Plant Physiol. (1991) 95, 628–635
0032-0889/91/95/0628/08/$01.00/0

Received for publication September 18, 1990
Accepted October 29, 1990

Effects of Salinity on Stomatal Conductance, Photosynthetic Capacity, and Carbon Isotope Discrimination of Salt-Tolerant (*Gossypium hirsutum* L.) and Salt-Sensitive (*Phaseolus vulgaris* L.) C_3 Non-Halophytes

Enrico Brugnoli*[1] and Marco Lauteri

C.N.R. Istituto per l'Agroselvicoltura, Via Marconi, 2, 05010 Porano (TR), Italy

ABSTRACT

The effects of salinity on growth, stomatal conductance, photosynthetic capacity, and carbon isotope discrimination (Δ) of *Gossypium hirsutum* L. and *Phaseolus vulgaris* L. were evaluated. Plants were grown at different NaCl concentrations from 10 days old until mature reproductive structures were formed. Plant growth and leaf area development were strongly reduced by salinity, in both cotton and bean. Stomatal conductance also was reduced by salinity. The Δ always declined with increasing external salinity concentration, indicating that stomatal limitation of photosynthesis was increased. In cotton plant dry matter, Δ correlated with the ratio of intercellular to atmospheric CO_2 partial pressures (p_i/p_a) calculated by gas exchange. This correlation was not clear in bean plants, although Δ showed a more pronounced salt induced decline in bean than in cotton. Possible effects of heterogeneity of stomatal aperture and consequent overestimation of p_i as determined from gas exchange could explain these results. Significant differences of Δ between leaf and seed material were observed in cotton and bean. This suggests different patterns of carbon allocation between leaves and seeds. The photon yield of O_2 evolution determined at rate-limiting photosynthetic photon flux density was insensitive to salinity in both species analyzed. The light- and CO_2-saturated rate of CO_2 uptake and O_2 evolution showed a salt induced decline in both species. Possible explanations of this observation are discussed. O_2 hypersensitivity was observed in salt stressed cotton plants. These results clearly demonstrate that the effect of salinity on assimilation rate was mostly due to the reduction of stomatal conductance, and that calculation of p_i may be overestimated in salt stressed plants, because of heterogeneity of stomatal aperture over the leaf surface.

Salinity causes large effects on higher plants, both halophytes and non-halophytes. In the latter, growth rate is generally reduced by salinity even at low salt concentration. However, within non-halophytes there is still large variability among species, ranging from extremely sensitive to tolerant species overlapping with halophytes (18). The reduction in growth is consequence of several physiological responses including modifications of ion balance, water status, mineral nutrition, stomatal behavior, photosynthetic efficiency, carbon allocation, and utilization (17, 18, 20).

The rate of photosynthetic CO_2 assimilation is generally reduced by salinity. This reduction is partly due to a reduced stomatal conductance (7, 12, 23) and consequent restriction of the availability of CO_2 for carboxylation.

Nonstomatal inhibition of photosynthesis, caused by direct effects of NaCl on photosynthetic apparatus independent of stomatal closure, has also been reported for several plant species, both halophytes and non-halophytes (1, 23, 24). This inhibition of photosynthetic capacity has been attributed to a reduced efficiency of RuBP[2] carboxylase when RuBP is in limiting supply (24), to a reduction of RuBP regeneration capacity (1, 24), or to the sensitivity of PSII to NaCl (2).

Recently, it has been argued (14, 26) that some of the effects of salinity on photosynthesis, previously referred to as nonstomatal effects, could be only apparent and actually caused by spatial heterogeneity of stomatal aperture over the leaf surface. It has been demonstrated in ABA-treated leaves (8, 28) that stomatal heterogeneity and consequent nonuniform photosynthesis cause a systematic overestimation of the intercellular CO_2 partial pressure (p_i) calculated by gas exchange. Nonuniform photosynthesis has also been observed in water-stressed plants (6, 9, 22, 25), and it has been concluded that the primary effect of water stress on photosynthesis is mediated by stomatal closure and that chloroplast reactions are not affected until after other plant processes have become strongly affected (26). Similar results have been recently obtained with the halophyte *Plantago maritima* exposed to increasing salinity (16).

Carbon isotope discrimination against the stable isotope ^{13}C may be useful for investigating the effect of stomatal heterogeneity. Theory predicts a linear relationship between Δ and p_i/p_a for C_3 plants (13, 15).

$$\Delta = a - d + (b - a)\, p_i/p_a, \quad (1)$$

[1] Present address: Carnegie Institution of Washington, Department of Plant Biology, 290 Panama Street, Stanford, CA 94035.

[2] Abbreviations: RuBP, ribulose-1,5-bisphosphate; Δ, carbon isotope discrimination; p_i/p_a, ratio of intercellular and atmospheric CO_2 partial pressures; A, assimilation rate; g, stomatal conductance; $A(p_i)$, response of A to varying p_i; PEP, phospho*enol*pyruvate; δ, carbon isotope composition; Φ_i, photon yield of O_2 evolution on the basis of incident photons; P_{max}, rate of O_2 evolution at light- and CO_2 saturation; J, electron transport rate; SPS, sucrose-phosphate-synthase.

where a ($= 4.4 \times 10^{-3}$) is the fractionation occurring during diffusion in air, b (about 27×10^{-3}) is the fractionation associated with RuBP and PEP carboxylations in C_3 plants, and d includes the effects of discrimination during CO_2 dissolution, liquid phase diffusion, and possible fractionations during respiration and photorespiration (15). This relationship has been demonstrated from correlations of p_i/p_a estimated by gas exchange and $^{13}C/^{12}C$ ratios in leaf dry matter (for recent reviews see refs. 15, 21), in leaf sugars and starch (4), and from correlation between concurrent short term measurements of p_i/p_a and the isotope composition of the air in gas exchange measurements (11). Measurement of Δ gives an estimation of the assimilation-rate-weighted value of p_i, whereas gas exchange gives a conductance-weighted value of p_i (15). When heterogeneity of stomatal opening occurs the two estimates will differ to some extent (15).

