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# Salinity Effects on Leaf Anatomy

## CONSEQUENCES FOR PHOTOSYNTHESIS[1]

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### ABSTRACT

**Increasing salinity led to substantially higher ratios of mesophyll surface area to leaf area ($A^{mes}/A$) for *Phaseolus vulgaris* and *Gossypium hirsutum* and a smaller increase for *Atriplex patula*, a salt-tolerant species. The increase in internal surface for $CO_2$ absorption did not lead to higher $CO_2$ uptake rates, since the $CO_2$ resistance expressed on the basis of mesophyll cell wall area ($r_{cell}$) increased even more with salinity. The differences among species in the sensitivity of photosynthesis to salinity in part reflect the different $A^{mes}/A$ and $r_{cell}$ responses.**

Increases in leaf thickness can be induced by exposure of roots to high concentrations of NaCl (6, 11, 17–20). Such salt-induced succulence could lower the resistance to $CO_2$ uptake and thus increase photosynthetic rates by increasing the amount of internal leaf surface area across which gaseous exchange can occur per unit leaf area. However, high concentrations of substrate NaCl generally reduce photosynthesis (2, 4, 12), although the photosynthetic rates of some species from saline habitats can be rather insensitive to high salinity (1, 8, 10).

At saturating irradiance, photosynthesis is generally limited by the rate of $CO_2$ diffusion into the leaf. The two most important components controlling this diffusion are stomatal resistance and mesophyll resistance (9). Using the ratio of mesophyll surface area to leaf surface area, the mesophyll resistance can be partitioned into effects of internal leaf anatomy and the inherent $CO_2$ diffusion resistance of the mesophyll cells (14, 16).

Here, the interaction between salinity-induced changes in leaf anatomy and net $CO_2$ exchange was studied for *Phaseolus vulgaris, Gossypium hirsutum,* and *Atriplex patula.* These species represent a wide range of salinity tolerance, since bean is salt-sensitive, cotton is moderately tolerant, and *Atriplex* grows in saline habitats (4, 5, 19). Using plants grown under different NaCl treatments, the relationship between NaCl-induced anatomical change and photosynthetic response at the mesophyll cell level was quantitavely analyzed using a resistance circuit analogy.

### MATERIALS AND METHODS

Seeds of *P. vulgaris* L. cv. Kentucky Wonder, *G. hirsutum* L. var. McNair 612, and *A. patula* ssp. *hastata* were germinated in wet sand and the young plants were transferred to nutrient solution after 10 days (bean and cotton) or 25 days (*Atriplex*). Plants were grown hydroponically in aerated nutrient solution (Hoagland No. 1, Hoagland minor solution, and 8 $\mu g\ g^{-1}$ iron in sequestered form [7]) for 7 days. Salinity was varied by adding NaCl (up to 0.4 molal) to the nutrient solution to yield a range of osmotic potentials from −0.05 MPa to −1.8 MPa (1 MPa = 10 bar). Salinity additions were made in daily increments of 0.025 molal for bean and 0.05 molal for cotton or *Atriplex* to reach the indicated levels. Predawn leaf xylem pressures determined with a PMS Instruments pressure bomb were similar to the osmotic potentials of the treatment solutions. Plants were maintained in environmental chambers using a 12-h day at 27 C with 300 $\mu E\ m^{-2}\ s^{-1}$ PAR provided by warm-white fluorescent lamps and a 12-h night at 21 C.

Leaves used for measurements developed under a particular salinity treatment for 19 to 25 days after full salinity had been reached. Rates of water vapor loss and $CO_2$ uptake were determined at 1,700 ± 200 $\mu E\ m^{-2}\ s^{-1}$ PAR on attached leaves of at least two plants in each salinity treatment using a null point, closed circuit flow system with circulating air containing approximately 1% $O_2$ (15). The low $O_2$ level minimized effects of respiration and photorespiration on measured $CO_2$ fluxes (9, 16). Leaf temperature was maintained at 29 ± 1 C as monitored by 36-gauge iron constantan thermocouples and the water vapor pressure difference between leaf and air was 1.5 ± 0.2 kPa.

Net $CO_2$ exchange ($J_{CO_2}$)[2] was represented by the $CO_2$ concentration difference between air and the site of carboxylation divided by a stomatal resistance plus a mesophyll resistance (9, 14, 16). Water vapor resistance ($r_{wv}$) was used as a measure of stomatal resistance and was set equal to the water vapor concentration drop from leaf to air divided by the transpiration rate (the water vapor concentration in the leaf was assumed to be the saturation value at the measured leaf temperature). $J_{CO_2}$ was plotted *versus* the $CO_2$ concentration in the intercellular air spaces next to the stomates ($c^{ias}_{CO_2}$), which was equated to the $CO_2$ concentration outside the leaf minus $J_{CO_2} \times 1.56\ r_{wv}$ (14); the reciprocal of the slope of the line connecting the $CO_2$ compensation point ($c^{ias}_{CO_2}$ at which $J_{CO_2}$ equals zero) and the $J_{CO_2}$ value at ambient $CO_2$ concentration (340 $\mu l\ l^{-1}$) was designated the mesophyll resistance ($r_{mes}$). Since $J_{CO_2}$ was generally linear with $c^{ias}_{CO_2}$ for the range considered here, using initial slopes to estimate $r_{mes}$ would have had little effect on the results.

