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Salinity Effects on Photosynthesis in Isolated Mesophyll Cells of Cowpea Leaves

Z. Plaut*, C. M. Grieve, and E. Federman

U.S. Salinity Laboratory, 4500 Glenwood Drive, Riverside, CA 92501 (C.M.G.), *and Institute of Soils and Water, Agricultural Research Organization, Bet Dagan, Israel* (Z.P., E.F.)

ABSTRACT

Mesophyll cells from leaves of cowpea (*Vigna unquiculata* [L.] Walp.) plants grown under saline conditions were isolated and used for the determination of photosynthetic CO_2 fixation. Maximal CO_2 fixation rate was obtained when the osmotic potential of both cell isolation and CO_2 fixation assay media were close to leaf osmotic potential, yielding a zero turgor pressure. Hypotonic and hypertonic media decreased the rate of photosynthesis regardless of the salinity level during plant growth. No decrease in photosynthesis was obtained for NaCl concentrations up to 87 moles per cubic meter in the plant growing media and only a 30% decrease was found at 130 moles per cubic meter when the osmotic potential of cell isolation and CO_2 fixation media were optimal. The inhibition was reversible when stress was relieved. At 173 moles per cubic meter NaCl, photosynthesis was severely and irreversibly inhibited. This inhibition was attributed to toxic effects caused by high Cl^- and Na^+ accumulation in the leaves. Uptake of sorbitol by intact cells was insignificant, and therefore not associated with cell volume changes. The light response curve of cells from low salinity grown plants was similar to the controls. Cells from plants grown at 173 moles per cubic meter NaCl were light saturated at a lower radiant flux density than were cells from lower salinity levels.

Several investigators concluded that chloroplasts rather than CO_2 diffusion processes are the primary control of photosynthesis under different kinds of environmental stress (2, 6, 14). However, several recent reports have suggested that photosynthesis of plants exposed to salinity, as measured under saturating CO_2 concentrations, was not greatly altered (4, 5, 22). Stomatal conductance, on the other hand, was significantly decreased, even under mild salt stress (8, 9, 15, 22). A simultaneous determination of water vapor conductance and CO_2 fixation by intact cowpea leaves indicated that stomatal conductance was much more sensitive than CO_2 fixation to salinity (20). However, conversely, other investigators demonstrated that nonstomatal factors were responsible for the inhibition of photosynthesis by salinity (8, 24, 25).

The effect of stomatal conductance on photosynthesis can be by-passed by studying photosynthetic fixation of isolated mesophyll cells. High rates of CO_2 fixation by isolated mesophyll cells from several plant species can be obtained easily (16, 18, 26) and these techniques could be applied to salt-stressed plants. Intact cells have several advantages over isolated chloroplasts for this purpose. Photosynthetically active cells may be isolated from a variety of plants, including salt-sensitive species, whereas, it is much more difficult in the case of chloroplasts. Second, the cells maintain activity for an extended period of time, while chloroplasts decay much faster. Third, the entire metabolic pathway of CO_2, including its release, is retained in the intact cell. Many salt-insensitive plants adjust osmotically when exposed to salinity, and this results in maintenance of turgor. The adjustment is achieved by both enhanced uptake and transport of ions and accumulation of low molecular weight assimilates (7). Such a change in osmotic potential of the cells may require a modification in the media used for cell isolation and for the assay of CO_2 fixation rates.

The purpose of this study was to develop adequate conditions required for CO_2 fixation in isolated mesophyll cells and to investigate the effect of NaCl salinity on photosynthesis of these cells. In appropriately prepared cells the effect of salinity on photosynthesis can be determined, avoiding the interference caused by diffusive conductance of CO_2. Photosynthesis was assayed by measuring $^{14}CO_2$ fixation by cells which were isolated from plants grown under a range of NaCl concentrations.

