

Growth, Phosphate Pools, and Phosphate Mobilization of Salt-stressed Sesame and Pepper

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ABSTRACT

The growth and phosphate mobilization of control and salt-stressed sesame (*Sesamum indicum* L.) and pepper (*Capsicum annuum* L.) plants were examined to ascertain whether or not translocation limits growth of salt-stressed plants. Plants were grown in a complete nutrient solution with and without excess salt. One-half of the control and salt-stressed plants were later transferred to phosphate-free culture solution ("–P" plants). Measurements of growth and phosphate pools in leaves indicated that with or without salinity "–P" plants utilized their phosphate reserves to support growth for a time at rates equaling those of plants supplied with phosphate. The results indicate that mobilization was not limiting for growth of salt-stressed plants.

Defoliation experiments were performed at a developmental stage when the import of assimilates by the youngest expanding leaves could be changed by removing certain source or sink leaves. These experiments also indicated that phloem transport was not limiting for leaf growth on salt-stressed plants.

Salinity suppresses plant growth by mechanisms that are not understood. Since young growing tissues must import metabolites, it is of interest to know if the rate of translocation limits growth of salt-stressed plants as has been suggested (6, 8, 10, 11). We investigated this possibility by ascertaining the effects of salinity on growth, phosphate mobilization, and on the dependence of young leaves on source leaves.

MATERIALS AND METHODS

Plant Culture. Seeds of sesame (*Sesamum indicum* L. cv. Long pod) and bell pepper (*Capsicum annuum* L. cv. Yolo wonder) were germinated in Vermiculite moistened with dilute (1:10) Hoagland solution No. 1 (5). This solution was further modified by reducing the Pi concentration to 0.02 mM for pepper and 0.1 mM for sesame and by supplying iron as the chelate, diethylenetriamine pentaacetate (Sequestrene 330). Seedlings 1 or 2 weeks old, depending on the experiment, were transferred to 15-liter polyethylene pots containing a 1:2 dilution of Hoagland solution; the Pi concentration was 0.02 mM for pepper and 0.1 mM for sesame. All experiments except one were conducted in a growth chamber at 25 C and between 60 and 80% RH. A light intensity of 72 w/m² at the plant tops was provided by a combination of fluorescent and incandescent lamps. The photoperiod was 16 hr/day. The defoliation experiment in which young sesame leaves were removed was performed between December and February in a heated

greenhouse with a day-night temperature range of 32 to 18 C and a natural light photoperiod of approximately 12 hr/day. The culture solutions were renewed weekly and/or adjustments were made to maintain the original concentrations. The pH was maintained between 5.5 to 6.5. The Pi concentration in the pepper cultures was closely controlled by circulating the solution from each set of four containers (replicate pots) through a 200-liter reservoir six times daily, each cycle lasting 0.5 hr, and periodically adding Pi to the reservoirs to avoid depletion of more than 30% of the initial concentration.

Culture solutions were salinized when plants were 2 to 4 weeks old. Salts were added to decrease the osmotic potential of culture solutions by 3 bars for pepper (2 bars NaCl + 1 bar CaCl₂) and 2 bars for sesame (1 bar NaCl + 1 bar CaCl₂ in the phosphate mobilization experiment and 2 bars of NaCl in defoliation experiments). The salts were added to the culture solution at the beginning of the dark period in increments of 1 bar/day.

Growth and Phosphate Mobilization Experiments. To determine the efficiency of plants at mobilizing their reserve phosphate, Pi was removed from half of the cultures 10 to 16 days after salination. Plants were sampled randomly, separated into roots and shoots, and their dry weight (oven-dried at 70 C) measured. In one experiment with pepper, growth increments were measured by successive weighings of intact plants after allowing their roots to drain for 30 sec to remove most of the surface-adsorbed water.

The acid-soluble phosphate fraction of leaf samples previously frozen and stored in liquid N₂ was extracted by grinding with cold, dilute perchloric acid (9). The Pi and total acid-soluble phosphate were measured by the Fiske and SubbaRow (4) procedure or by the Bartlett (1) modification of this procedure. In some samples from "–P" plants, the Pi concentration was too low to be determined, so only the total soluble phosphate (Pi + phosphate-esters) is reported.