The aim of the present study was to investigate the effects of salinity on stomatal behavior and photosynthesis of non-halophytes, taking into account possible implications of spatial heterogeneity of stomatal aperture. Photosynthetic responses to salinity of two C_3 non-halophytes, upland cotton and bean, showing marked differences in sensitivity to salinity, were compared. Several experimental approaches were used including conventional gas exchange, O_2 evolution at saturating CO_2, and carbon isotope analysis. Some of the present results have appeared as an extended abstract (5).

MATERIALS AND METHODS

Plant Material

Cotton (*Gossypium hirsutum* L.), cv. Deltapine and cv GSC 20 (supplied by COMES, Catania, Italy) and bean (*Phaseolus vulgaris* L.), cv Strike (Quadrisem, Asgrow, Italy) were grown in 10 L pots containing a sterilized and washed mixture of sand and garden soil. Plants were grown in a growth room. Temperature was maintained at 30°C during day and 23°C during night. RH was about 70% and 85% during day and night, respectively. The photoperiod was 12 h. Light was provided by metal halide lamps (Power Star, HQI, 400 W each, Osram, F.R.G.) and the irradiance at plant level was about 900 μmol of photons m^{-2} s^{-1}.

Some of the O_2 evolution and photon yield measurements were repeated on plants grown inside a greenhouse. In this set of experiments, temperature varied from 20 to 32°C and RH from 45 to 80%. Plants were grown under full sunlight and natural photoperiod of the spring season in Porano (47°21′ north latitude).

Salt treatments were started when plants were 10 d old, and salinity level in the medium was increased by steps of 50 mM per day, until the final NaCl concentration was reached. Cotton plants were grown at 0, 50, and 250 mM NaCl, and bean plants at 0, 50, and 150 mM NaCl (250 mM NaCl was lethal to bean). All other growth conditions were identical for cotton and bean. All plants were watered daily with the appropriate NaCl solution. A modified Hewitt nutrient solution (19) with ammonium nitrate in place of potassium nitrate was used.

Cotton and bean plants were harvested on d 52 and d 37 after start of salt treatments, respectively. All photosynthesis measurements were started 10 d after start of salt treatments.

Gas Exchange

Gas exchange was measured on youngest fully expanded attached leaves, using an open-flow gas exchange system.

Compressed air was passed through soda lime columns to remove CO_2. O_2 partial pressure was either kept at 210 mbar or varied by injecting appropriate proportions of N_2 into the CO_2-free air stream. The gas stream was humidified and then passed through a glass condenser at known temperature and pressure. The condenser temperature was regulated by circulating water from a temperature controlled water bath. Pure CO_2 or mixtures of CO_2 in air were injected into the air stream after the condenser. Flow rates of the various gases were controlled with mass-flow controllers (models MFTV-24; MFTV-11; MFTV-14, Matheson, Union Carbide, Oevel, Belgium).

Absolute partial pressure of CO_2 in the system was continuously monitored by an absolute IR gas analyzer (IRGA) (model SB-MK2, Analytical Development Company, Hoddesdon, UK). CO_2 and H_2O exchange by leaf was detected by differential IRGAs (Binos 2, Leybold-Heraeus, Hanau, FRG). The air streams were passed through ice traps before entering the CO_2 IRGAs.

Flow rate of air entering the leaf chamber was monitored with a mass-flow meter (Matheson, model MFM-14U).

Single leaves were placed in a ventilated Peltier-temperature controlled leaf chamber (model GK22, Walz, Effeltrich, FRG). Leaf temperature was monitored by a fine wire copper-constantan thermocouple (diameter = 0.2 mm) appressed against the lower leaf surface. Light was provided by a metal halide lamp (HQI, 400 W, Osram, Munich, FRG) filtered through a heat and UV filter (KG5, Schott, Mainz, FRG). PPFD was varied by changing the distance of the lamp from the leaf chamber and/or by inserting neutral-density screens. PPFD inside the chamber was monitored by a silicon photocell sensor calibrated against a quantum sensor (Li-190SB, Li-Cor, Lincoln, NE).

The various sensor signals were recorded by a data acquisition system (model DataTaker 100, Data Electronics, Melbourne, Australia). On-line calculations of gas exchange parameters were made according to von Caemmerer and Farquhar (29).

Spot gas exchange measurements were taken inside the growth room on several days to detect variations in gas exchange parameters during ontogeny. A, transpiration, and intercellular CO_2 partial pressure were determined with a portable gas exchange system (LCA2 and Parkinson leaf cuvette, Analytical Development Company, Hoddesdon, UK). To avoid the effect of peak broadening due to H_2O and CO_2 cross-sensitivity, air leaving the leaf cuvette was passed through a magnesium perchlorate column before entering the CO_2 IRGA. Calculation of gas exchange parameters were made according to von Caemmerer and Farquhar (29) allowing for the above modification.

O_2 Evolution

Rates of O_2 evolution were measured using a leaf-disc oxygen electrode (Hansatech, Kings Lynn, Norfolk, UK). To verify CO_2-saturation for cotton and bean leaves the CO_2

Table I. *Total Leaf Area and Shoot Dry Weight of Cotton Plants Grown at Different Salinity Levels*

Mean values and analysis of variance. Values followed by different capital letters are significantly different per $P \leq 0.01$. $n = 12$.