MATERIALS AND METHODS

Cowpea (*Vigna unquiculata* [L.] Walkp. cv 'California Blackeye' [CB-5]) seeds were germinated on paper towels saturated with 0.5 mol·m^{-3} $CaSO_4$. Twelve 5-d-old seedlings were transplanted into containers filled with 28 L continuously aerated nutrient solution. In some experiments smaller containers, with 15 L, were used for eight seedlings each. The composition of the nutrient solution was as follows: 2.5 mol·m^{-3} $Ca(NO_3)_2$, 3 mol·m^{-3} KNO_3, 1.5 mol·m^{-3} $MgSO_4$, 0.17 mol·m^{-3} KH_2PO_4, 5 mol·m^{-6} Fe as sodium ferric diethylenetriamine pentaacetate, 23 mol·m^{-6} H_3BO_3, 10 mol·m^{-6} $MnSO_4$, 0.4 mol·m^{-6} $ZnSO_4$, 0.3 mol·m^{-6} $CuSO_4$ and 0.1 mol·m^{-6} H_2MoO_4. The pH of the solution was maintained between 5.5 and 6.5. Plants were grown in a naturally illuminated glasshouse in which a rise in temperature above 27°C and a drop below 17°C were prevented with pad and fan and heating systems, respectively.

Three weeks after germination, the cultures were salinized. At this time the primary leaves were fully expanded, the first trifoliated leaf was partly expanded and the second was at an initial expanding stage. NaCl was added to the nutrient solutions over a 3-d period; final NaCl concentrations were 0, 43, 87, 130 and 173 mol·m^{-3}. The salt was added shortly before

sunset, as this timing was found to minimize damage. Solutions were changed every 5 to 7 d. In one experiment plants were transferred after 9 d from the various saline solutions within a 3-d period to a nonsaline nutrient solution for an additional 6 d.

The second or third trifoliated leaf was at full expansion when sampled for cell isolation 7 to 14 d after salination. Leaves were removed 3 to 4 h after sunrise and floated on distilled water in the light for approximately 1 h. Leaves were osmotically adjusted and the floating was not required to regain turgor. It had no effect on CO_2 fixation rates but improved the isolation of cells. Samples of leaf blades (1–3 g) were then cut lengthwise into approximately 1-mm wide strips, discarding major veins, and perpendicularly every 8 to 10 mm. The strips were vacuum infiltrated for 45 s with 10 to 30 mL (depending on size of sample) cell-separation medium in a suction flask; vacuum was released slowly to prevent damage to the tissue.

The cell separation medium was based on that used by Paul and Bassham (18), and contained 300 to 700 $mol \cdot m^{-3}$ sorbitol (as indicated for individual experiments) 1 $mol \cdot m^{-3}$ KNO_3, 0.5 $mol \cdot m^{-3}$ KH_2PO_4, 0.5 $mol \cdot m^{-3}$ $MgSO_4$, 0.125 $mol \cdot m^{-3}$ H_3BO_3, 25 $mol \cdot m^{-6}$ $MnSO_4$, 2.5 $mol \cdot m^{-6}$ $ZnCl_2$, 1 $mol \cdot m^{-6}$ $CuSO_4$, and 50 $mole \cdot m^3$ MES. The pH was adjusted to 5.8 with 1 M Tris. Immediately before use, the following compounds were added: 1 $mol \cdot m^{-3}$ DTT, 10 mM cellobiose, 0.2% methyl-cellulose, 10 mg/ml Macerase (Calbiochem). The strips were shaken constantly and gently at room temperature (24 ± 2°C) for 90 min. The medium was then filtered through a 100 μm nylon screen, the residual leaf strips were discarded and the suspension was kept at 2°C until assay. Intact cells were separated by centrifugation for 1 min at 100*g*, the pellet was washed in a suspension medium recentrifuged for 1 min at 100*g* and resuspended. Suspension and assay media contained 300 to 700 $mol \cdot m^{-3}$ sorbitol (as indicated for individual experiments), 3 $mol \cdot m^{-3}$ KNO_3, 2 $mol \cdot m^{-3}$ $MgSO_4$, 1 $mol \cdot m^{-3}$ KH_2PO_4, 4 $mol \cdot m^{-3}$ $Ca(NO_3)_2$, and 50 $mol \cdot m^{-3}$ MOPS; pH was adjusted to 7.5 with 1 M Tris, and then 1 $mol \cdot m^{-3}$ $MnSO_4$ was added. Shortly before use 1 $mol \cdot m^{-3}$ DTT was added, and the medium was flushed with N_2 for approximately 5 min. Assays were conducted at 20°C in serum-stoppered test tubes which were slightly shaken and illuminated from both sides with fluorescent lamps (240 $\mu E \cdot {}^{-2}s^{-1}$). Cells in suspension were added to the assay medium and illuminated for 6 min prior to the addition of 5 mM $NaH^{14}CO_3$ (0.12 MBq/mole unless otherwise outlined). This concentration of HCO_3^- was equivalent to a CO_2 concentration of 0.9% under the assay conditions. The final volume of the medium (1 mL) contained 10 to 20 μg Chl. At 6-min intervals 0.2-mL samples were transferred into 0.1 mL 50% acetic acid and counted by liquid scintillation.