Defoliation Experiments. Defoliation experiments were performed 10 to 21 days after salination and at a developmental stage when it was expected that the supply of assimilates to the youngest unfolded leaf could be decreased (by removal of old source leaves) or increased (by removal of competing young leaves). All leaves that attained two-thirds of their full size (as determined from separate but similarly treated plants) were regarded as exporting or source leaves and those less than two-thirds full size were considered as importing or sink leaves (3). In all experiments, the most recently unfolded leaf was retained and its rate of expansion measured. Leaf measurements and diagrams of representative patterns of defoliation and locations of the measured leaves are shown in Figures 4 and 5.

RESULTS

GROWTH AND PHOSPHATE MOBILIZATION

¹ Permanent address: Department of Biology, College of Arts and Sciences, Pahlavi University, Shiraz, Iran. Downloaded from www.plantphysiol.org on March 15, 2018. Published by www.plantphysiol.org. The growth (shoot or root) rates of either control or salinized plants
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during the following 16 days even though plant weights increased 2- to 5-fold (Fig. 1). However, during the next 15 days without Pi growth (shoot or root) declined more on control than on saline treatments (as compared with their respective "+P" treatments). This might be expected since nonsalinized plants grew faster and used their reserve phosphate faster than the salt-treated plants.

The phosphate analysis of leaves sampled before and after transfer to Pi-free media (Fig. 2) indicated that both the salinized and control plants mobilized and exported their phosphate reserves from old leaves to young leaves. The phosphate concentration in older leaves declined to comparable values on control and saline treatments. The concentration in young leaves also declined, but less on the saline treatment. The difference was particularly evident on the 47th day. By the 62nd day, the phosphate reserves were virtually depleted on both treatments. The older leaves of salt-stressed plants, but not of controls, became senescent and abscised 7 to 16 days after the transfer to Pi-free media, suggesting that, for these leaves, salinity intensified the phosphate deficiency.

The phosphate concentration in roots of both control and salinized plants decreased from about 10 to 2 $\mu\text{mol/g}$ fresh weight after the transfer to Pi-free media. Since root weight was only a minor fraction of the total plant weight (Fig. 1) and the phosphate concentration of roots was severalfold less than that of the leaves, it is unlikely that the root system was important in supplying phosphate for shoot growth.

Before sesame plants were transferred to Pi-free media, Pi constituted 63 and 66% of the total phosphate in mature leaves of control and salinized plants, respectively (Fig. 3), indicating similar Pi pool sizes. Seven days after removing nutrient Pi, the Pi percentages in leaves had decreased considerably, but similarly, in salinized and control plants. Thereafter, Pi was not distinguished from ester-phosphate; however, the per cent of acid-soluble phosphate did not seem to be affected by salinity in young sesame leaves.

Pepper. The results obtained with pepper were qualitatively the same as those obtained with sesame. Removal of nutrient Pi promoted phosphate mobilization from old to young leaves on both control and saline treatments; about one-half of the phosphate of old leaves was exported within 10 days on both treatments. Growth without nutrient Pi decreased more on control than on saline treatments, suggesting that mobilization was not limiting for growth of salt-stressed plants. Pepper differed from sesame in that salinity about doubled the phosphate concentration in older leaves both before and up to 10 days after removal of nutrient Pi. Salinity had no consistent effect on the distribution of phosphate into Pi, ester-phosphate, and acid-insoluble pools of old leaves but it seemed to increase the per cent ester-phosphate in young leaves.

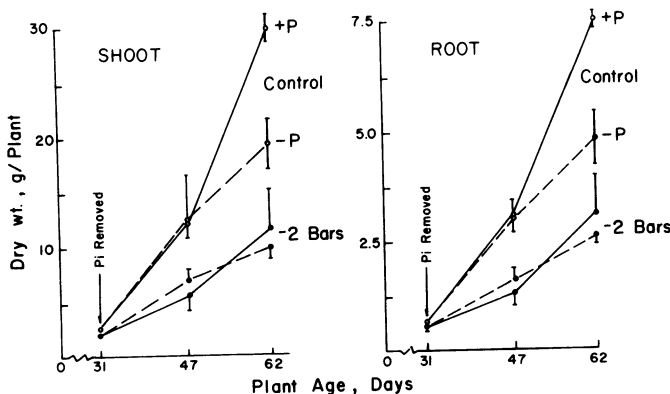


FIG. 1. Effect of Pi removal from culture solution on growth of control (O) and salinized (●) sesame plants. (—): complete culture solution; (---): Pi-free culture. Values are means of three replicates. Vertical lines indicate standard errors.