Leaf thickness and $A^{mes}/A$ were determined for each leaf used in the gas exchange analysis. Fresh sections cut from each side of the leaf midvein were infiltrated with distilled $H_2O$ and examined using a Zeiss microscope with a camera lucida. Cell surface areas were calculated assuming that palisade cells were cylindrical with

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[2] Abbreviations: $A^{mes}/A$: surface area of mesophyll cells per unit leaf surface area; $c^{ias}_{CO_2}$: $CO_2$ concentration in the intercellular air spaces next to the stomates; $J_{CO_2}$: net $CO_2$ exchange rate per unit leaf area; $r_{cell}$: cellular $CO_2$ resistance expressed on a mesophyll surface area basis; $r_{mes}$: $CO_2$ mesophyll resistance; $r_{wv}$: water vapor resistance (principally stomatal).

hemispherical ends and spongy cells were spheres (13, 16). The ratio of mesophyll cell surface area to leaf surface area ($A^{mes}/A$) was derived from the leaf anatomical measurements and used to calculate $r_{cell}$ (14):

$$r_{cell} = r_{mes} \times A^{mes}/A \quad (1)$$

Fresh and dry leaf weights were also determined, and fresh weight/cm$^2$ − dry weight/cm$^2$ was designated succulence (12, 17). To estimate plant dry matter production, the dry weight of the whole plant was determined 30 days after full salinity had been reached.

## RESULTS

Salinity had a marked effect on dry matter production per plant (Fig. 1A). Plant biomass of the salt-sensitive bean declined sharply with salinity up to 0.1 molal, cotton biomass declined sharply above 0.1 molal, and the biomass of *Atriplex*, the salt-tolerant species, declined gradually from 0.0 to 0.4 molal NaCl (bean and cotton did not survive salinities 0.1 molal above those indicated in Fig. 1). Leaf succulence increased with increasing NaCl concentration for all three species (Fig. 1B).

Mesophyll thickness also increased with salinity in all three species (Table I), due to an increase in length of palisade cells and an increased number of spongy cell layers. Diameters of palisade cells of bean and cotton remained fairly constant in all salinity treatments, but were greater in the *Atriplex* palisade cells of longer lengths. Spongy cell diameters tended to increase with salinity for all three species (Table I). The surface area of a spongy mesophyll was 33 to 34% of the total $A^{mes}/A$ for bean, was 37 to 40% for cotton, and increased from 45 to 53% as the salinity was raised to 0.4 molal for *Atriplex*. Greater palisade cell lengths and more spongy layers resulted in a higher $A^{mes}/A$ for bean and cotton (Fig. 2). $A^{mes}/A$ for *Atriplex* varied little with increasing salinity, because palisade cells increased in diameter as well as length (Fig. 2 and Table I).

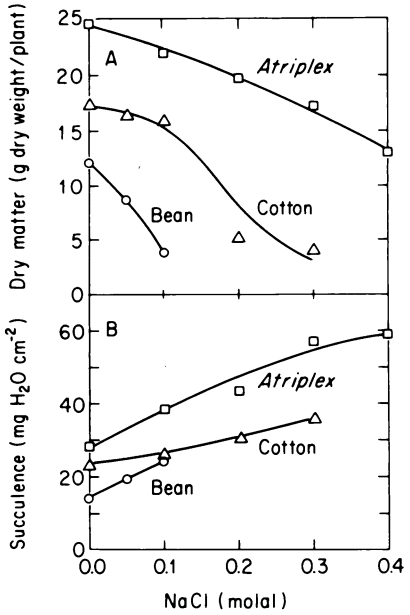


FIG. 1. Effects of NaCl treatments on plant dry matter production (A) and leaf succulence (B) for bean (○), cotton (△), and *Atriplex* (□). Standard errors averaged 5% of the mean.

Net $CO_2$ exchange retes decreased markedly at 0.05 molal NaCl for bean, at 0.2 molal for cotton, while *Atriplex* appeared to be affected only at 0.4 molal (Fig. 3A). Correlated with salinity-induced reductions in net $CO_2$ exchange rates were increases in resistance to water vapor diffusion (Fig. 3B). Over the ranges of NaCl concentrations used, salinity had little effect on $r_{mes}$ for bean, a small effect for *Atriplex*, and an appreciable effect for cotton (Fig. 3C).