Cell intactness was measured by exclusion of Evans blue dissolved in solutions of sorbitol (12). Chl content was determined on aliquots of cell suspensions (1).

Uptake of sorbitol by cells during leaf strip incubation for the isolation of cells was determined by using [^{14}C]sorbitol at known specific activities in the medium. Cells were then separated as outlined and washed twice in 2 mL ^{14}C-free sorbitol medium for 20 s. The final pellet was resuspended in 2 mL water and aliquots were counted. The possibility of sorbitol uptake by cells during suspension or CO_2 fixation assay was determined similarly using [^{14}C]sorbitol. In this case, after centrifugation, the packed cells were suspended and shaken in 300 $mol \cdot m^{-3}$ [^{14}C]sorbitol in the standard suspension medium for 3 to 12 min. The cells were then centrifuged for 30 s at 5000*g* and the pellet resuspended in H_2O and counted.

Leaf water potential (ψ_w) was determined with a pressure chamber and leaf osmotic potential (ψ_s) by freezing point depression osmometry of leaf sap following freezing and thawing.

RESULTS

Digestion of the leaf strips with the macerase enzyme system produced three fractions: A, intact cell; B, ruptured cells; and C residual strips. Determination of cell distribution into the three fractions based, on Chl assays, showed that fraction A contained approximately 22 to 25% of the total mesophyll leaf cells of plants grown at 0, 43 and 87 $mol \cdot m^{-3}$ NaCl (Table I). Based on the exclusion of Evans blue, this fraction contained over 80% intact cells at all salinity levels. The size of this fraction could be increased by extending the incubation time, yielding a better representation of the actual mesophyll. This resulted in reduced photosynthetic activity of the isolated cells, and was thus abandoned. There was a decrease in relative Chl content in the intact cell fraction at 130 $mol \cdot m^{-3}$ NaCl in addition to the reduction in Chl content per gram fresh weight. This decrease implies that the yield of intact cells was markedly decreased, probably due to inhibited separation of cells from the intact tissue and increased rupture of isolated cells. Fewer isolated cells could result from the lower efficiency of the digestion at the high leaf Na^+ and/or Cl^- content; while enhanced cell rupture may have been a consequence of suboptimal sorbitol concentrations in the digestion medium at this salinity level. While the intact cell fraction fixed CO_2 at high rates, the fraction of ruptured cells, although high in Chl content, exhibited very low fixation activity (results not presented).

The optimal sorbitol concentrations in the cell isolation and assay media may depend on the osmotic properties of the tissue which will be a function of the salinity level in the growth medium. This was studied by changing sorbitol concentrations in both cell isolation and assay media (Table II). The rates of CO_2 fixation of the cells were affected by the concentration of sorbitol in the cell isolation and in the suspension and CO_2 fixation assay media depending on the salinity level during plant growth. A clear interaction between the concentration of sorbitol in the medium and salinity level can be seen. When sorbitol concentration was raised, fixation rates increased in cells from plants grown at increasing salinity levels. The optimal sorbitol concentration was thus shifted from 300 $mol \cdot m^{-3}$ for the control cells to 400 $mol \cdot m^{-3}$ for the 43 $mol \cdot m^{-3}$ NaCl and to 500 $mol \cdot m^{-3}$ for the 87 and 130 $mol \cdot m^{-3}$ NaCl-grown plants. The lowest sorbitol concentration (200 $mol \cdot m^{-3}$) gave the lowest rates, and only with the control cells was a significant fixation rate detected. On the other hand, the higher sorbitol concentration resulted in

Table I. *Distribution of Chl between Fractions of Digested Cowpea Leaves*

Leaves were taken from plants grown for 8 d at different NaCl concentrations. Strips were cut out of leaves and digested enzymically for separation of intact mesophyll cells. Sorbitol was added to digestion media at concentrations between 300 and 700 mM, depending on salt concentration in the growth medium. SD of means in parentheses.

