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Salinity and Nitrogen Effects on Photosynthesis, Ribulose-1,5-Bisphosphate Carboxylase and Metabolite Pool Sizes in *Phaseolus vulgaris* L.[1]

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ABSTRACT

Salinity (100 millimolar NaCl) was found to reduce photosynthetic capacity independent of stomatal closure in *Phaseolus vulgaris*. This reduction was shown to be a consequence of a reduction in the efficiency of ribulose-1,5-bisphosphate (RuBP) carboxylase (RuBPCase) rather than a reduction in the leaf content of photosynthetic machinery. In control plants, photosynthesis became RuBP-limited at approximately 1.75 moles RuBP per mole 2-carboxyarabinitol bisphosphate binding sites. Salinization caused the RuBP pool size to reach this limiting value for CO_2 fixation at much lower values of intercellular CO_2. Plants grown at low nitrogen and ± NaCl became RuBP limited at similar RuBP pool sizes as the high nitrogen-grown plants. At limiting RuBP pool sizes and equal values of intercellular CO_2 photosynthetic capacity of salt-stressed plants was less than control plants. This effect of salinity on RuBPCase activity could not be explained by deactivation of the enzyme or inhibitor synthesis. Thus, salinity reduced photosynthetic capacity by reducing both the RuBP pool size by an effect on RuBP regeneration capacity and RuBPCase activity by an unknown mechanism when RuBP was limiting.

The photosynthetic capacity of many plant species is known to be reduced by growth in the presence of NaCl. A portion of this reduction is a direct consequence of stomatal closure and a reduction in the C_i,[2] as demonstrated by changes in carbon isotope fractionation (7, 15). Reductions in photosynthesis which are a consequence of nonstomatal inhibition of photosynthesis by salt have also been observed (3, 6, 11, 15, 22, 23), but the biochemical basis for such alterations in photosynthetic capacity are as yet not understood. However, these alterations in capacity must be the result of either a change in the leaf content of photosynthetic machinery and/or an alteration in the efficiency with which this machinery operates (15, 17).

Seemann and Critchley (15) demonstrated that in *Phaseolus vulgaris* the initial slope of the photosynthetic CO_2 assimilation *versus* C_i (A/C_i) response was reduced with increasing leaf chloride concentration. They showed that this reduction in photosynthetic capacity by salt was a consequence of a reduction in the apparent efficiency (*in vivo* turnover number) of RuBPCase rather than a reduction in the leaf content of the enzyme. This was consistent with their finding that chloride levels in the chloroplast/cytosol were quite high in salt-stressed *P. vulgaris*. The basis for this reduction in the efficiency of RuBPCase was not determined.

Many natural and agricultural areas which are saline are also nitrogen poor. Limiting nitrogen availability during plant growth is known to cause both a reduction in the absolute levels of components of the photosynthetic apparatus and changes in their relative ratios (4). The consequence of such effects of nitrogen limitation in the face of salinity stress are not known.

This paper examines the biochemical basis for the salt-induced reduction in photosynthesis which is independent of stomatal closure in *P. vulgaris* L. The additional effect of limiting nitrogen availability was also examined.

MATERIALS AND METHODS

Plants. *Phaseolus vulgaris* L. (var Tendergreen) was grown essentially as described by Seemann and Critchley (15). Plants were grown in pots in artificial media (vermiculite:perlite, 1:1, v/v) and stood in trays containing nutrient solution (12). 'High' nitrogen plants received a solution containing 10 mM NO_3^-, 6 mM Cl^-, 2 mM SO_4^{2-}, 1 mM $H_2PO_4^-$, 7 mM K^+, 5 mM Ca^{2+}, 2 mM Mg^{2+}, micronutrients, and FeEDTA. 'Low nitrogen' plants received 1.0 mM NO_3^-, 3.5 mM K^+, 3 mM Ca^{2+}, and 1.0 mM Mg^{2+}, with all other ion concentrations similar to the high nitrogen solution. Salt-stressed plants had 100 mM NaCl added to either the high or low nitrogen-containing nutrient solutions.