Treatment	Total Leaf Area	Shoot Dry Weight
mM NaCl	*cm²*	*g*
0 (control)	1339 A	44.6 A
50	868 B	32.5 B
250	547 C	18.6 C

response of O_2 evolution was previously measured using a gas cylinder at known CO_2 concentration and a gas diluter (GD-600, Analytical Development Company, UK).

Measurements of light response of O_2 evolution and of photon yield of O_2 evolution were performed according to Björkman and Demmig (3). CO_2 concentration inside the leaf-disc chamber was kept at 10% by flowing a mixture of 10% CO_2 in air from a cylinder. Then, the chamber was closed and the rate of O_2 evolution was recorded. Light was provided by a quartz-lamp housing as previously described (3). Irradiance was varied by interposing neutral density filters (Balzers, Liechtenstein).

Carbon Isotope Analysis

Carbon isotope composition was measured on different parts of cotton and bean plants and carbon isotope discrimination was calculated as

$$\Delta = (\delta_a - \delta_p)/(1 + \delta_p) \quad (2)$$

where δ_a and δ_p are the carbon isotope composition of source air and plant material, respectively, relative to the international standard Pee Dee Belemnite (15). Measurements were taken of δ_a of the greenhouse and of growth room. Typically, δ_a was -8.4×10^{-3}. Carbon isotope analysis of air and of plant material was as previously described (4). Samples were collected from plants at the end of experiments. Oven-dried samples were combusted in a Dumas-combustion elemental analyzer (model NA1500, Carlo Erba Instruments, Milan, Italy). CO_2 from combusted samples or from air was purified by cryogenic traps. Water vapor was removed by a −100°C trap and CO_2 was then frozen in liquid N_2 traps. Purified CO_2 was then analyzed by a dual-inlet isotope ratio mass spectrometer (VG SIRA Series II, VG Isotech, Middlewich, UK). Carbon isotope ratio of sample CO_2 was compared with that of a working standard reference CO_2 previously calibrated against Pee Dee Belemnite. Internal precision of individual measurements was always greater than 0.01×10^{-3}.

Table II. *Growth Response of Bean Plants to Increasing Salinity*

Plant dry weight determined at the end of the experiment. Mean values and analysis of variance. Values followed by different capital letters are significantly different per $P \leq 0.01$. $n = 8$.

Treatment	Dry Weight
mM NaCl	*g*
0 (control)	26.0 A
50	6.1 B
150	2.3 C

Table III. *Stomatal Conductance of Cotton and Bean Plants Grown at Different Salinity Levels*

Mean values and analysis of variance of spot gas exchange measurements taken during the lifespan of plants (see "Materials and Methods"). Values followed by different capital letters are significantly different per $P \leq 0.01$. For cotton, $n = 280$; for bean, $n = 40$.

Species	Treatment	Stomatal Conductance
	mM NaCl	*mol m⁻² s⁻¹*
Cotton	0 (control)	0.31 A
Cotton	50	0.25 B
Cotton	250	0.18 C
Bean	0 (control)	0.25 A
Bean	50	0.13 B
Bean	150	0.08 C

Statistical Analysis

Statistical analysis was based on one-way analysis of variance and on Student-Newman-Keuls test for multiple comparisons among means (27). Regression analysis was performed according to the least squares method.

RESULTS

Growth Responses

Growth responses of cotton plants to different salinity concentration in the medium are shown in Table I. Shoot dry weight and total leaf area, measured at the end of experiments, were strongly reduced by salinity. Total leaf area was reduced by 35% in plants grown in 50 mM NaCl medium and by 60% in those grown in 250 mM NaCl, relative to control plants (Table I). These decreases in leaf area were accompanied by 27 and 58% decreases in shoot dry weight, compared to control plants.

Growth of bean was more severely affected by salinity than was growth of cotton (Table II). Shoot dry weight was reduced by 77% in bean plants grown in 50 mM NaCl and by 91% in 150 mM NaCl, compared to control plants (Table II). Growth of bean plants was completely inhibited by salinity, whereas growth of cotton was reduced but not stopped by the salinity concentrations tested.

When salt-stressed bean plants were harvested most of their leaves were shrunk and dry, causing the leaf area measurements to be unreliable. For this reason leaf area data for bean are not presented.

Photosynthesis and Stomatal Conductance

The effects of salinity on g of cotton and bean leaves are shown in Table III. Stomatal conductance always declined with increasing salinity concentration. The effect of salinity on g was more dramatic in bean than in cotton. In bean, 150 mM NaCl caused a greater depression of g than did 250 mM

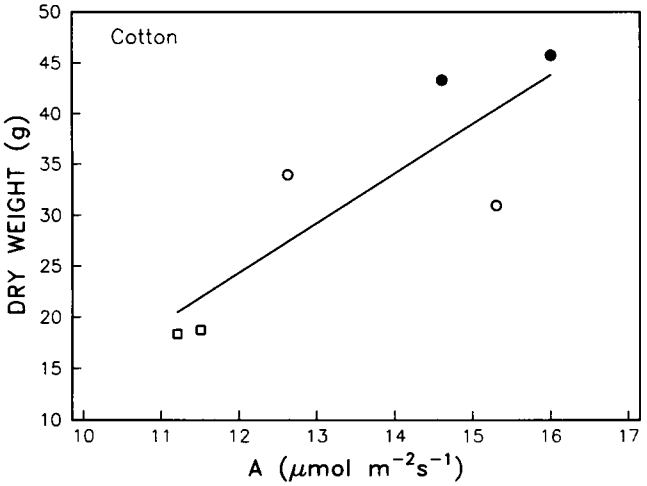

Figure 1. Relationship between shoot dry weight and average assimilation rate determined by spot gas exchange measurements in cotton. The straight line represents the fitted linear regression (r^2 = 0.70). Each *A* value is the average of 140 measurements. Symbols: (●), control; (○), 50 mM NaCl; (□), 250 mM NaCl.