Table I. Effects of NaCl on Leaf Thickness and Mesophyll Cell Dimensions. Epidermal thickness is the sum of both lower and upper epidermis; mesophyll thickness is the sum of both palisade and spongy layers. Each entry is the mean of 16 measurements. Standard errors averaged 3% of the mean.

| | NaCl (molal) | | | | | |
|---|---|---|---|---|---|---|
| | 0.0 | 0.05 | 0.1 | 0.2 | 0.3 | 0.4 |
| Epidermal thickness (μm) | | | | | | |
| Bean | 26 | 28 | 31 | | | |
| Cotton | 31 | 35 | 33 | 37 | 36 | |
| Atriplex | 27 | -- | 44 | 41 | 41 | 51 |
| Mesophyll thickness (μm) | | | | | | |
| Bean | 150 | 165 | 260 | | | |
| Cotton | 209 | 256 | 329 | 373 | 422 | |
| Atriplex | 210 | -- | 210 | 212 | 260 | 340 |
| Palisade cell length (μm) | | | | | | |
| Bean | 88 | 103 | 129 | | | |
| Cotton | 85 | 106 | 113 | 118 | 124 | |
| Atriplex | 80 | -- | 80 | 82 | 87 | 115 |
| Palisade cell diameter (μm) | | | | | | |
| Bean | 19 | 18 | 20 | | | |
| Cotton | 20 | 23 | 21 | 23 | 20 | |
| Atriplex | 29 | -- | 28 | 23 | 34 | 43 |
| Spongy cell diameter (μm) | | | | | | |
| Bean | 25 | 22 | 32 | | | |
| Cotton | 28 | 23 | 27 | 33 | 34 | |
| Atriplex | 36 | -- | 41 | 41 | 42 | 63 |

Resistance per unit mesophyll cell surface ($r_{cell}$) approximately doubled over the range of salinity used for each species (Fig. 4). The rate of increase in $r_{cell}$ was inversely correlated with salt tolerance, *e.g.* from 0.0 to 0.1 molal NaCl, $r_{cell}$ increased 39% for bean, 28% for cotton, and 13% for *Atriplex*. The minimum cellular resistance of 38 s $cm^{-1}$ for *Atriplex* is apparently the lowest one so far reported, and approaches the predicted lower limit of about 20 s $cm^{-1}$ for $r_{cell}$(14).

To see whether salinity effects on $A^{mes}/A$ and $r_{cell}$ were reversible, cotton was kept in 0.0 molal NaCl, kept in 0.3 molal NaCl, or placed in 0.3 molal NaCl and then transferred to 0.0 molal NaCl after the normal development period of 19 days. Two days after transfer, $J_{CO_2}$ recovered 22% of the salinity-induced inhibition and after 6 days recovered 59% of the difference between 0.0 and 0.3 molal NaCl (Fig. 3A). The increase in $J_{CO_2}$ upon transfer from 0.3 to 0.0 molal NaCl was due to a 44% decrease in $r_{wv}$ and a 35%

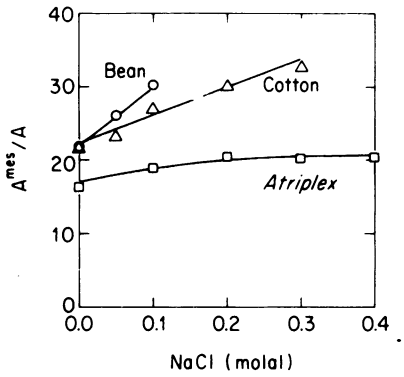


FIG. 2. Mesophyll cell surface area per unit leaf surface area *versus* NaCl treatments for bean (○), cotton (△), and *Atriplex* (□).

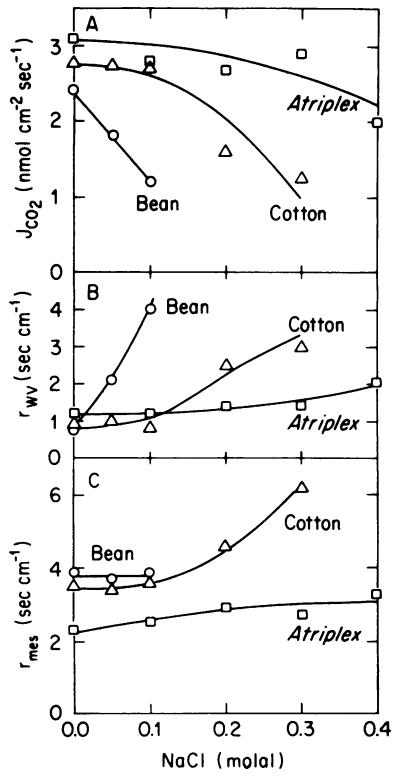


FIG. 3. Net $CO_2$ exchange (A), stomatal resistance (B), and mesophyll resistance (C) for bean (○), cotton (△), and *Atriplex* (□). $J_{CO_2}$ and $r_{wv}$ were determined at an external $CO_2$ concentration of 340 $\mu l\ l^{-1}$, while $r_{mes}$ was calculated from curves of $J_{CO_2}$ *versus* $c_{CO_2}^{ias}$.