NaCl	Sorbitol Concentration in Medium	Chl in Fractions			Chlorophyll in Fresh Strips
		A Intact cells	B Ruptured cells	C Residual strips	
$mol \cdot m^{-3}$	$mol \cdot m^{-3}$		%		*mg/g fresh wt*
0	300	25	38	37	1.41 (.12)
43	300	24	38	38	1.44 (.12)
87	500	22	39	39	1.37 (.11)
130	700	14	44	42	0.97 (.09)

Table II. *CO_2 Fixation Rates of Leaf Cells Isolated from Cowpea Plants Grown at Different Salinity Levels for 11 d*

Cells were isolated, resuspended, and assayed at a series of sorbitol concentrations. Rates were calculated for the initial 6 min of exposure to light and CO_2. The sorbitol concentrations were varied in the cell isolation suspension and assay medium.

Sorbitol Concentration	CO_2 Fixation Rates with NaCl in Nutrient Solution (mol m^{-3}):			
	0	43	87	130
$mol \cdot m^{-3}$	$\mu mol \cdot mg\ Chl^{-1} \cdot h^{-1}$			
200	39.0	4.8	0.9	0.4
300	91.7	96.5	62.6	34.4
400	76.8	108.9	86.2	55.0
500	48.5	74.2	98.0	61.8
700	18.5	22.2	36.3	52.7

a relatively high rate in cells from 130 $mol \cdot m^{-3}$ NaCl grown plants.

The optimal sorbitol concentration, required for maximal rates, may be related to leaf water and osmotic potentials (ψ_{wl} and ψ_{sl}). A plot of ψ_{wl} and ψ_{sl} *versus* osmotic potential of the nutrient solution (ψ_{se}) was thus determined, yielding a linear relationship ($r \geq 0.95$) with slopes close to unity (Fig. 1). The two lines were nearly parallel down to a ψ_{se} of −0.8 MPa, which would imply that a very similar turgor potential (ψ_p) was maintained at least until a leaf water potential ψ_{wl} of approximately −1.4 MPa, and that at the tested salinity levels cells encountered full osmotic adjustment.

A maximal CO_2 fixation rate by control cells was obtained at 300 $mol \cdot m^{-3}$ sorbitol (Table II), which is equivalent to a ψ_{se} of approx. −0.7 MPa, very close to the control leaf ψ_{sl} (Fig. 2). This implies that a zero cell turgor was required to yield a maximal fixation rate, provided no sorbitol was taken up, as indicated later, and that cells were at steady state. Reduced ψ_{se}, for instance, in the control cells at an external sorbitol concentration of 200 $mol \cdot m^{-3}$, resulted probably in swelling of cells depending on wall extensibility, and this decreased the rate of photosynthesis. The increase in sorbitol concentration for cells from plants grown at elevated salinities was thus required for maximal CO_2 fixation in order to accomplish loss of cell turgor. It is of interest that at sorbitol concentrations producing zero turgor the relative amount of ^{14}C found in the medium outside the cells, regardless of salinity level, was less than at elevated turgor or at reduced cell ψ_s (data not shown). This indicates that non-isotonic conditions enhanced leakage of photosynthetic intermediates out of the cells.

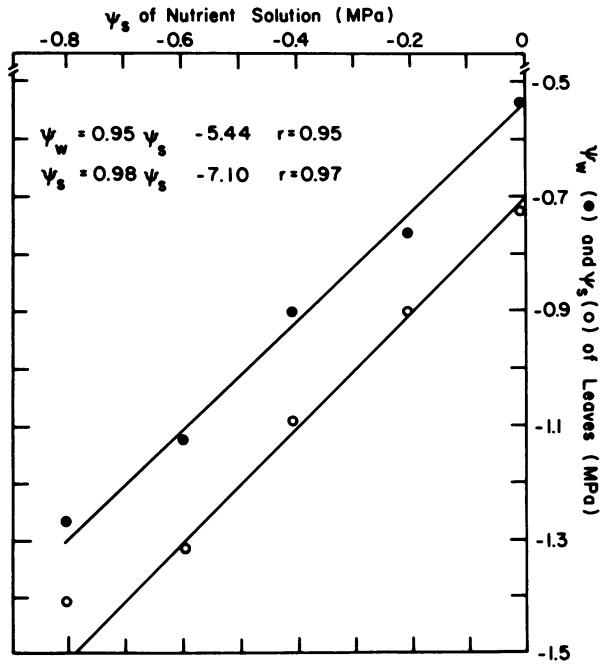

Figure 1. Leaf water potential (ψ_{wl}) and osmotic potential (ψ_{sl}) as a function of external salinity level. Plants were grown at the different salinity levels for 10 d prior to leaf sampling. Water potential was determined with a pressure chamber and osmotic potential by freezing point depression osmometry.