NaCl in cotton (Table III). The average of assimilation rate measured by gas exchange during the life cycle of plants, was positively correlated (r^2 = 0.70) with the shoot dry weight of cotton plants measured at the end of experiment (Fig. 1).

The response of CO_2 assimilation to varying p_i ($A[p_i]$ relationship) was measured several times during the experiment. Figure 2 shows typical $A(p_i)$ relationships for different salinity treatments in cotton and bean. The initial slope and the CO_2 saturated region of $A(p_i)$ curves were reduced by salinity, both in cotton and bean (Fig. 2), although the effect was much more pronounced in bean than in cotton. The operational p_i corresponding to 345 μbar of ambient $p(CO_2)$ (indicated by arrows in Fig. 2) was reduced by salinity in cotton leaves, whereas it remained unaffected (50 mM NaCl) or increased

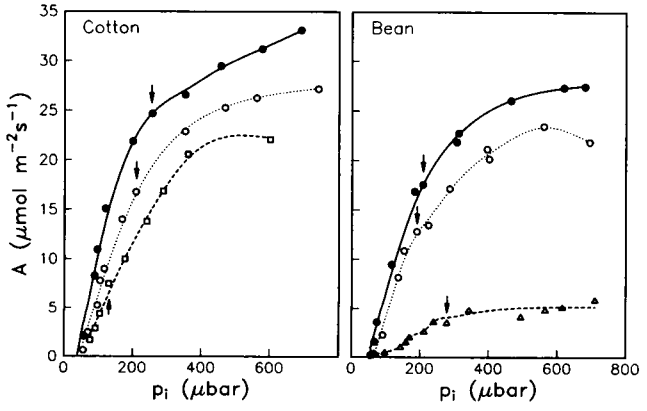

Figure 2. Effect of salinity on $A(p_i)$ relationships of cotton and bean plants. Cotton plants (left) grown at 0 mM (●), 50 mM (○), and 250 mM NaCl (□). Bean plants (right) grown at 0 mM (●), 50 mM (○), and 150 mM NaCl (△). Arrows indicate measurements made under normal atmospheric CO_2 partial pressure of 345 μbar.

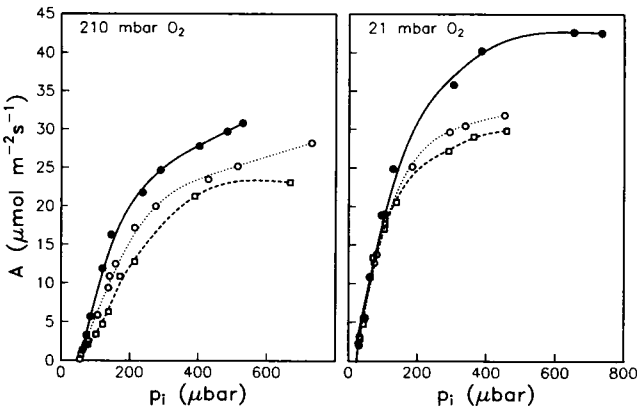

Figure 3. Effect of O_2 partial pressure on $A(p_i)$ relationships in cotton grown at 0 mM (●), 50 mM (○), and 250 mM NaCl (□). Measurements made at 210 mbar O_2 (left) and at 21 mbar O_2 (right).

(250 mM NaCl) in salt stressed bean compared to control plants.

Cotton leaves showed a high oxygen sensitivity (Fig. 3), particularly in stressed plants. At 21 mbar O_2, the initial slope of $A(p_i)$ curve did not show significant differences among salinity treatments (Fig. 3). This is in contrast with the salt-induced decline observed at 210 mbar O_2 (Fig. 3). Hence, the effect of salinity on the initial slope of $A(p_i)$ curve was overcome by switching to low O_2 concentration. This effect was less evident at CO_2 saturation (Fig. 3), the ratio of *A* at 21 mbar to that at 210 mbar calculated at 500 μbar CO_2 being 1.3 in control plant compared to 1.2 in both salinity treatments. Such high O_2 sensitivity was not apparent in bean plants (data not shown).

Photon Yield and Light Response of O_2 Evolution

Rate of O_2 evolution was measured on leaf discs taken either from leaves on which gas exchange and $A(p_i)$ relationship had been previously analyzed or from sample collected directly from the greenhouse. The apparent photon yield of O_2 evolution (Φ_i) (mol O_2 evolved/mol incident photons) measured at rate-limiting PPFDs for cotton and bean plants grown at different salinity levels are shown in Table IV. Φ_i

Table IV. *Apparent Photon Yield of O_2 Evolution (mol O_2 evolved mol^{-1} incident photon) of Cotton and Bean Plants Grown at Different Salinity Concentrations*

Mean values ± SE; *n* = 5.

Species	Treatment	Photon Yield ± SE
	mM NaCl	
Cotton	0 (control)	0.082 ± 0.004
Cotton	50	0.080 ± 0.003
Cotton	250	0.080 ± 0.003
Bean	0 (control)	0.078 ± 0.001
Bean	50	0.082 ± 0.010[a]
Bean	150	0.078 ± 0.012

[a] *n* = 4.

was near 0.080 mol of O_2/mol of incident photons in all treatments, both in cotton and bean (Table IV). It should be pointed out that Φ_i of cotton leaves remained constant during the life cycle, in all salinity treatments.