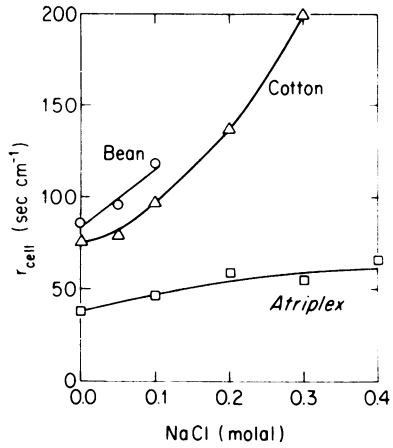


FIG. 4. Influence of NaCl treatments on cellular resistance for bean (○), cotton (△), and *Atriplex* (□).

decrease in $r_{mes}$, which was accompanied by no significant change in $A^{mes}/A$.

## DISCUSSION

Raising the concentration of NaCl in hydroponic solutions resulted in greater leaf succulence (mg $H_2O$ $cm^{-2}$) and greater mesophyll thickness for bean, cotton, and *Atriplex*. Similar effects on succulence and leaf thickness have been reported previously for bean (11, 20) and cotton (18), as well as other species (6, 12, 17). A substantial increase in $A^{mes}/A$ also occurred with increased salinity for bean and cotton, but not for *Atriplex* (Fig. 2). Palisade cell length increased and diameter remained relatively constant with salinity for bean and cotton (Table I), accounting for the increases in $A^{mes}/A$. Both length and diameter of *Atriplex* palisade cells increased, resulting in little change in $A^{mes}/A$ with salinity. Thus $A^{mes}/A$ increased more rapidly with salinity as the salt tolerance of the species decreased.

Salinity can affect photosynthesis at stomatal and/or mesophyll levels, depending on type of salinity, duration of treatment, species, and plant age (2, 4, 5, 8, 10, 12). Here, stomatal closure substantially reduced photosynthesis for bean, while for cotton and *Atriplex* increases in both $r_{wv}$ and $r_{mes}$ were responsible for the decreases in photosynthesis. Although the major focus of this study was on anatomical changes and their impact on mesophyll resistance, a significant effect of salinity was on stomatal resistance.

The increase in $A^{mes}/A$ with salinity could have reduced $r_{mes}$ because there is then more internal cell surface for gas exchange. Such a relationship has previously been shown for illumination effects on *Plectranthus parviflorus* and *Hyptis emoryi* (13, 14, 16). The increases found in $r_{mes}$ (Fig. 3C) together with the increases in $A^{mes}/A$ (Fig. 2) showed that resistance on a mesophyll cell surface basis ($r_{cell}$) increased substantially with salinity, especially for the less salt-tolerant species (Fig. 4). The influence of increasing salinity on $CO_2$ uptake at the mesophyll cell level would not have been apparent if only mesophyll resistance had been measured, since the increases in $A^{mes}/A$ compensated for much of the increases in $r_{cell}$.

Lowering substrate salinity after a high $A^{mes}/A$ had developed could result in a lower $r_{mes}$, if $r_{cell}$ declined in response to the reduction in salinity and there was no change in $A^{mes}/A$. Indeed $A^{mes}/A$ here did not change upon transferring cotton from 0.3 to 0.0 molal NaCl after leaf development and $r_{cell}$ did decline. However, it went only from 200 to 130 s $cm^{-1}$ after 6 days, and hence did not reach the low value of 78 s $cm^{-1}$ appropriate for a plant maintained in 0.0 molal NaCl (Fig. 4). The photosynthetic rate of the transferred plant was not increased above that of the

plant maintained continuously in 0.0 molal NaCl, although most of the salinity inhibition was overcome.

Components of $r_{cell}$ (14) are both physical (cell walls, membranes, intracellular distances) and chemical (reactions of photosynthesis). Although the methods used here do not allow quantitative assessment of each component, calculations based on known ranges of some cellular properties (14) indicate that physical dissimilarities probably could not account for the changes in $r_{cell}$ with salinity or the differences in $r_{cell}$ among species (Fig. 4). The constancy of $r_{cell}$ for *Atriplex* over a wide NaCl range as compared to the variation for bean and cotton presumably indicates differences among species at the chemical level. Such differences in the response of $r_{cell}$ may reflect different degrees of shielding of the photosynthetic mechanism from harmful NaCl effects, rather than inherent dissimilarities in enzyme properties (3).

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