Plants were grown at approximately 1000 μmol quanta m^{-2} s^{-1} provided by water-cooled mixed metal arc-sodium vapor lamps (Sunbrellas, EGC, Chagrin Falls, OH). Air temperature was 25/15°C day/night with a day length of 14 h. The growth performance of plants grown under either control or salt-stressed conditions at high nitrogen was very similar to those described by Seemann and Critchley (15).

Gas Exchange. Measurements of the rate of whole leaf photosynthetic CO_2 assimilation were made using a gas exchange system and freeze-clamp cuvette described by Sharkey *et al.* (18). The leaf could be rapidly frozen (<250 ms to 0°C) during steady state photosynthesis under a defined atmosphere. This allowed simultaneous measurements of photosynthesis and metabolite pool sizes in leaves of *Phaseolus*. A separate leaflet was used for each data point in the A/C_i curves shown. Calculation of evaporation, conductance to gas exchange, photosynthesis, and inter-

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[2] Abbreviations: C_i, intercellular CO_2 partial pressure; RuBP, ribulose-1,5-bisphosphate; RuBPCase, RuBP carboxylase; CABP, 2-carboxyarabinitol bisphosphate; A, net photosynthetic CO_2 assimilation.

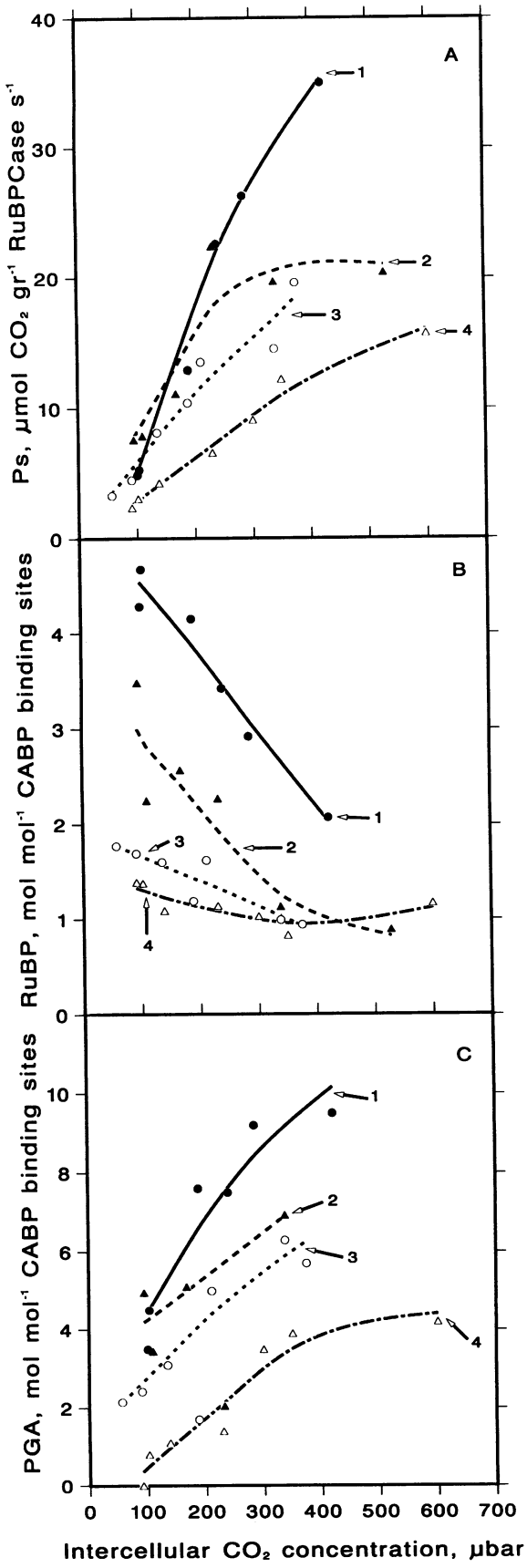

FIG. 1. Photosynthetic and biochemical characteristics of control (●, ▲) and salinized (○, △) *P. vulgaris* grown at high nitrogen (10 mM NO_3) as a function of intercellular CO_2 concentration (C_i). A, The rate of whole leaf photosynthesis per unit RuBPCase (Ps) *versus* C_i. Each point represents a determination of photosynthetic rate, RuBPCase content, and C_i for an individual leaflet which was then freeze-clamped. Points for each numbered curve are data obtained at one time from plants grown together under identical conditions. See "Results" for further details. B, The pool size of RuBP expressed as a function of the RuBPCase catalytic site content (CABP binding sites). Each point corresponds to a point in panels A and C. C, The pool size of PGA expressed as a function of the RuBPCase catalytic site content (CABP binding sites). Each point corresponds to a point in panels A and B.

cellular CO_2 partial pressure were made according to von Caemmerer and Farquhar (19). Leaf temperature was maintained at 25°C and the leaf to air VPD was 10 kPa. Light intensity was 1000 μmol quanta m^{-2} s^{-1} which was saturating for photosynthesis.

