ●, control; □, 26% seawater; ▽, 55% seawater

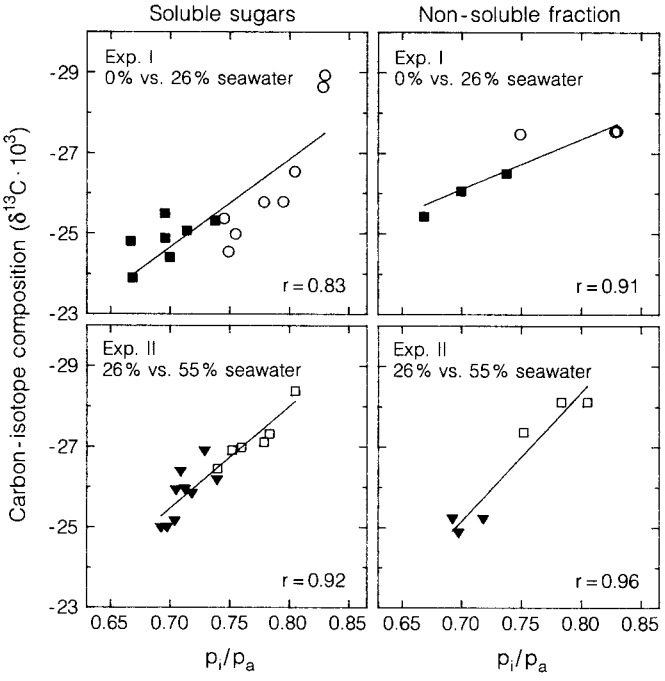

Fig. 5. Relationship between carbon-isotope composition of leaf soluble sugars and non-soluble fraction and p_i/p_a measured by gas exchange in cotton grown at different salinity levels. Gas-exchange measurements were taken inside the growth room; samples for $\delta^{13}C$ were collected at evening of the same day. Air temperature was 30° C; relative humidity 35–40%; PFD incident on leaves used for experiments was 658 ± 8 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Each value of p_i/p_a is the mean of five or six measurements taken on the day when leaves were collected. $\delta^{13}C$ values for soluble sugars are single determinations, while those for the non-soluble fraction represent mean of two sub-samples from the same leaf. Symbols: ○, control; □, ■, 26% seawater; ▼, 55% seawater

et al. 1989; Brugnoli et al. 1988) these acids were removed by Dowex treatment. As shown in Fig. 5, in each comparison the leaves from the higher salinity treatment (closed symbols) had the less negative $\delta^{13}C$ values. Moreover, there was a good correlation between the $\delta^{13}C$ values in both the soluble sugars and the non-soluble fraction and the p_i/p_a ratio determined from gas-exchange measurements on the day when the samples were taken. As noted above, there was a significant difference in both $\delta^{13}C$ and p_i/p_a between the two 26% seawater treatments and, therefore, the data from the two experiments are presented separately. The slopes of the relationship between $\delta^{13}C$ and p_i/p_a from different experiments and from different leaf fractions were similar with the exception of that for non-soluble fraction in Exp. I in which $\delta^{13}C$ was less sensitive to change in p_i/p_a.

Effect on intrinsic photosynthetic efficiency and capacity, pigment and nitrogen contents. The above results show that the effect of salinity on photosynthesis rates in cotton in large part is caused by a decrease in stomatal conductance. Nevertheless, as shown in Table 4 the calculated rate of CO_2 uptake at a constant p_i of 277 µbar CO_2 (i.e. the mean p_i in leaves of control plants) was marginally lower in the 26% seawater than in the control plants and as much as 42% lower in the 55% seawater plants. There were similar effects of salinity on the calculated rate of energy flow via the PCRO cycles at the actual p_i value (Table 4). This indicates that either the intrinsic light-conversion efficiency or the intrinsic capacity for light-saturated photosynthesis (or both) was reduced by high salinity.