Proper comparison of photosynthesis by cells isolated from plants grown at different salinity levels would therefore require different ψ_s of the cell isolation suspension and assay media. In fact, no decrease in CO_2 fixation rate of leaf cells was obtained up to a salinity level of 87 $mol \cdot m^{-3}$ NaCl (Fig. 2a), while a decrease of approx. 40% was found when these cells were prepared and assayed like the controls (Fig. 2B). At 130 $mol \cdot m^{-3}$ NaCl the decrease was only ≃25%, as compared with 70% (Figs. 2a,b). A nearly linear fixation rate was obtained for the initial 24 min for the control and all salinity

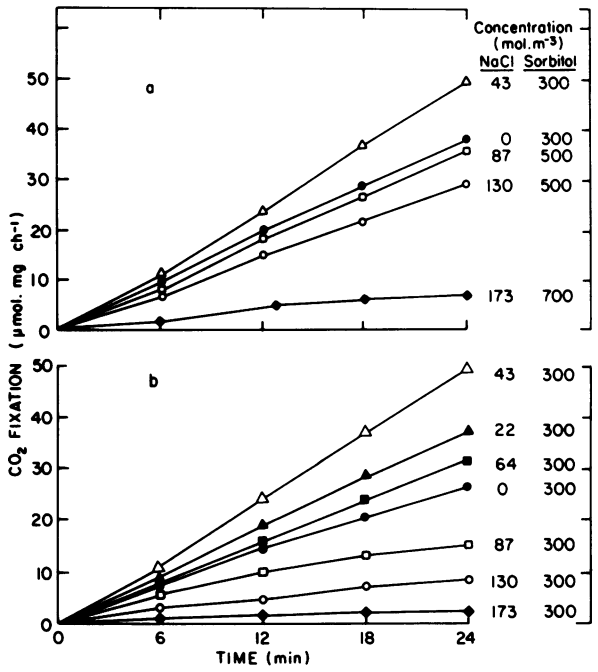

Figure 2. CO_2 fixation of leaf cells isolated from cowpea leaves as function of time. Plants were grown at different salinity levels for 7 d. Cell isolation, suspension and CO_2 fixation were conducted under isotonic conditions (a); or isolated, resuspended and assayed at a constant sorbitol concentration of 300 mol·m^{-3} (b).

Table III. *Concentrations of Cl^- and Na^+ in First and Second Trifoliated Leaves (L-1 and L-2) 7 d after Salinization*

Values are of six replicates.

NaCl	Cl^-		Na^+	
	L-1	L-3	L-1	L-3
mol·m^{-3}	*mmol·kg^{-1} dry wt*			
0	6	7	3	1
43	296	190	207	126
87	388	283	278	94
130	356	226	232	99
173	809	586	678	360

levels both in ψ_s adjusted (Fig. 2a) and unadjusted (Fig. 2b) cells. The rates were enhanced in cells isolated from plants grown at NaCl concentrations up to 43 mol·m^{-3} in both cases; and there was no need for a ψ_s adjustment of cells from plants of these salinity levels. Cells from plants grown at 173 mol·m^{-3} NaCl exhibited a markedly reduced ability to fix CO_2 and the decrease in medium ψ_s had little effect on the inhibition.

The concentration of Cl^- and Na^+ were not much different in the range of external NaCl between 43 and 130 mol·m^{-3} (Table III). This must be ascribed to exclusion of these ions, which was more effective in younger leaves and more predominant in the case of Na^+. It seems that this exclusion was not so sufficient at 173 mol·m^{-3} NaCl, at which the inhibition of CO_2 fixation was very high.