Biochemical Determinations. The activity and activation state of RuBPCase were determined as described by Seemann *et al.* (14) on one-half (3 cm^2) of the leaf disc whose metabolism was stopped in the freeze-clamp chamber. Activities were corrected for any unlabeled HCO_3^- in the assay and for the 5% slower rate of catalysis caused by fixation of $^{14}CO_2$ rather than $^{12}CO_2$. The leaf content of RuBPCase was determined in aliquots of the same extracts by radio-labeling each catalytic site of the enzyme with ^{14}CABP and precipitation of the enzyme-CABP complex with rabbit antibodies prepared against purified RuBPCase, as described by Collatz *et al.* (5) and Evans and Seemann (9). It has been determined that no proteolysis of enzyme occurred during the incubation phase of this procedure with *Phaseolus* extract (JR Seemann, unpublished data). Collatz *et al.* (5) have previously shown that 7.55 mol of CABP were bound per mol of purified spinach RuBPCase using this technique, very close to the expected 8 mol binding sites per mol enzyme. The k_{cat} of RuBPCase (mol CO_2 fixed mol^{-1} RuBPCase s^{-1}) was calculated using the CO_2-Mg^{2+} activated activity of the enzyme, a mol wt of 550,000 and assuming 8 mol catalytic sites mol^{-1} RuBPCase.

Metabolite pool sizes were determined on the other half of the leaf disc which had been stored in liquid N_2. The disc was ground to a fine powder in a mortar precooled with liquid N_2, and then 0.5 ml of 3% $HClO_4$ was added and ground in with the leaf powder. Upon thawing, the mixture was centrifuged for 3 min in a microcentrifuge. Of the supernatant, 0.4 ml was combined with 0.135 ml of neutralizing agent (1.67 N KOH + 0.133 M Hepes) plus 0.02 ml of 100 mg ml^{-1} charcoal suspension. The pH of this mixture was checked and adjusted to 7 if necessary. After standing on ice for 10 min, the mixture was centrifuged for 3 min in a microcentrifuge to remove the $KClO_4$ and charcoal. The supernatant was stored for up to 3 d at −80°C before assay. RuBP concentration in the extract was determined as ^{14}C incorporation into acid stable counts using purified spinach RuBPCase. In order that this determination reflect only RuBP concentration and not other pentose phosphates (because of contamination of purified RuBPCase with phosphoriboisomerase and phosphoribulokinase), the extract was preincubated for 10 min with hexokinase and 1 mM Glc to remove ATP.