As shown in Table 5, the photon yield of O_2 evolution (φ_a), determined at strictly rate-limiting photon flux densities, was unaffected by the salinity treatments as was also the ratio of variable to maximum PSII fluorescence, F_v/F_m, determined under conditions where all PSII reaction centers were fully oxidized. Moreover, as shown in Table 6 the amount of chlorophyll per leaf area was 30–40% higher in the saline treatments than in the control and the effect of salinity on the Chl a/b ratio was very small. The carotenoid composition of the leaves was unaffected by salinity and the total carotenoid content per unit leaf area was approx. 20% higher inh the two salinity treatments than in the control. The xanthophyll cycle also was fully operational in the high-salinity leaves (see below). These results demonstrate conclusively that even the highest salinity had no adverse effect on the light-harvesting capacity, nor on the intrinsic efficiency of photochemical energy conversion. However, as shown

Table 4. Effect of salinity on calculated rates of CO_2 uptake at a constant p_i of 277 µbar CO_2 (A_c), rates of energy flow through the PCRO cycle (P[ET]) at the actual p_i levels, compensation point in the absence of dark respiration (Γ_*) and nitrogen contents of cotton leaves. A_c and P(ET) values were calculated from the A and p_i values given in Table 3. In the calculation of A_c it was assumed that A is directly proportional to $(p_i-\Gamma)$, where $\Gamma=\Gamma_*+b$ with $b=5$ µbar CO_2. Values in parentheses are percentages of control

Seawater (%)	Γ_* (µbar)	A_c (µmol · m^{-2} · s^{-1})	P(ET) (µmol · m^{-2} · s^{-1})	N (mg · m^{-2})	N (mg · (g DW)$^{-1}$)
0	54.7±0.3	14.1±0.4 (100)	26.4±0.7 (100)	3.14±0.13 (100)	49.5±1.5 (100)
26	56.3±0.2	13.8±0.4 (98)	23.9±0.4 (91)	2.86±0.19 (91)	44.1±1.3 (89)
55	57.7±0.2	8.2±0.3 (58)	14.4±0.4 (55)	1.76±0.14 (56)	32.4±2.0 (65)

Table 5. Photon yield of oxygen evolution on the basis of incident photons (φ_i) and absorbed photons (φ_a), absorptance (α) and ratio of variable (F_v) to maximum (F_m) fluorescence emission of cotton leaves grown at different salinity concentrations

Seawater (%)	φ_i (mol O_2 · (mol photons)$^{-1}$)	α	φ_a (mol O_2 · (mol photons)$^{-1}$)	F_v/F_m
0	0.090±0.001 (n=3)	0.796	0.113±0.012	0.831±0.005 (n=8)
26	0.089±0.001 (n=3)	0.823	0.108±0.002	0.834±0.003 (n=10)
55	0.089±0.002 (n=3)	0.783	0.113±0.002	0.832±0.003 (n=8)

Table 6. Pigment composition and epoxidation state (EPS) of xanthophyll-cycle components in leaves of cotton grown at different salinity concentrations. Samples for HPLC analysis were collected in situ

Parameter	Pigment composition		
	0% Seawater ($n=10$)	26% Seawater ($n=10$)	55% Seawater ($n=10$)
Chl a+b (µmol · m^{-2})	743 ±3	1037 ±26	983 ±17
Chl a/b ratio	3.34±0.04	3.20±0.03	3.54±0.03
Lutein (µmol · m^{-2})	86 ±1	109 ±2	103 ±2
β-Carotene (µmol · m^{-2})	85 ±6	113 ±4	110 ±3
Neoxanthin (µmol · m^{-2})	26.3 ±0.3	32.0 ±0.8	27.6 ±0.5
V+A+Z (µmol · m^{-2})	60 ±1	68 ±2	63 ±2
EPS (V+0.5 A)/(V+A+Z)	0.98±0.01	0.96±0.01	0.95±0.02

in Table 4, one factor that shows a remarkably close relationship with A_c and P(ET) is organic nitrogen per unit leaf area which was 9% lower in 26% seawater and 44% lower in 55% seawater plants than in the control. It is well known that the pigment-protein complexes of the thylakoid membranes together with the soluble enzyme of the PCRO cycles, especially ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco), constitute the bulk of leaf protein. The fact that the chlorophyll and carotenoid contents were *higher* in the saline than in the control leaves indicates that the salinity-induced reduction in leaf nitrogen content was not caused by a reduction in the amount of thylakoid membrane protein. Therefore, it is probable that it was caused by reduction in the amount of PCRO-cycle enzymes.