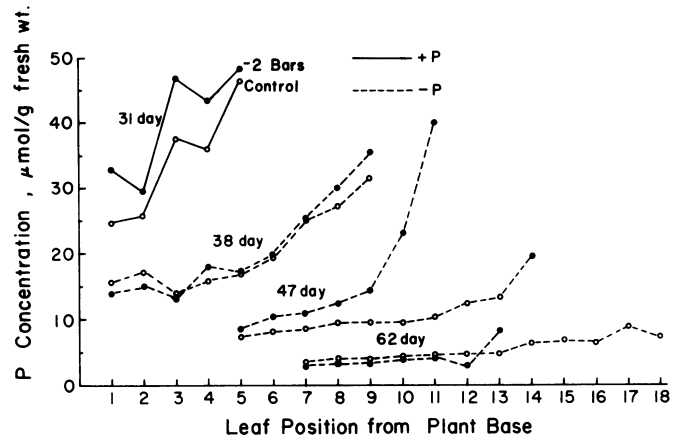


FIG. 2. Phosphate concentration in leaves of control (O) and salinized (●) sesame plants before (—) and after (---) transfer to Pi-free culture. Pi was removed on the 31st day. Values are means of duplicates. On the saline, -P treatment leaves 1 to 4 of 47-day-old plants and leaves 1 to 6 of 62-day-old plants were necrotic or abscised. These and their comparable control leaves were not analyzed.

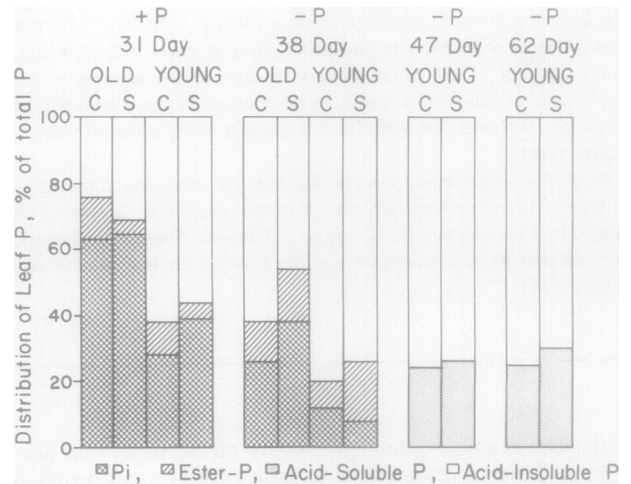


FIG. 3. Distribution of phosphate in various fractions of leaves of control (C) and salinized, -2 bars (S) sesame grown in complete culture solution up to 31 days (+P) and after their transfer to Pi-free media (-P). Old leaf values are means of leaves 1 and 2 and young leaf values are the means of the two youngest unfolded leaves.

DEFOLIATION EXPERIMENTS

Sesame. Removal of all lower leaves more than two-thirds full size suppressed growth of young leaves (Fig. 4), presumably by decreasing the assimilate supply and increasing competition among sinks (roots and the remaining young leaves). The degree of dependency of young leaves on the older ones seemed similar in control and salinized plants.

Removal of sesame leaves less than two-thirds full size stimulated growth of the youngest unfolded leaf of control plants (Fig. 5), presumably by increasing its supply of assimilates. This treatment seemed without effect on growth of comparable leaves of salt-stressed plants.

Pepper. Removal of immature pepper leaves had little or no effect on expansion of the youngest unfolded leaf of either control salinized plants.

DISCUSSION

The apparent absence of an inhibitory salt effect on phosphate mobilization in sesame and pepper is of interest for two reasons.

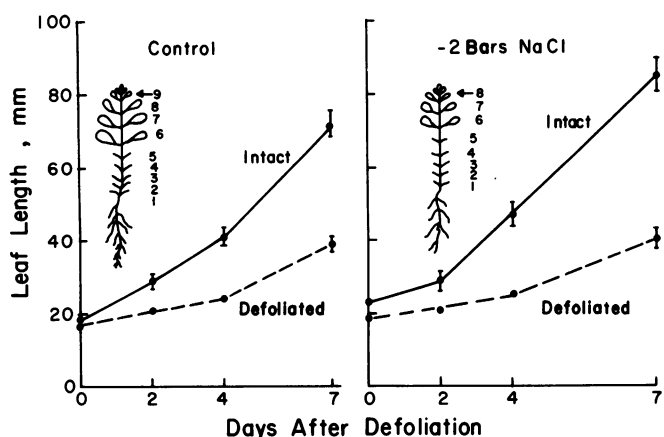


FIG. 4. Effect of removing lower source leaves on growth of young leaves of control (leaf pair 9) and salinized (leaf pair 8) sesame plants. Values are means of eight measurements (opposite leaves of four replicate plants). Vertical lines indicate standard errors.