All other metabolite pool sizes were determined spectrophotometrically by NADH oxidation or NADP reduction in sequential fashion using a Sigma ZFP22 dual wavelength filter photometer (Sigma Instruments, West Germany). Filters were used to select the 334 and 405 nm mercury lines and an extinction coefficient of 6180 was used. An oxidation of 0.1 nmol NADH could be detected. The assay buffer (0.8 ml) was 50 mM Bicine (pH 8.2), 10 mM $MgCl_2$, 0.5 mM EDTA, 4 mM ATP, and 100 μM NADH to which the sample (up to 0.15 ml) was added. The assay was initiated in the photometer cuvette by addition of a mixture of α-glycerol-P dehydrogenese/triose-P isomerase to determine triose-P (glyceraldehyde 3-P plus dihydroxyacetone-P)

Table I. *Nitrogen, RuBPCase Content and Kinetics and Chl Content of Leaflets of Control and Salt-stressed P. vulgaris used for Experiments in Figures 1 and 2 and Leaflets for which Only the Measurements Shown Below Were Made*

Values are ± SE.

	Total N	RuBPCase	Chl	Chl *a/b*	RuBPCase[a]	RuBPCase[b]
	$mmol \cdot m^{-2}$	$g \cdot m^{-2}$		*ratio*	*% Act*	$k_{cat} \cdot s^{-1}$
Controls, high N						
$n = 15$	75 ± 4	0.77 ± 0.05	0.38 ± 0.01	4.08 ± 0.10	64 ± 2	29.0 ± 0.0
100 mM NaCl, high N						
$n = 14$	70 ± 2	0.73 ± 0.04	0.36 ± 0.01	4.01 ± 0.10	71 ± 3	26.0 ± 0.9
Controls, low N						
$n = 16$	45 ± 1	0.53 ± 0.03	0.25 ± 0.01	3.54 ± 0.09	82 ± 3	22.0 ± 0.6
100 mM NaCl, low N						
$n = 11$	37 ± 1	0.43 ± 0.01	0.20 ± 0.01	3.68 ± 0.13	NA[c]	NA[c]

[a] Initial (*in vivo*)/total (CO_2-Mg^{2+} activated) activity. [b] mol $CO_2 \cdot mol^{-1}$ RuBPCase protein $\cdot s^{-1}$. [c] Not available.

Table II. *Pool Sizes of Various Chloroplastic and Cytosolic Metabolites Determined on High Nitrogen Grown (10 mM NO_3) Phaseolus (same samples as those for Fig. 1)*

As there was no significant increase or decrease with C_i for these metabolites, the value given is the average for all points on a particular A/C_i curve.

A/C_i Response	Triose-P	Fru 1,6-P_2	Fru 6-P	Glc 6-P	Glc 1-P	UDPGlc
	mol metabolite/mol CABP binding sites ± SE					
Control curve 1						
$n = 6$	2.67 ± 0.45	0.91 ± 0.22	2.86 ± 0.16	2.86 ± 0.16	0.87 ± 0.10	3.80 ± 1.24
Control curve 2						
$n = 6$	2.45 ± 0.28	1.33 ± 0.23	3.17 ± 0.72	2.45 ± 0.28	1.29 ± 0.25	7.05 ± 1.28
Salt curve 3						
$n = 7$	2.17 ± 0.28	1.22 ± 0.09	1.83 ± 0.28	2.21 ± 0.23	1.35 ± 0.11	3.43 ± 0.63
Salt curve 4						
$n = 7$	2.12 ± 0.20	1.16 ± 0.10	1.75 ± 0.19	2.21 ± 0.29	1.83 ± 0.25	7.66 ± 1.32

concentration. The P-glycerate concentration was determined by addition of a mixture of 3-phosphoglyceric phosphokinase/glyceraldehyde 3-P dehydrogenase. Two NADH molecules are oxidized for every P-glycerate molecule in this reaction. To measure hexose phosphates the assay buffer was made up to 10 μM NADP and the filter photometer rezeroed. Glc 6-P concentration was determined by addition of Glc 6-P dehydrogenase. Fru 6-P concentration was determined by addition of phosphoglucose isomerase. Fructose bisphosphatase was added to determine the Fru 1,6-bisP concentration. This measurement may not be sensitive to sedoheptulose bisphosphate as is the more common aldolase-linked measurement of Fru 1,6-bisP, but this was not tested. Glc 1-P concentration was measured by addition of phosphoglucomutase. UDPGlc was measured by simultaneously adding pyrophosphate (final concentration of 10 μM) and UDP glucose pyrophosphorylase. In each case, 2 units of enzyme were added to the cuvette and the reaction was allowed to go to completion. All chemicals and enzymes were from Sigma with the exception of RuBPCase (purified from spinach) and RuBP for RuBPCase assays, which was generated from ribose 5-P.