Factors relating to dissipation of excess excitation energy. The finding that the calculated rate of energy flow through the PCRO cycle was strongly reduced in 55% seawater implies that there was an increased excess of excitation energy. From the photon yield (Table 5) and the P(ET) values (Table 4) it can be calculated that the excess energy increased from 56% of the total excitation energy in the control to 59% and 75% in 26% and 55% seawater, respectively. The finding that no sign of photoinhibitory damage could be detected even in 55% seawater indicates that this increased excess energy must have been safely dissipated by some other path or, possibly, repair processes were fully capable of keeping up with any photoinhibitory damage. Non-radiative energy dissipation in the pigment bed is now generally considered to be a major means of 'safe' dissipation of excessive energy; it is estimated from the degree of non-photochemical fluorescence quenching (NPQ) (Bilger and Björkman 1990; for a review see Krause and Weis 1991). It is also well established that the development of excess energy and NPQ is accompanied by de-epoxidation of violaxanthin to zeaxanthin and there is much evidence that zeaxanthin is causally linked to NPQ (for a review, see Demmig-Adams 1990). Therefore, a series of experiments was conducted on the responses of chlorophyll fluorescence and zeaxanthin formation to salinity to asses the extent to which, if any, non-radiative energy dissipation in the pigment bed accounted for the dissipation of the salinity-induced increase in excess energy.

The induction kinetics of fluorescence parameters upon illumination are shown in Fig. 6. The photochemical (q_P) and non-photochemical (NPQ) fluorescence quenching together with the photon yield of PSII photochemistry were analyzed in different treatments. Non-photochemical quenching calculated from the Stern-Volmer equation (Fig. 6, top panel) showed no significant differences between treatments, indicating that there was no salinity-induced increase in non-radiative energy dissipation in the pigment bed. However, q_P was slightly lower in 55% seawater than in the control and 26% seawater treatments (Fig. 8, center panel), i.e. the reduction state of the PSII reaction centers was slightly higher in the 55% than in the 26% and 0% seawater. Accordingly, the efficiency of PSII electron flow at the actual reduction state of PSII reaction centers (Fig. 6, bottom panel) also was slightly reduced in 55% seawater. As noted above, the efficiency of PSII photochemistry determined under conditions where all reaction centers were open (F_v/F_m) was unaffected by salinity.

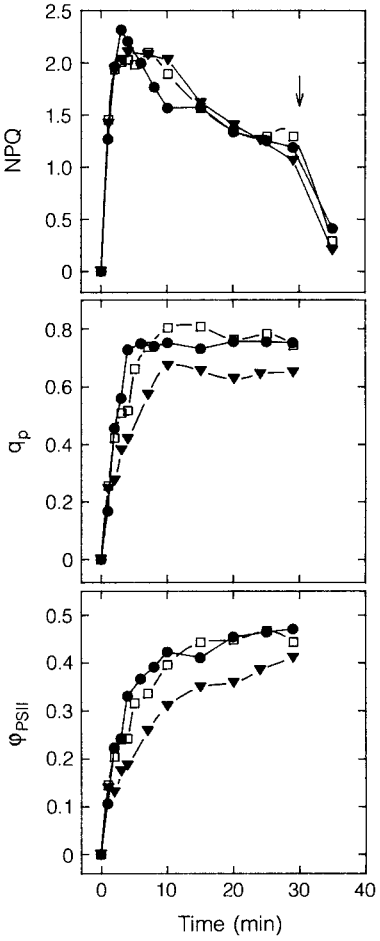

Fig. 6. Effect of salinity on the kinetics of fluorescence-quenching parameters (NPQ, φ_{PSII} and q_P) in cotton plants grown at different salinity concentrations. Experimental conditions were 30° C, 340 µbar $p(CO_2)$, and 210 mbar $p(O_2)$; PFD was 1100 µmol · m^{-2} · s^{-1}. ↓ = light off. Symbols: ●, control; □, 26% seawater; ▼, 55% seawater