The CO_2 fixation rate of cells from control plants was about 50% of that obtained with intact leaves, based on leaf chlorophyll content (Table IV). In contrast to cells, the fixation rate of intact leaves decreased even in the range of 0–87 mol·m^{-3} NaCl. This decrease can be attributed to the increase in Chl content, which was not necessarily fully light saturated and to a decrease in stomatal conductance.

When plants were transferred from saline to non-saline nutrient solutions, the photosynthetic capability of mesophyll cells from plants grown previously in 87 mol·m^{-3} NaCl, recovered completely, when assayed at 300 mol·m^{-3} sorbitol, and no increase in sorbitol concentration was required to obtain this rate (Table V). Photosynthesis of cells from plants grown at 130 mol·m^{-3} NaCl recovered only partly. Maximum activity was obtained at 300 mol·m^{-3} sorbitol even with these cells, suggesting that adjustment was complete, although the recovery in photosynthesis was not complete. Leaf ψ_w and ψ_s were also reversal within several days after this transfer.

The possibility that sorbitol was taken up by the intact cells during their separation, suspension or CO_2 fixation assay, causing changes in cell ψ_s and volume and thus directly affecting photosynthetic activity was assessed using [^{14}C]sorbitol. The rate of ^{14}C sorbitol uptake during leaf digestion in 400 mol·m^{-3} sorbitol was approx. 50 μmol/mg Chl·h for the first 30 min of incubation, 20 μmol/mg Chl·h for the following 30 min, and negligible thereafter (Table VI). This uptake could not reduce significantly cell ψ_s. In cell suspension media sorbitol was barely accumulated with time even at 20°C. It was therefore concluded that sorbitol served mainly as an osmoticum that maintained the cell at a specific ψ_w during cell isolation, suspension and assay. Changes in cell volume were thus caused primarily by water movement across cell membranes and was not a result of sorbitol uptake by the cells.

Cells isolated from plants grown at the highest salinity level and assayed at appropriate sorbitol concentrations were light-saturated at 160 μE m^{-2}s^{-1}, at a maximal flux density CO_2 fixation was even somewhat reduced (Fig. 3). In cells from lower salinity levels and in the control, the rate of CO_2 fixation was not fully light saturated at the maximal radiant flux density. The similarity in the light response at rate-limiting radiation intensities of cells from the lower salinity levels indicates that mainly nonlight driven reactions were inhibited by salinity.

DISCUSSION

When the isolation of leaf cells and CO_2-fixation assay were conducted in a hypotonic or hypertonic medium, CO_2 fixation was inhibited regardless of salinity level during plant growth (Table II). Hypotonic conditions will induce a marked increase in cell volume and produce excessive turgor pressure resulting in partial rupture of cells which are probably more fragile when in suspension than when packed in the intact leaf. This will result in a decrease in measured CO_2 fixation rates on a Chl basis, since the fraction of ruptured cells (Table I) retained a very low fixation rate. The decrease could also be caused by leakage of intermediates, which seems to be minimal at isotonic conditions. Hypertonic conditions will lead to plasmolysis and shrinkage of the entire cell and its compartments. Such an inhibition of photosynthesis was shown with isolated chloroplasts by several investigators (3,

Table IV. *CO_2 Fixation Rates and Chl Content of Intact Cowpea Leaves Grown at Different Salinity Levels for 12 d*

Fixation rates were determined (Z Plaut, CM Grieve, EV Maas, unpublished data) and expressed on Chl basis. Values are means ± SE of the means.

Measurement	NaCl in Nutrient Solution ($mol \cdot m^{-3}$):				
	0	43	87	130	173
CO_2 fixation rate ($\mu mol \cdot mg\ Chl^{-1}\ h^{-1}$)	195 ± 17	146 ± 14	124 ± 10	108 ± 12	61 ± 9
Chl content ($mg \cdot m^{-2}$)	326	380	364	284	229

Table V. *CO_2 Fixation Rates of Leaf Cells from Plants Grown at Different Salinity Levels for 9 d (A) and from Plants which Were Transferred from Saline to Control Nonsaline Nutrient Solutions for an Additional 6 d (B)*

Sorbitol concentrations were varied with the cell isolation, suspension, and assay medium.