Total leaf nitrogen was determined by high temperature digestion in a sulfuric-selenous acid mixture and subsequent colorimetric analysis using an Autoanalyzer (Technicon, Tarrytown, NY). Chl content was determined as described by Arnon (1).

RESULTS

Salinity Stress at High Nitrogen. Photosynthetic CO_2 assimilation at various partial pressures of CO_2 for control and salt-stressed (100 mM NaCl) leaflets of *Phaseolus vulgaris* are shown in Figure 1A. Each point represents a different leaflet from plants grown under either of two treatment conditions. In order to reduce variability in the A/C_i plot which would result from differences between leaflets in the concentration of photosynthetic components, we have expressed the rate of CO_2 assimilation as a function of the leaf RuBPCase content (μmol CO_2 g^{-1} RuBPCase s^{-1}). For studies of the biochemistry of CO_2 assimilation, this is the most appropriate form of expression for photosynthesis, as the capacity for whole leaf CO_2 assimilation is then based directly upon the amount of enzyme which catalyzes the carboxylation reaction. The relationship between photosynthetic capacity and RuBPCase content is known to be quite consistent over a wide range of many environmental variables (*i.e.* light intensity, nitrogen nutrition) (4, 8, 20). In contrast, Chl is a poorer standard, as the RuBPCase/Chl ratio in leaves may vary up to 7-fold in *Phaseolus* grown under different conditions (JR Seemann, unpublished data).

The A/C_i responses for control (curves 1 and 2) and salt-stressed plants (curves 3 and 4) are shown in Figure 1A. Plants used for curves 1 and 4 were 1 to 2 weeks older than those used for curves 2 and 3. Photosynthetic capacity was reduced by salinization at all but the lowest values of C_i, with an increasing effect with time of growth in salt-containing nutrient solution. Since photosynthesis is expressed on the basis of RuBPCase content, these results indicate that salinity caused a reduction in the efficiency of the carboxylation reaction (similar to the result found by Seemann and Critchley [15]).

Salinization did not greatly affect the total nitrogen, RuBPCase or Chl content per unit leaf area (Table I). The Chl *a/b* ratio was also independent of salinization. The CO_2-Mg^{2+} activation state of RuBPCase was significantly higher in the salt-stressed leaflets while the measured k_{cat} was slightly lower in the salt-stressed leaflets. The recently described potent inhibitor of RuBPCase (14, 16) can be detected by a change in the k_{cat} of RuBPCase (14). None of these measures of RuBPCase activity could account for the up to 60% reduction in photosynthetic capacity in response to salt stress in *Phaseolus* leaflets.