The unexpectedly small influence of salinity on NPQ (Fig. 6, top panel) was matched by a similarly small influence on zeaxanthin formation as shown in Fig. 7 (closed symbols) which gives the time courses of the light-induced increases in ΔA_{510}. This absorbance change is caused by the de-epoxidation of violaxanthin to zeaxanthin and is directly proportional to the decrease in the epoxidation state (Bilger and Björkman 1991). ΔA_{540}, adjusted for the direct contribution of zeaxanthin formation to the absorbance change at this wavelength (Bilger et al. 1989), showed similar induction and relaxation kinetics among the different treatments, while the maximum level of ΔA_{540} tended to increase with increasing salinity (Fig. 7, open symbols). Since ΔA_{540} is considered to result from an increased light scattering caused by ΔpH-induced conformational changes in the thylakoid membranes (e.g. Kobayashi et al. 1982), this in-

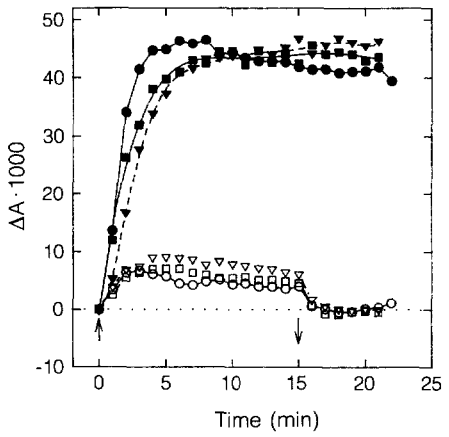

Fig. 7. Time courses of ΔA_{510} *(closed symbols)* and net ΔA_{540} *(open symbols)* in cotton plants grown in 0% seawater (○, ●), 26% seawater (□, ■) or 55% seawater (▽, ▼). ↑ = light on, ↓ = light off. The net values of ΔA_{540} were calculated by subtracting the contribution of the tail of the 510-nm peak to the 540-nm absorbance change. Leaf temperature was 30° C and PFD was 1100 μmol · m^{-2} · s^{-1}

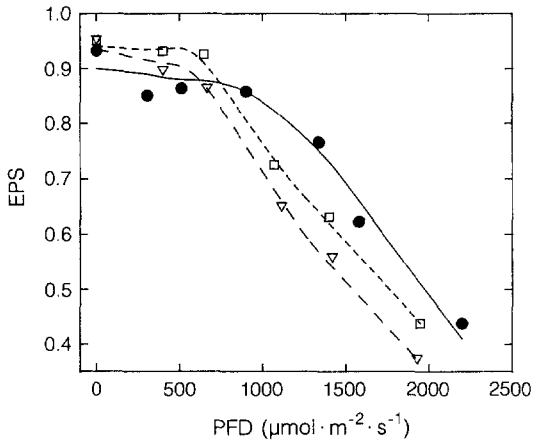

Fig. 8. Light response to the epoxidation state (EPS) of the xanthophyll-cycle components in leaves of cotton grown at 0% seawater (●), 26% seawater (□) and 55% seawater (▽). Leaf temperature = 30° C, $p(CO_2)$ = 340 μbar and $p(O_2)$ = 210 mbar. Each datum point represents the average of two individual measurements on different leaves

creased A_{540} might indicate that the ΔpH was somewhat higher in the saline than the control leaves. However, it is also possible that the differences in the amplitude of ΔA_{540} are caused by differences in leaf optical characteristics.

The influence of salinity on the epoxidation state of the xanthophyll-cycle components was also determined by direct measurements of pigment composition both in situ under the growth light (Table 6) and over a range of different PFDs (Fig. 8). In all cases the EPS of leaves kept at the growth PFD or below was high and little affected by salinity. However, at PFDs higher than 1200 μmol · m^{-2} · s^{-1} the EPS was consistently lower in the saline than in the control leaves at any given PFD (Fig. 8). Thus the xanthophyll cycle was fully operational even in the 55% seawater treatment. Nevertheless, little zeaxanthin was formed at the PFD at which the leaves had developed.

Comparison of the effects of acute salinity stress and water stress. The absence of any marked effect of salinity on EPS and NPQ in cotton is in contrast with the strong effect of water stress on these parameters in *Nerium oleander* (Demmig et al. 1988). Previous observations in this laboratory on cotton (not shown) also indicated that water stress induced strong fluorescence quenching and reductions in EPS. This prompted us to compare the effects of salinity and short-term water stress on cotton plants grown under similar conditions. Water stress was induced by withholding water and salinity stress was induced by sudden increase in salinity. In both cases, changes in leaf orientation were prevented by restraining the leaves in a horizontal position and the normal 14 h light–10 h dark cycle was maintained. Imposition of water stress by withholding water obviously required a longer time than salinity stress.