The rate of O_2 evolution measured at light saturation was lower in salt stressed than in control plants (Table V). It declined by 28% in cotton in the range 0 to 250 mM NaCl and by 27% in bean in the range 0 to 150 mM NaCl. However, the observed difference between control and 50 mM NaCl treatments (Table V) was not statistically significant in both species. Subsequently, no further declines of the rate of O_2 evolution were observed in cotton, whereas the photon yield and the maximum photosynthesis of salt stressed bean quickly declined. This decline coincided with visible damage to the leaves.

Carbon Isotope Discrimination

Carbon isotope discrimination was analyzed in cotton leaves and seed epidermal hair (fiber). Plants grown under salinity were always enriched in the heavier isotope ^{13}C compared to control plants. The carbon isotope discrimination in leaves and fiber was compared to the average p_i/p_a, weighted for CO_2 assimilation, as obtained from spot gas exchange measurements during the lifespan of plants. Figure 4 shows the relationships between Δ in leaves and in fiber and p_i/p_a. A positive correlation was found between p_i/p_a and Δ in both leaves ($r^2 = 0.87$) and fiber ($r^2 = 0.69$).

Cotton fibers were always isotopically heavier with respect to leaves. Thus, the discrimination was lower in the fiber than in the leaf material. The difference between Δ of leaves and that of fiber increased with increasing salinity (*i.e.* with decreasing p_i/p_a) (Fig. 4).

Bean plants showed a response of carbon isotope discrimination to salinity (Table VI) similar to that of cotton. The discrimination in leaf dry matter strongly decreased in salt stressed beans compared to control plants. The effect of salinity on Δ was higher in bean than in cotton. In bean, differences in Δ among treatments (Table VI) were always highly statistically significant ($P \leq 0.01$). Carbon isotope discrimination in stems matched that of leaves in all treatments. Carbon isotope composition was also analyzed in bean pods and seeds. However, this analysis could not be made on plants grown in 150 mM NaCl since they failed to produce any flowers and fruits. In the treatments analyzed, the value of Δ of pods was very close to that of leaves and stems. In contrast, Δ of seeds was indeed lower than that in leaves by approximately 3×10^{-3}, both in control and 50 mM NaCl treatment.

Table V. *Rate of O_2 Evolution (μmol m^{-2} s^{-1}) at Light- and CO_2-Saturation (P_{max}) of Leaf Discs of Cotton and Bean Grown at Different Salinity Levels*

Mean values and standard errors; $n = 5$.

Species	Treatment	P_{max} ± SE
	mM NaCl	
Cotton	0 (control)	36.6 ± 2.2
Cotton	50	30.4 ± 2.4
Cotton	250	26.4 ± 2.8
Bean	0 (control)	32.3 ± 2.6
Bean	50	28.9 ± 3.0[a]
Bean	150	24.0 ± 3.7

[a] $n = 4$.

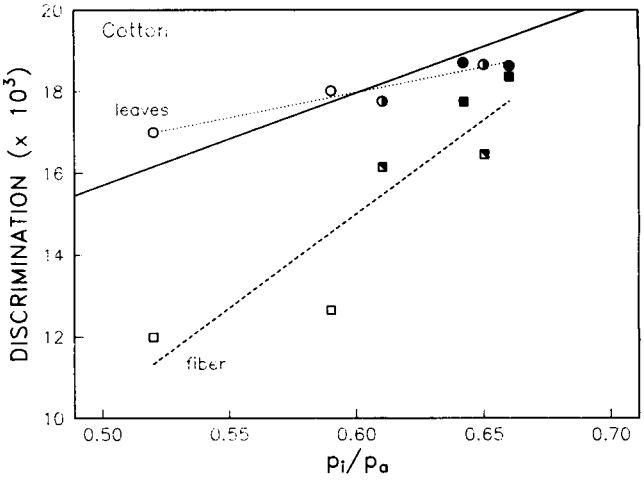

Figure 4. Relationships between p_i/p_a and carbon isotope discrimination (Δ) measured in leaf (circles) and seed epidermal hair (squares) dry matter. Open symbols: 250 mM NaCl treatment; half-closed symbols: 50 mM NaCl treatment; closed symbols: control plants. Solid line: relationship from Eq. 1 with $b = 27 \times 10^{-3}$ and $d = 0$. Dotted line: regression equation for Δ in leaves, $y = 11.9 + 9.7x$ ($r^2 = 0.87$). Broken line: regression equation for Δ in fiber, $y = -7.4 + 37.3x$ ($r^2 = 0.69$).

DISCUSSION

Plant growth was strongly reduced by salinity (Tables I and II), but bean showed a greater reduction of growth than cotton. In bean, the growth was severely inhibited even at 50 mM NaCl after a few days from the start of the treatments, whereas the growth of cotton was reduced but not stopped even at the highest NaCl concentration used. Bean is known to be highly sensitive to salinity, whereas cotton has been reported to as tolerant non-halophyte (18).

Assimilation rate and stomatal conductance always declined when cotton and bean plants were exposed to salinity (Fig. 2, Table III). This is consistent with previous observations on the effect of salinity on photosynthesis of non-halophytes (7, 14, 23). Similarly, declines of g and A have been reported in several halophytes (1, 12, 16).

In cotton plants p_i declined with increasing salinity (Fig. 2), indicating that the salt-induced decrease of A was at least partly caused by stomatal closure. In contrast, bean plants grown under salinity showed a marked decline of A and g, but not of p_i (Fig. 2, Table VI) which remained almost constant, or increased in the 50 and 150 mM NaCl treatments respectively.