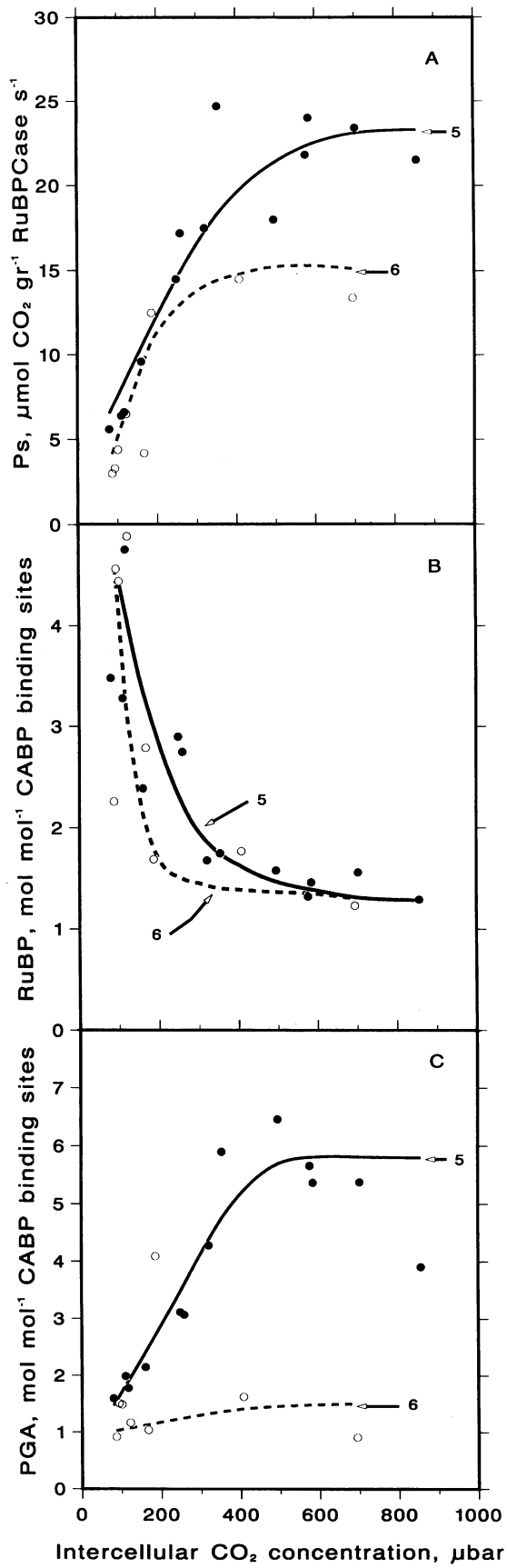

FIG. 2. Photosynthetic and biochemical characteristics of control (●) and salinized (○) *Phaseolus* (as in Fig. 1) except that plants were grown at low nitrogen (1.0 mM NO_3). A, The rate of whole leaf photosynthesis per unit RuBPCase (Ps) *versus* C_i; B, RuBP pool size as a function of CABP binding sites; C, PGA pool size as a function of CABP binding sites. Data at any particular C_i are from the same leaflet in each panel.

After the measurement of photosynthetic capacity at a particular C_i for each leaflet (Fig. 1A), metabolism of that leaflet was rapidly quenched in the cuvette and metabolite pool sizes in the freeze-clamped leaflet piece determined. All metabolite pools were calculated on the basis of the RuBPCase catalytic site content (determined by ^{14}CABP binding); thus, a metabolite pool size of 1 indicates that the pool size of that metabolite in the leaflet was equal to the content of RuBPCase catalytic sites.

The pool size of RuBP in a number of leaves killed over a range of C_i are shown in Figure 1B. RuBP pool size was inversely proportional to C_i (seen previously by Badger *et al.* [2] and Mott *et al.* [13]) for control and salt-stressed *Phaseolus*, but more strongly C_i dependent in leaflets from control plants than in leaflets from stressed plants. At C_i <300 μbar, the RuBP/CABP binding site ratio was significantly lower for the salt-stressed beans than for the control plants, as was photosynthetic capacity (Fig. 1A). The larger reductions in photosynthesis were associated with larger reductions in RuBP pool size (*e.g.* curve 4 *versus* curve 3).

Control A/Ci curve 1 exhibited no saturation of photosynthesis with increasing CO_2, which is consistent with the RuBP-saturated kinetics of RuBPCase (10). The RuBP pool size in these leaflets fell from over 4.5 times binding sites to just over 2 times binding sites. Curve 2, again a control A/Ci curve, was very similar to curve 1 below 200 μbar C_i but above 200 μbar appeared strongly CO_2 limited. Because our measure of photosynthesis is based on the amount of RuBPCase present, a variation in the CO_2 response of assimilation indicates that either RuBPCase was less active at high CO_2 in leaflets used for curve 2 *versus* those for curve 1 (however, the CO_2-Mg^{2+} activation state and k_{cat} were not reduced, data not shown) or that RuBP became limiting at roughly 200 to 300 μbar C_i. If the latter were true, the pool size where RuBP became limiting was between 1.5 and 2.0 times the CABP binding site concentration.