The effects of transfer from 0% to 26% seawater on the rate of CO_2 uptake (A), stomatal conductance (g_s) and pool size (V + A + Z) and epoxidation state (EPS) of the xanthophyll-cycle components are shown in Fig. 9 (left panels). The changes in rate of CO_2 uptake and stomatal conductance that took place upon transferring to 26% transferring to 26% seawater had similar time courses (Fig. 9, left top panel). The rate of CO_2 uptake declined to half of the initial value (21.1 μmol · m^{-2} · s^{-1}) in about 20 min and continued to decline in the following hours down to level as low as 4.2 μmol · m^{-2} · s^{-1}. By comparison, the rate of plants continuously grown in 26% seawater was 11.3 μmol · m^{-2} · s^{-1} (dashed line, left top panel in Fig. 9). Stomatal conductance also declined quickly reaching a steady-state value of about 0.1 mol · m^{-2} · s^{-1} · bar^{-1} in 50–60 min, compared with 0.22 mol · m^{-2} · s^{-1} · bar^{-1} in plants continuously grown in 26% seawater (dotted line, left top panel in Fig. 9). In spite of the dramatic change of A and g_s, the EPS showed a surprisingly small decrease, and eventually fully recovered (Fig. 9, left bottom panel). After 24 hours from the increase in salinity the A and g_s values were 5.1 μmol · m^{-2} · s^{-1} and 0.1 mol · m^{-2} · s^{-1} · bar^{-1}, respectively, i.e. about one-half of the mean values of plants continuously grown in 26% seawater. Nevertheless, the EPS value was as high as in plants continuously grown in 26% seawater (0.96). No significant changes were detected in the pool size of xanthophyll-cycle components or in chlorophyll content during the treatment (Fig. 9, left bottom panel).

Water stress likewise caused a strong decline in g_s (Fig. 9, right top panel) while the fall in leaf water potential was not very large. At noon on day 2 when g_s had decreased to about 0.12 mol · m^{-2} · s^{-1} · bar^{-1}, EPS had fallen to the very low value of 0.18, and zeaxanthin content had risen from almost zero to 108 mmol · (mol Chl)$^{-1}$ (Fig. 9, right bottom panel). Water stress also resulted in a gradual increase in the pool size of xanthophyll-cycle components (V + A + Z). There was no appreciable effect on chlorophyll content