Sorbitol Concentrations	CO_2 Fixation Rates with NaCl Concentration in Nutrient Solution ($mol \cdot m^{-3}$):			
	0	43	87	130
$mol \cdot m^{-3}$	$\mu mol \cdot mg\ Chl^{-1} \cdot h^{-1}$			
A 300	96.3	95.5	39.3	18.4
500	71.7	93.7	91.3	48.3
700	46.5	67.9	65.7	48.4
B 300	101.5	104.6	106.8	72.4
500	74.8	93.5	81.0	56.5
700	38.2	50.3	63.2	43.1

Table VI. *Sorbitol Uptake by Intact Cells of Cowpea Leaves during Leaf Digestion or during Cell Incubation in Suspension Media*

Uptake was determined on a cell Chl basis using [^{14}C]sorbitol (400 mM) of known specific activities.

A. Uptake during digestion

Expt. No.	Sorbitol Accumulation		
	30 min	60 min	90 min
	$\mu mol \cdot mg\ Chl^{-1}$		
1	20.6	33.2	36.2
2	27.6	36.1	35.2

B. Uptake by suspending cells

Expt. No.	Duration of Suspension	Temperature during Suspension (°C)	
		2	20
	min	$\mu mol \cdot mg\ Chl^{-1}$	
1	3	7.7	10.0
	12	7.3	11.5
2	3	12.0	17.5
	12	12.4	16.3

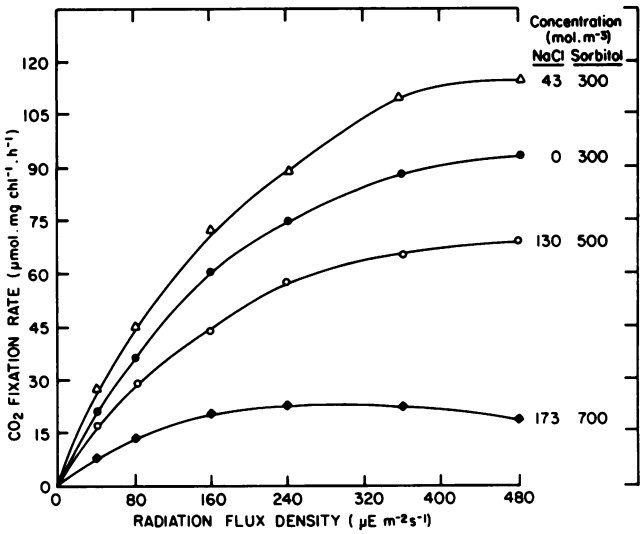

Figure 3. CO_2 fixation rates as a function of radiation flux density for leaf mesophyll cells isolated from control and salt-stressed plants. Plants were grown for 7 d with varying NaCl in the growth media; leaf strips were digested, and cells were assayed for CO_2 fixation at isotonic sorbitol concentrations.

17, 20, 21). It should thus be noted that in intact cells an optimal osmotic potential for the determination of photosynthesis which produced a turgor potential close to zero was required. When sorbitol concentrations were used to produce a ψ_{se} close to the cells ψ_s photosynthesis was not inhibited by NaCl concentration up to 87 $mol \cdot m^{-3}$ and was only moderately inhibited at 130 $mol \cdot m^{-3}$. Kaiser *et al.* (10) have shown that a similar decrease in osmotic volume of cells from control and salt-stressed plants exhibited similar rates of photosynthesis. In this case, such a volume change also required a higher sorbitol concentration for cells from salinized plants.

The ψ_s of the cell separation and CO_2 fixation media has to be set according to the *in vivo* cell ψ_s at the time of isolation and is unrelated to previous conditions. When plants were transferred from saline to non-saline conditions, the salt-adapted cells probably readjusted osmotically, and exhibited maximal activity at 300 $mol \cdot m^{-3}$ sorbitol (Table V). Moreover, the inhibitory effect of salinity on the CO_2 fixation apparatus in cells from salinity grown plants mostly diminishes, suggesting that no permanent injury occurred.