Both A/C_i curves from the salt treatment (Nos. 3 and 4) exhibited less assimilation for the same amount of RuBPCase and CO_2 above 150 to 200 μbar C_i (Fig. 1A). Below 150 μbar the data are difficult to interpret and, in any case, are not diagnostic since the CO_2 compensation point should be almost independent of the RuBP pool size. The reduced assimilation in salt-treated plants was not caused by reduced activation of RuBPCase (Table I). The pool size of RuBP in these plants was below the controls at all but the highest values of C_i and was at or below 1.75 times CABP binding sites over the entire range of C_i measured (Fig. 1B).

P-glycerate pool sizes, expressed as mol mol^{-1} CABP binding sites, are given for a range of values of C_i (Fig. 1C). The P-glycerate pool size increased with C_i for both control and salt-stressed beans and was higher at equal values of C_i for control *versus* salinized plants. The relative levels of P-glycerate for curves 1 to 4 corresponded to the photosynthetic capacity of those plants (Fig. 1A).

Pool sizes of other metabolites involved in photosynthetic carbon metabolism showed no significant change with increasing C_i as was observed by Badger et al. (2). Table II contains average values for each of the four curves. The pool sizes of metabolites given in Table II are the sum of the chloroplastic and cytosolic pools of each metabolite.

Metabolite pool sizes in control and salt-stressed plants (Table II) exhibited few significant differences. Fru 6-P was higher in control than treated plants but this is difficult to interpret because Fru-6 occurs in both the cytosol and stroma and it participates in starch, sucrose, and RuBP synthesis. The largest differences

Table III. *Pool Sizes of Various Chloroplastic and Cytosolic Metabolites Determined on Low Nitrogen Grown (1.0 mM NO_3) Phaseolus (same samples as those for Fig. 2)*

The value given is the average for all points on a particular A/C_i curve.

A/C_i Response	Triose P	Fru 1,6-P_2	Fru 6-P	Glu 6-P	Glu 1-P	UDPGlc
	mol metabolite/mol CABP binding sites ± SE					
Control curve 5						
$n = 13$	2.21 ± 0.17	0.40 ± 0.09	1.92 ± 0.17	2.15 ± 0.14	0.37 ± 0.08	7.78 ± 0.94
Salt curve 6						
$n = 8$	2.10 ± 0.27	0.80 ± 0.17	1.40 ± 0.22	1.67 ± 0.23	0.55 ± 0.13	5.50 ± 1.00

were in UDPGlc concentration, but the variation did not appear to be directly related to salinization.

Salinity Stress at Low Nitrogen. Control and salt-stressed plants grown at low nitrogen (1.0 mM NO_3) had significantly lower nitrogen, RuBPCase, and Chl contents than the high nitrogen grown plants (Table I), with salinized plants slightly lower than control plants. Control plants grown at low nitrogen never achieved as high photosynthetic capacities on an RuBPCase basis (Fig. 2A, curve 5) as control plants sometimes did at high nitrogen (Fig. 1A, curve 1). Photosynthetic capacity of low nitrogen salt-stressed plants was reduced little from the controls at low values of C_i (<200 μbar). At higher values of C_i photosynthetic capacity was reduced by salt to about the same extent as in the high nitrogen beans used to produce curve 2.

Both the control and salt-stressed leaflets exhibited a distinct transition between the initial slope of the A/C_i response at low CO_2 and the CO_2-saturated portion of the curve at higher CO_2 (Fig. 2A). In the salt-stressed leaves this transition occurred at a lower C_i than in the control leaves (approximately 200 *versus* 350 μbar). As for the high nitrogen treatment, the transition in gas exchange characteristics corresponded to the point where the RuBP pool size fell below approximately 1.75 mol mol^{-1} binding sites (Fig. 2B).

P-glycerate pool sizes in the low nitrogen control plants (Fig. 2C) were similar to the lower values found for control plants grown at high nitrogen (curve 2), reaching 5 to 6 mol mol^{-1} CABP binding sites in high CO_2. P-glycerate pool sizes in the salt-stressed low N plants were generally very low. We have no explanation for this.