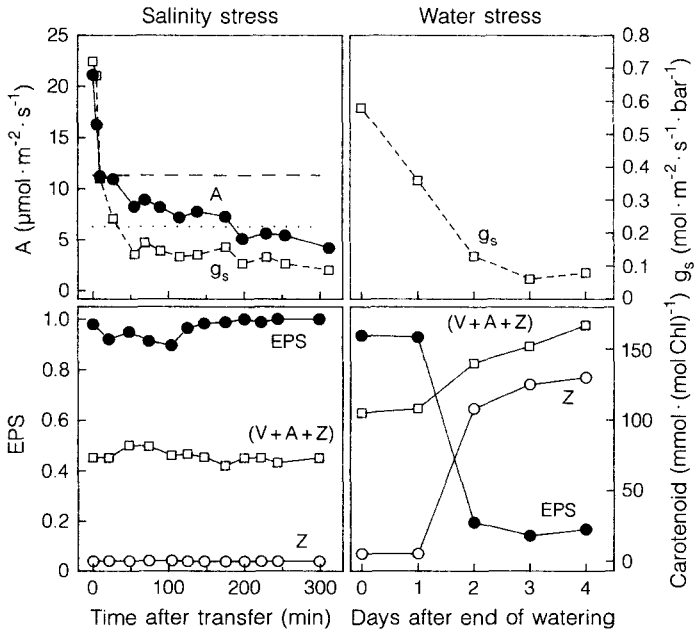

Fig. 9. Comparisons of the effects of acute salinity stress and water stress on stomatal conductance (g_s), epoxidation state (EPS) and pool size of xanthophyll-cycle components (V + A + Z) in leaves of cotton grown at different salinity concentrations. In the acute-salinity-stress experiment, plants were transferred from 0% to 26% seawater *(left panels)*. In the water-stress experiment *(right panels)*, plants were grown in a 1:1 perlite-vermiculite mixture. Water was withheld starting on day 0, and the leaf water potential decreased from −6 to −10 bar on day 2, recovered to −7.5 bar on day 3 following stomatal closure, and then decreased to −12 bar on day 4. Both experiments were conducted inside the growth room at 30° C air temperature and 35–40% relative humidity. The PFD was 700 and 800 μmol · m^{-2} · s^{-1}, in the salinity- and water-stress experiments, respectively. Chlorophyll content showed no change in either experiment (759 ± 21 and 671 ± 13 μmol Chl a + b · m^{-2} in the salinity-stress and water-stress experiments, respectively). The *broken* and *dotted horizontal lines* in the *upper left panel* represent the mean net CO_2 uptake and g_s, respectively, for plants continuously grown at 26% seawater (compare Table 3)

(671 ± 13 · μmol · m^{-2}) nor on the contents of other carotenoids. Mean values for neoxanthin, lutein and β-carotene were, in this order, 41 ± 1, 161 ± 3 and 125 ± 4 mmol · (mol Chl)$^{-1}$. The increase in zeaxanthin content was closely correlated with an increase in non-photochemical fluorescence quenching (data not shown).

Discussion

Growth and photosynthesis. Cotton plants grown under salinity accumulated large amount of NaCl into leaves. Nevertheless, the plants were able to maintain the leaf K^+ concentration above 100 mM even at the highest salinity concentration tested. In part, this was probably aided by the fact that the K^+ concentration in the culture solution increased with increasing salinity. However, the main cause must be an increase in the selectivity for uptake of K^+ relative to uptake of Na^+ (Table 2). The maintenance of a high K^+ content may be crucial to sustaining the integrity of the photosynthetic system under high salinity (Chow et al. 1990) and thus an important pre-requisite for salinity tolerance.

Although all plants remained healthy, dry weight accumulation (RGR) was markedly reduced in the 26% seawater treatment and dramatically reduced in 55% seawater (Figs. 1, 3). In 26% seawater the larger part of the reduction of RGR was caused by an increased allocation to root growth at the expense of leaf growth and the remainder was caused by a reduction in NAR. The ratio of leaf-to-root growth and the leaf-area ratio were reduced even more in 55% seawater but because of the strong decrease in NAR in these plants the latter factor contributed more to the overall reduction in RGR than did the reduction in leaf area ratio (Fig. 3). These results show that even in the same genotype and under otherwise identical conditions the relative contributions of a reduced allocation to leaf growth and a reduction in NAR depend on the salinity level. This may in part explain some of the conflicting results reported in the literature. For example, Shennan et al. (1987) contended that LAR but not NAR was affected by salinity in *Aster tripolium*. Similar conclusions were made for the Malvacean species *Hibiscus cannabinus* by Curtis and Läuchli (1986), whereas Cramer et al. (1990), working with salt-stressed barley, found the opposite to be true.

The salt-induced reductions in NAR were matched by quantitatively similar reductions in leaf photosynthesis rates measured by gas exchange (A). In both salinity treatments the reduction in A was accompanied by an even greater reduction in stomatal conductance (g_s). As was mentioned in the *Introduction* such salt-induced concurrent reductions in A and g_s have been previously reported for a number of species, although it is often difficult to assess the extent to which the reduction in A was caused by partial stomatal closure and the extent to which it may have been caused by a reduction in non-stomatal factors. In the present study non-stomatal factors contributed little, if at all, to the decrease in A in 26% seawater. However, in 55% seawater, non-stomatal photosynthetic capacity, calculated from gas-exchange measurements as P(ET), decreased to 55% of the control values (Table 4). These results are in close agreement with previous results obtained with different cotton cultivars. Brugnoli and Lauteri (1991) found that 250 mM NaCl in the culture solution caused a 45% reduction of P(ET) and Longstreth and Nobel (1979) found that 0.1 molal NaCl had little or no effect on the "mesophyll conductance" in cotton while 0.3 molal NaCl caused this conductance to decline to 56% of the non-saline control. This decrease took place in spite of a salt-induced increase of approx. 50% in the ratio of cell-surface area to leaf-surface area. Although no direct measurements of this ratio were made in the present study, it is most probable that such changes did occur since the specific leaf weight, adjusted for the NaCl contents of the leaves, increases by 18% in 26% seawater and by 46% in 55% seawater. Leaf thickness and the content of chloroplast pigments per unit leaf area (Table 6) also increased with increasing salinity.