A decline of A without a corresponding decline of p_i usually has been interpreted as a direct effect of stress on photosynthetic capacity. However, it has become evident that such interpretation might be incorrect if patchy photosynthesis does occur during stress (28). Salinity might cause patchy

Table VI. *Comparison of Carbon Isotope Discrimination (Δ) of Bean Plant Material in Different Plant Organs (Leaves, Stems Pods, and Seeds) and p_i Calculated from Gas Exchange Measurements*

Sample for isotope analysis were taken from six plants for each treatment. Measurements on the same sample were repeated four times.

Treatment	Discrimination ($\Delta \times 10^3$) ± SE				p_i ± SE
	Leaves	Stems	Pods	Seeds	
mM NaCl					
0 (control)	20.6 ± 0.1	20.4 ± 0.2	19.4 ± 0.2	17.6 ± 0.3	220 ± 10
50	17.9 ± 0.1	17.3 ± 0.3	17.4 ± 0.4	15.5 ± 0.4	210 ± 6
150	16.7 ± 0.4	16.4 ± 0.2			242 ± 9

stomatal aperture and, if so, the salt-induced decrease of the initial slope and of the CO_2 saturated region of $A(p_i)$ relationships in both cotton and bean leaves (Fig. 2) would be overestimated to some extent (14).

This view is confirmed by the analysis of carbon isotope discrimination in plant material. On the basis of Equation 1, one would expect a positive relationship between Δ in plant material and p_i/p_a measured by gas exchange. In bean plants, Δ measured in different plant organs, always decreased with increasing salinity (Table VI), although p_i/p_a measured by gas exchange was unaffected or even, increased in 150 mM NaCl grown plants (Fig. 2, Table VI). This would appear to imply that p_i/p_a measured by gas exchange in salt stressed bean leaves was overestimated to different extents, increasing with salinity concentration.

From the analysis of Δ in plant material, one can estimate the average p_i/p_a, weighted by assimilation rate, in different salinity treatments or, at least, the variation of p_i among treatments (15). Values of Δ in leaves of 20.63×10^{-3} in control plants, of 17.96×10^{-3} in 50 mM grown plants, and of 16.67×10^{-3} in 150 mM NaCl treatment would correspond to values of p_i/p_a, in this order, 0.72, 0.60, and 0.54. The observed range of variation of p_i is substantial and seems to indicate that the effect of salinity on photosynthetic CO_2 assimilation is mediated by partial stomatal closure.

The analysis of carbon isotope discrimination in cotton leaves and seed epidermal hair (fiber), is consistent with a salt-induced increase of stomatal limitation of A, also evident from gas exchange results. The lower values of Δ in stressed cotton plants compared with control plants, indicate a decrease of g and hence of p_i (10, 15). In cotton leaves and fiber, Δ correlated with the average p_i/p_a measured by gas exchange ($r^2 = 0.87$ for Δ in leaves and $r^2 = 0.69$ for Δ in fiber).

It is noteworthy that seed material was always enriched in ^{13}C compared to leaf material, both in cotton (*cf.* Δ of fiber with that of leaves; Fig. 4) and bean (*cf.* Δ of seeds with that of leaves or shoots; Table VI). These differences in Δ may have several explanations. A fractionation in favor of ^{13}C during translocation of carbon toward the growing seeds is possible, but not likely. Seeds and leaves might also contain different proportions of products of PEP carboxylation. However, it seems unlikely that such differences would cause such large variations in Δ, particularly in cotton fiber which consists of almost pure cellulose. Another possibility is that the carbon of seed material (*e.g.* cotton fiber) is derived from CO_2 assimilated later during ontogeny with respect to that of leaf organic matter, with decreasing p_i/p_a. This seems to be reasonable, since the decline of p_i/p_a was more dramatic in stressed plants. The p_i/p_a compared with Δ in Figure 4 is the assimilation weighted average measured by spot gas exchange during the entire lifespan of plants. The observed deviation of these relationships from that predicted from Equation 1 could be explained if the carbon of leaves and fiber were assimilated under p_i/p_a ratios different from the averages obtained by gas exchange measurements. This effect would be more important in stressed cotton plants, in which p_i/p_a decreased from 0.70 at the beginning of salt application to 0.40 at the end of experiment (data not shown). Possible variations of liquid phase and cell wall resistances could also contribute to the observed differences (11). However, carbon isotope discrimination in cotton and bean plants indicates that stomatal closure is the main cause of the observed reduction of A under salinity stress, and that calculations of p_i may be incorrect in salt stressed plants.

That stomatal aperture in stressed leaves is heterogenous is also indicated by the analysis of $A(p_i)$ curves obtained with cotton leaves at 20 mbar O_2. The salt-induced inhibition of photosynthetic capacity as detected by a decline of the initial slope of $A(p_i)$ relationships in 200 mbar O_2 was largely overcome in 20 mbar O_2 (Fig. 3). Hypersensitivity to O_2 may be interpreted as indicating that heterogeneity of stomatal aperture is limiting photosynthesis (26). In fact, CO_2 limited A, as would occur in leaf compartments with tightly closed stomata, is very sensitive to varying O_2. Therefore, hypersensitivity shown by salt stressed cotton leaves would support the view that the salt induced inhibition of photosynthetic capacity is partly apparent, being indeed caused by patchy photosynthesis.

The study of O_2 evolution partly confirmed the above observation. The insensitivity of the apparent photon yield of O_2 evolution to salinity in both cotton and bean plants (Table IV) seems to demonstrate that the photochemical efficiency was unaffected by salinity. Salt-induced reduction of photon yield of CO_2 assimilation has been previously reported in bean plants by Seemann and Critchley (23), and this is not consistent with the present results. This could be due to different plant age or exposure time to NaCl when photon yield measurements were made. We also observed a decline of Φ_i in bean leaves, but only when other damages (as Chl bleaching) were clearly evident. On the other hand, Seemann and Critchley (23) measured the photon yield by gas exchange at high CO_2 partial pressure, but probably not exceeding 1 mbar that

is usually the maximum allowed by IRGA systems. Therefore, it is also possible that their results were affected by heterogeneity of stomatal opening (28).