Robinson (21) has recently shown that sorbitol was taken up at high rates by spinach chloroplasts and lowered their ψ_s, causing water uptake, an increase in chloroplast volume and, as a result, in photosynthetic activity. Our data in Table VI indicate clearly that this is not the case with intact cells. The fraction of intact mesophyll cells was found to be approximately 25% (Table I), specific mass of cells is probably close to unity, and the volume of mesophyll in the entire cowpea leaf volume (excluding main veins) was approximately 60% cells. This implies that from 1 g of leaf material we obtained 150 μL of cell containing approximately 350 μg Chl (based on Table I). The amount of sorbitol taken up by such a cell population would thus be around 12 μmol within 90 min of

incubation (Table VI). This would be equivalent to a concentration of 80 mol·m^{-3} in the cells, provided sorbitol was uniformly distributed throughout the cell, as shown by Robinson and Loveys for barley protoplasts (23). Thus only 20% of the sorbitol concentration in the medium (400 mol·m^{-3}) was found in the cells, which is also similar to that found by Robinson and Loveys (23). Moreover, the highest rate of sorbitol uptake during the initial 30 min, as compared with that found later, indicates that most of the sorbitol was probably taken up by free chloroplasts released from ruptured cells into the medium and much less by intact cells. The negligible uptake of sorbitol during suspension between 3 and 12 min, even at a sorbitol concentration of 500 mol·m^{-3}, and the lack of a temperature response, also suggests that very little sorbitol was taken up. Uptake of sorbitol by free chloroplasts does not interfere with the osmotic effect on intact cells, since CO_2 fixation by chloroplasts was practically nil. We therefore conclude that sorbitol served mainly as an osmoticum to control water flow into and out of the cells. The possibility that inhibition of photosynthesis by salinity in the growth medium could be restored during cell separation and suspension by uptake of sorbitol followed by uptake of water must thus be denied. Additional evidence for this is the decline of photosynthesis by control and low salinity cells at elevated sorbitol concentrations (Table II), as was shown also by Mawson and Colman (16).

The fact that there was no response of photosynthesis to mild salinity even at low light intensities (Fig. 3) suggests that quantum yield was hardly affected and that dark reactions were limiting photosynthesis under salinity. Water-stressed plants in contrast, exhibited a decline in quantum yield (17).

The decrease in CO_2 uptake by intact leaves in contrast to isolated mesophyll cells at 0 to 87 mol·m^{-3} NaCl (Table IV), verifies other findings which also show that the decrease in photosynthesis of cowpea plants under low salinity levels could mainly be attributed to reduced stomatal conductance (Z Plaut, CM Grieve, EV Maas, unpublished data). Moreover, the more intense decline in stomatal conductance compared with photosynthesis was interpreted as a stimulation of photosynthetic activity. This can in fact be seen at present with mesophyll cells. In semihalophytic plants, *e.g.* spinach, which accumulate Cl^- and Na^+ in their leaves when grown under salinity, the rate of photosynthesis was not inhibited at NaCl concentrations of 200 mol·m^{-3} (4) or even at 350 mol·m^{-3} (11). In sugar beets, photosynthesis of intact leaves was even enhanced under low salinity levels (9, 19). A significant increase in CO_2 fixation per unit leaf area was also found for a more sensitive crop, eggplant, at mild salinity levels under field conditions (27). Dry matter production of mature leaves which were used for cell preparation was barely affected by salinity, while the growth of young leaves and buds was strongly inhibited (Z Plaut, CM Grieve, EV Maas, unpublished data). This also suggests that even in a salt sensitive plant, as cowpeas, photosynthesis is not the rate limiting factor of photosynthesis, as long as salinity is below a critical level at which it is not excluded from the leaves (Table III).

Water stress, in contrast to salinity, markedly inhibited photosynthesis, as outlined by several investigators (3, 13, 16, 17). This inhibition was mainly by reactions taking place in the chloroplast. PSII activity of the coupling factor and photophosphorolation appeared to be sensitive to water stress (13, 17, 28). Reduced activity of several Calvin cycle enzymes was also shown to be stress-sensitive (3, 10, 20).

The salinity adaptation of leaf cells and the minimal inhibition of photosynthetic activity took place up to a salinity level which caused damage to cells. This level in the case of cowpeas grown under the present environmental conditions was approx. 170 mol·m^{-3} NaCl. Photosynthetic activity was drastically inhibited at this level (Fig. 2); $NaHCO_3$ and light saturation were obtained at lower levels (Figs. 3 and 4). The damage to the photosynthesis apparatus can be related to the sharp increase in accumulation of Na^+ and Cl^- in the leaves at this salinity. Although the compartmentalization of these ions inside the cell is difficult to determine, it is speculated that beyond the point at which exclusion is halted direct toxic effects on photosynthesis may occur.

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