Estimates of non-stomatal photosynthetic capacity from gas exchange are based on the average intercellular

CO_2 partial pressure (p_i) calculated from simultaneous determinations of the atomospheric CO_2 partial pressure (p_a), A and g_s, and therefore do not take into account the effect of possible heterogeneity of g_s resulting from „patchy" stomatal closure across the leaf surface. Such patchiness could lead to an underestimate of non-stomatal photosynthetic capacity (Terashima et al. 1988). In order to explain all of the decrease of P(ET) in the present study, "patchy" photosynthesis should have caused overestimations of p_i by 57 μbar and 106 μbar in 26% and 55% seawater, respectively. However, the relationship obtained between p_i/p_a ratio and carbon-isotope ratio of the leaf sugars (Fig. 5) indicates that the effect of "patchy" stomatal closure, if present, was not large enough to explain such dramatic effect on p_i and therefore the values of A_c and P(ET) in Table 4 probably are reasonably accurate.

The relatively greater effect of salinity on g_s than on A results in an improved photosynthetic water-use efficiency (A/E ratio). This may be considered an adaptive response to salinity since it reduces the amount of solution, and hence, the amount of salt that the plant is forced to take up per amount of carbon fixed. The finding that the $\delta^{13}C$ values of the non-soluble fraction were less negative in the seawater treatments than in the control indicates that the water-use efficiency was improved by salinity also in the long term. The $\delta^{13}C$ of the soluble-sugar fraction and of the non-soluble fraction can be considered to represent integrations of the A/E ratio over a period of about 1 d and over the entire life of the leaf or plant, respectively (Brugnoli et al. 1988; for a review, see Farquhar et al. 1989).

The relationship between p_i/p_a and $\delta^{13}C$ had roughly similar slopes in the different experiments and in the different leaf fractions analyzed (Fig. 5). Thus the carbon in soluble-sugar fraction and in non-soluble fraction had similar isotopic signatures, although the carbon was derived from CO_2 assimilated over very different time periods. This indicates that the p_i/p_a ratio was remarkably constant over time and in all treatments. These results differ from those obtained by Brugnoli and Lauteri (1991) who observed a decline of p_i/p_a during ontogeny in salinity-stressed plants reflected in differences between the $\delta^{13}C$ values of leaf dry matter and cotton-boll fiber. The difference between the results of the two studies can probably be explained by the fact that in the previous study the plants were not transferred from non-saline to saline conditions until day 10 after emergence. This caused a decline in p_i/p_a from the start of the salinity treatment ($p_i/p_a = 0.70$) to the end of the experiment ($p_i/p_a = 0.40$). In the present study, the plants developed under constant salinity throughout the period of autotrophic growth.

A salinity-induced reduction in A_c (or in "mesophyll conductance") could be caused by detrimental effects on the photosynthetic system, including photoinhibitory damage. No such effect could be detected in this study in spite of the fact that the leaf sodium concentration reached at least 360 mM and light clearly was excessive in the 55% seawater treatment. The intrinsic photon yield of O_2 evolution and the ratio of variable-to-maximum fluorescence were unaffected by salinity (see also Brugnoli and Lauteri 1990, 1991), and the content of photosynthetic pigments per unit leaf area actually increased under high salinity. The leaves also exhibited normal induction and relaxation kinetics of chlorophyll fluorescence and of the light-induced 540-nm light-scattering change which is thought to reflect changes in the trans-membrane proton gradient (Kobayashi et al. 1982).