Pool sizes of other chloroplastic and cytosolic metabolites in low nitrogen-grown control and salt-stressed *Phaseolus* (Table III) tended to be slightly lower than high nitrogen-grown plants (Table II). As with the high nitrogen plants, there was also a small reduction in the pool size of these metabolites in the salt-stressed low nitrogen plants relative to the controls. The exceptions were Fru 1,6-bisP and Glc 1-P which were higher in salt-stressed plants, similar to the high nitrogen plants.

DISCUSSION

The photosynthesis and RuBP pool size data from control *Phaseolus* leaflets (Figs. 1 [A, B], 2[A, B]) provides evidence that the rate of CO_2 fixation may be limited by RuBP regeneration capacity. The pool size of RuBP which began to limit photosynthesis in these plants was approximately 1.75 mol mol^{-1} CABP binding sites (Figs. 1, 2; Refs. 2, 21). This value is different from the value of 1 RuBP per RuBPCase catalytic site value originally proposed (10). This discrepancy has been investigated by von Caemmerer and Edmundson (21) who have shown that when Mg^{2+} binding to RuBP and ionization are considered, a pool of RuBP of between 1.5 and 2 mol mol^{-1} binding sites should be required to saturate RuBPCase, since not all RuBP is in a form which can be utilized in the carboxylation reaction. Our results are consistent with this revised view of limiting RuBP levels.

Salinization of high nitrogen *Phaseolus* plants caused the RuBP pool at low C_i to drop until it was limiting over almost the entire range of C_i measured. Such a limitation should reduce the *in vivo* turnover number (efficiency) of RuBPCase, and in fact the initial slope of the A/C_i curve (adjusted for RuBPCase content) was reduced by salinity (Fig. 1A; Ref. 15). However, in low nitrogen leaves the RuBP pool at low C_i was above 1.75 mol mol^{-1} binding site for both control and salinized plants (Fig. 2B). When RuBP was above 1.75 mol mol^{-1} binding sites there was no effect of salinization on the initial slope of the A/C_i response (Fig. 2A). Therefore, we conclude that salinization does not affect the RuBP-saturated kinetics of RuBPCase.

Salinization generally lowered the RuBP pool size. The biochemical basis for reduced RuBP regeneration capacity resulting from salt stress is unknown, but we hypothesize that it may result from a salinity effect on the capacity for ATP formation. In addition, the rate of photosynthesis at any given pool size of RuBP was lower for leaflets from salinized plants than control leaves except at low C_i in the low nitrogen treatment. This is most evident at values of C_i above the transition region in the A/C_i response, where RuBP pool sizes became equal (approximately 1.1 mol mol^{-1} CABP binding sites in Fig. 1A, approximately 1.3 mol mol^{-1} in Fig. 2A), but a dramatic difference in photosynthetic capacity remained. A salt-related reduction in RuBP regeneration capacity cannot account for this finding. This effect of salinity on photosynthetic capacity must be due to a reduction in the activity of RuBPCase. However, the mechanism for this reduction in activity was not a reduced CO_2-Mg^{2+} activation state or a reduced k_{cat} of RuBPCase caused by the inhibitor (Table I). Clearly, salinity has some other effect on RuBPCase which cannot at present be assessed by *in vitro* techniques. This effect apparently does not occur when RuBP is in saturating supply, as evidenced by the similar initial slopes of the low nitrogen control and salt-stressed plants (Fig. 2A). We have seen similar results after feeding the plant stress hormone abscisic acid to leaves (JR Seemann, TD Sharkey, unpublished data). We conclude that factors other than CO_2-Mg^{2+} activation and the inhibitor of RuBPCase may influence the *in vivo* activity of this enzyme and consequently the photosynthetic capacity of intact leaves.

In summary, we have shown that salinity reduces the photosynthetic capacity of leaves by (a) reducing the pool of RuBP by an effect on RuBP regeneration capacity and (b) reducing the activity of RuBPCase by an unknown mechanism when RuBP is in limiting supply.

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