It has been shown that PSII in isolated thylakoids from both halophytes and glycophytes is highly sensitive to NaCl *in vitro* (2). However, an effect of NaCl on PSII should cause a reduction of photon yield and this was not observed in the present study.

The light- and CO_2-saturated rate of O_2 evolution was somewhat reduced by salinity, in cotton and bean plants (Table V). To compare this effect with that on the RuBP limited region of $A(p_i)$ relationships, the rate of linear electron transport was calculated from the maximum rate of O_2 evolution assuming that 4.5 mol of electrons are moved through the electron carriers chain per mol of O_2 evolved (29). This estimation of the rate of electron transport was strongly correlated ($r^2 = 0.95$) with that calculated from CO_2 assimilation at p_i of about 500 μbar (29) in both the species analyzed (Fig. 5). However, a 1:1 relationship would be expected, whereas the slope of the regression equation was 1.56 (Fig. 5). The deviation of observed data from the expected relationship increased with increasing salinity. A possible explanation of this deviation is that the electron transport rate calculated from gas exchange in stressed leaves is underestimated because of patchy stomatal closure. This effect would increase with increasing salinity, causing the observed shift of the slope of the regression of Figure 5. Cell wall resistance could also increase in stressed plants, and such an increase would eventually contribute to underestimate the calculation of electron transport based on p_i, since in such case the $p(CO_2)$ inside the chloroplast actually available for RuBP carboxylase would be significantly lower than p_i.

The results obtained in the present experiment with tolerant and sensitive nonhalophytes, demonstrate that (a) plant growth and leaf area development are much more salt sensitive than the photosynthetic apparatus; (b) stomatal conductance and intercellular CO_2 partial pressure also are reduced by salinity, as indicated by gas exchange and carbon isotope discrimination; (c) the photon yield, measured at rate-limiting PPFDs, was unaffected by the NaCl concentrations tested; and (d) the light-saturated rate of O_2 evolution was somewhat reduced by salinity.

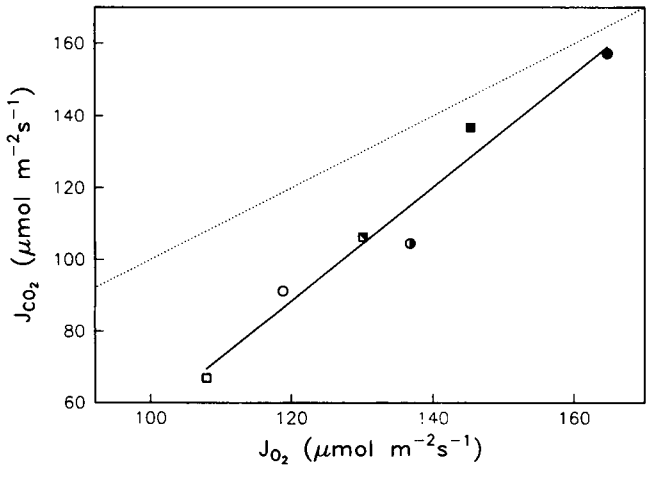

Figure 5. Relationship between the electron transport rate calculated from CO_2 assimilation at p_i of about 500 μbar and that calculated from O_2 evolution measured at CO_2 saturation. All measurements were performed at a PPFD of 1200 μmol photons m^{-2} s^{-1}. Solid line: regression equation $y = -98.96 + 1.56x$ ($r^2 = 0.95$). The broken line denotes the relationship assuming $y = x$. Symbols: Cotton: (●), control; (◑), 50 mM NaCl; (○), 250 mM NaCl; Bean: (■), control; (◪), 50 mM NaCl; (□), 150 mM NaCl.

There are several possible explanation of the latter observation. It has been recently reported that starch synthesis (22, 25, 30) and extractable SPS activity are both reduced by mild water stress (26, 30). The reduction of starch synthesis would results from effects which generally limits starch formation at low CO_2 assimilation such as low level of 3-phosphoglycerate (25, 30). Similarly, the reduced SPS activity has been attributed to the CO_2 limited photosynthesis due to stomatal closure (26; but see also 22). Feedback inhibition of photosynthesis could also occur under salt stress, causing the observed variation of photosynthetic capacity.

However, it is also possible that the observed reduction of photosynthetic capacity is caused by modification of the pattern of resource allocation, such as a reduced investment of resources in the photosynthetic apparatus, consequent to the CO_2 limited photosynthesis, in favor of other plant organs such as roots or reproductive organs, as suggested by carbon isotope discrimination data. Further investigations are needed to understand if this observation is a consequence of an adaptive response to reduced CO_2 availability rather than a deleterious effect of stress on biochemistry.

ACKNOWLEDGMENTS

We thank Prof. Olle Björkman for valuable discussion and criticism on the manuscript. We also wish to thank Luciano Spaccino for skillful assistance in sample preparation and carbon isotope analysis.