Reduction in A_c could also be caused by a reduction in amount or activity of photosynthetic enzymes, especially ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) (for reference see Seemann and Sharkey 1986; von Caemmerer and Evans 1991), and or a decrease in g_w, the conductance to CO_2 transfer from the intercellular spaces to the chloroplasts (Evans 1983; von Caemmerer and Evans 1991). In those studies, different levels of Rubisco were obtained by varying the nitrogen availability in the culture medium. In the present study, the salinity-induced decrease in A_c was closely matched by a similar decrease in nitrogen content per leaf area. This decrease took place despite of concomitant increases in specific leaf weight, parenchyma thickness and chloroplast-pigment content. It is therefore probable that most of the decrease in nitrogen content was caused by a decrease in the content of enzymes of the PCRO cycle of which Rubisco is a major component. Although we cannot exclude the possibility that g_w decreased in response to increased salinity and the concomitant decrease in nitrogen content, any effects on photosynthetic rate is likely to be small. Wheat leaves used by Evans (1983), having nitrogen contents equal to the cotton leaves in the 0, 26 and 55% seawater treatments, had g_w values of approx. 0.56, 0.54 and 0.43 $mol \cdot m^{-2} \cdot s^{-1} \cdot bar^{-1}$. If we assume that the cotton leaves had g_w values similar to those of wheat then the CO_2 gradient between the intercellular spaces and the CO_2 fixation sites ($p_i - p_c$) would have been 25, 22, and 15 μbar; the rates of CO_2 fixation at 252 μbar (the calculated p_c in control leaves) would have been 14.1, 13.8 and 8.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (see Table 4).

We conclude, therefore, that the main effects of salinity on "mesophyll conductance" at the growth PFD probably is attributable to a reduction in the content of PCRO-cycle enzymes. Such a reduction need not be considered an undesirable response to salinity; instead, such a response may reflect a reallocation of resources that enables the plant to complete its life cycle under high salinity.

Energy dissipation. The reduced rate of photosynthetic electron flow through the PCRO cycle caused by high salinity inevitably would result in an increased excess of excitation energy at the growth PFD which must be dissipated by some other process. Contrary to expectation, there was no indication that non-photochemical fluorescence quenching (NPQ) was greater in the salinity treatments than in the control (Fig. 6). Similarly, the epoxidation state (EPS) as well as the pool size of the xanthophyll-cycle components remained remarkably constant among the different salinity treatments (Table 6).

These results indicate that the salinity-induced excess energy at the growth PFD was dissipated by a process other than non-radiative dissipation in the pigment bed. It should be noted that any contribution of energy dissipation via the Mehler-Peroxidase reaction (Schreiber and Neubauer 1990) or by ATP consumption in salt pumping (Yeo 1983) were not included in our calculated values of P(ET). Although there is little information on the amount of energy that may be dissipated via these processes, it is apparent that in the present study, the total rate of electron transport at the growth PFD was sufficient to preclude an accumulation of excessive energy in PSII which would have triggered non-radiative energy dissipation in the pigment bed and zeaxanthin formation. That the total capacity for electron transport nevertheless decreased with increased salinity is indicated by the finding that the PFD at which the EPS began to fall decreased with increased salinity as did also the extent of the decrease in EPS at any given PFD beyond this point (Fig. 8). This conclusion is also consistent with the finding that at a PFD of 1100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ a somewhat larger fraction of the PSII reaction centers were in the reduced state in the 55% seawater plants than in the control and 26% seawater plants (Fig. 6, center panel).

The experiment in which plants were suddenly transferred from non-saline to saline conditions also shows that EPS was relatively insensitive to salinity, provided that the PFD was kept constant (Fig. 9). This is in sharp contrast with the results obtained when plants grown in 0% seawater were subjected to short-term water stress by stopping the supply of water to the roots. As in the salinity treatments, these water-stress treatments resulted in partial stomatal closure but in the latter treatment the decline in g_s was accompanied by a dramatic initial reduction in EPS and increase in NPQ, followed by a substantial increase in the pool size of the xanthophyll cycle components (V + A + Z). This response to short-term water stress is similar to that found with leaves of *Nerium oleander* (Demmig et al. 1988). However, contrary to the results with *N. oleander*, in which the increase in V + A + Z was accompanied by a decrease in β-carotene, no such decrease could be detected in the cotton leaves used by us.

We can offer no obvious explanation for the different responses of cotton to *salinity* stress (short-term or long-term) and to short-term *water* stress. Factors that may be considered in future studies are differences in the opportunity for osmotic adjustment, the extent of increase in excessive excitation energy caused by the two treatments, and rates of dissipation via the Mehler-Peroxidase reaction and salt pumping.

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