LITERATURE CITED

1. **Ball MC, Farquhar GD** (1984) Photosynthetic and stomatal response of two mangrove species, *Aegiceras corniculatum* and *Avicennia marina* to long term salinity and humidity conditions. Plant Physiol **74:** 1–6
2. **Ball MC, Anderson JM** (1986) Sensitivity of photosystem II to NaCl in relation to salinity tolerance. Comparative studies with thylakoids of the salt-tolerant mangrove, *Avicennia marina*, and the salt-sensitive pea, *Pisum sativum*. Aust J Plant Physiol **13:** 689–698
3. **Björkman O, Demmig B** (1987) Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. Planta **170:** 489–504
4. **Brugnoli E, Hubick KT, von Caemmerer S, Wong SC, Farquhar GD** (1988) Correlation between the carbon isotope discrimination in leaf starch and sugars of C_3 plants and the ratio of intercellular and atmospheric partial pressures of carbon dioxide. Plant Physiol **88:** 1418–1424
5. **Brugnoli E, Lauteri M** (1990) An evaluation of the effect of salinity on photosynthesis. *In* M Baltscheffsky ed, Current Research in Photosynthesis, Vol IV. Kluwer Academic Publishers, The Netherlands, pp 741–744
6. **Cornic G, Le Gouallec L, Briantais JM, Hodges M** (1989) Effect of dehydration and high light on photosynthesis of two C_3 plants (*Phaseolus vulgaris* L. and *Elatostema repens* (Lour.) Hall f.). Planta **177:** 84–90
7. **Downton WJS, Grant WJR, Robinson SP** (1985) Photosynthetic

and stomatal responses of spinach leaves to salt stress. Plant Physiol **77:** 85–88

8. **Downton WJS, Loveys BR, Grant WJR** (1988) Stomatal closure fully accounts for the inhibition of photosynthesis by abscisic acid. New Phytol **108:** 263–266
9. **Downton WJS, Loveys BR, Grant WJR** (1988) Non-uniform stomatal closure by water stress causes putative non-stomatal inhibition of photosynthesis. New Phytol **110:** 503–509
10. **Ehleringer JR** (1990) Correlations between carbon isotope discrimination and leaf conductance to water vapor in common beans. Plant Physiol **93:** 1422–1425
11. **Evans JR, Sharkey TD, Berry JA, Farquhar GD** (1986) Carbon isotope discrimination measured concurrently with gas exchange to investigate CO_2 diffusion in leaves of higher plants. Aust J Plant Physiol **13:** 281–292
12. **Farquhar GD, Ball MC, von Caemmerer S, Roksandic Z** (1982) Effect of salinity and humidity on δ^{13} values of halophytes—evidence for diffusional fractionation determined by the ratio of intercellular/atmospheric partial pressure of CO_2 under different environmental conditions. Oecologia **52:** 121–124
13. **Farquhar GD, O'Leary MH, Berry JA** (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Aust J Plant Physiol **9:** 121–137
14. **Farquhar GD, Masle J, Hubick KT, von Caemmerer S, Terashima I** (1987) Effects of drought, salinity and soil compaction on photosynthesis, transpiration and carbon isotope composition of plants. Curr Top Plant Biochem Physiol **6:** 147–155
15. **Farquhar GD, Ehleringer JR, Hubick KT** (1989) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Physiol Plant Mol Biol **40:** 503–537
16. **Flanagan LB, Jefferies RL** (1989) Effect of increased salinity on CO_2 assimilation, O_2 evolution and $\delta^{13}C$ values of leaves of *Plantago maritima* L. developed at low and high NaCl levels. Planta **178:** 377–384
17. **Flowers TJ, Troke PF, Yeo AR** (1977) The mechanisms of salt tolerance in halophytes. Annu Rev Plant Physiol **28:** 89–121
18. **Greenway H, Munns R** (1980) Mechanisms of salt tolerance in non-halophytes. Annu Rev Plant Physiol **31:** 149–190
19. **Hewitt EJ, Smith TA** (1975) Plant Mineral Nutrition. English University Press, London
20. **Munns R, Termaat A** (1986) Whole plant responses to salinity. Aust J Plant Physiol **13:** 143–160
21. **O'Leary MH** (1988) Carbon isotopes in photosynthesis. Bioscience **38:** 328–356
22. **Quick P, Siegel G, Neuhaus E, Feil R, Stitt M** (1989) Short term water stress leads to a stimulation of sucrose synthesis by activating sucrose phosphate synthase. Planta **177:** 535–546
23. **Seemann JR, Critchley C** (1985) Effects of salt stress on the growth, ion content, stomatal behaviour and photosynthetic capacity of a salt sensitive species, *Phaseolus vulgaris* L. Planta **164:** 151–162
24. **Seemann JR, Sharkey TD** (1986) Salinity and nitrogen effects on photosynthesis, ribulose-1,5-bisphosphate carboxylase and metabolite pool size in *Phaseolus vulgaris* L. Plant Physiol **82:** 555–560
25. **Sharkey TD, Seemann JR** (1989) Mild water stress effects on carbon-reduction-cycle intermediate, ribulose-1,5-bisphosphate carboxylase activity, and spatial homogeneity of photosynthesis in intact leaves. Plant Physiol **89:** 1060–1065
26. **Sharkey TD, Loreto F, Vassey T** (1990) Effects of stress on photosynthesis. *In* M Baltscheffsky, ed, Current Research in Photosynthesis, Vol IV. Kluwer Academic Publishers, The Netherlands, pp 549–556
27. **Sokal TD, Rohlf FJ** (1981) Biometry. Freeman and Co, San Francisco
28. **Terashima I, Wong SC, Osmond CB, Farquhar GD** (1988) Characterisation of non-uniform photosynthesis induced by abscisic acid in leaves having different mesophyll anatomies. Plant Cell Physiol **29:** 385–395
29. **von Caemmerer S, Farquhar GD** (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta **153:** 376–387
30. **Vassey TL, Sharkey TD** (1989) Mild water stress causes a reduction in the extractable activity of sucrose phosphate synthase. Plant Physiol **89:** 1066–1070