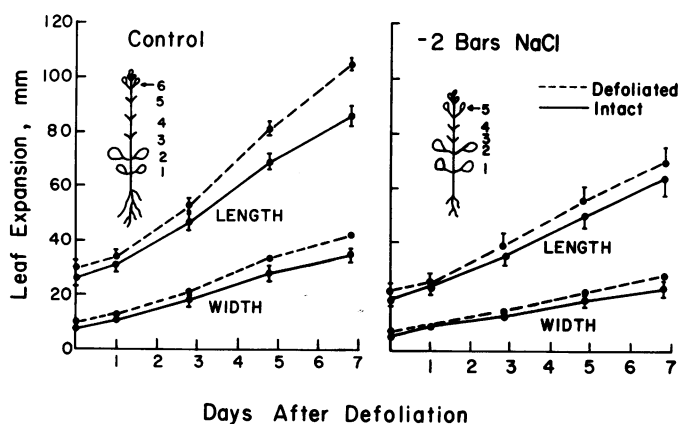


FIG. 5. Effect of removing some upper leaves on growth of young leaves of control (leaf pair 6) and salinized (leaf pair 5) sesame plants. Values are means of six measurements (opposite leaves of three replicate plants). Vertical lines indicate standard errors.

First, phosphate mobilization is a common phenomenon in plants, playing a significant role when phosphate is limiting (2, 13). Second, phloem tissue is the primary pathway for phosphate mobilization (2, 12). Thus, with certain precautions, it may be inferred that in these experiments salinity did not cause phloem transport to be limiting for growth.

The defoliation experiments supplement the phosphate mobi-

lization experiments and provide further evidence that translocation was not limiting for growth of salt-stressed plants. Since in our experiments we measured the effect of source size and sink competition on expansion of the youngest unfolded leaves, we do not know if the same conclusion applies to leaf primordia. It is of interest in this regard that salinity suppressed leaf initiation much more than later stages of expansion growth. There still remains the question of the import by young leaves or primordia of specific metabolites that may be limiting under salt stress (6, 8).

If the transport in salt-stressed plants is diminished because the activity of source and/or sink is suppressed, the problem is of a different nature and does not involve the question of phloem function. Starck *et al.* (11) observed a decreased rate of export of ^{14}C photosynthates from blades of old leaves on salt-stressed bean plants. Since most of the ^{14}C that was exported by these leaves remained in the stem and petioles, the authors suggested that translocation may have been limited in this case by a weak, slow growing sink.

The faster response of control plants to the removal of nutrient Pi is presumably due to their faster growth and phosphate utilization. Nassery (7) observed that the growth of plants on Pi-deficient media was directly related to their phosphorus reserve and inversely related to their rate of phosphate utilization for growth.

LITERATURE CITED

- BARTLETT GR 1959 Phosphorus assay in column chromatography. *J Biol Chem* 234: 466-468
- BIELESKI RL 1973 Phosphate pools, phosphate transport and phosphate availability. *Annu Rev Plant Physiol* 24: 225-252
- CRAFTS AS, CE CRISP 1971 Phloem Transport in Plants. WH Freeman & Co, San Francisco
- FISKE CH, Y SUBBAROW 1925 The colorimetric determination of phosphorus. *J Biol Chem* 66: 375-400
- HOAGLAND DR, DI ARNON 1950 The water-culture method for growing plants without soil. *Calif Agric Exp Sta Circ* No 347
- MAAS EV, RH NIEMAN 1978 Physiology of plant tolerance to salinity. In GA Jung, ed, *Crop Tolerance to Suboptimal Land Conditions*. Am. Soc Agron Spec Publ. In press
- NASSERY H 1970 Phosphate absorption by plants from habitats of different phosphate status. II. Absorption and incorporation of phosphate by intact plants. *New Phytol* 69: 197-203
- NIEMAN RH, RA CLARK 1976 Interactive effects of salinity and phosphorus nutrition on the concentration of phosphate and phosphate esters in mature photosynthesizing corn leaves. *Plant Physiol* 57: 157-161
- OBENDORF RL, A MARCUS 1974 Rapid increase in adenosine 5'-triphosphate during early wheat embryo germination. *Plant Physiol* 53: 779-781
- PRISCO JT, GHF VIEIRA 1976 Effect of NaCl salinity on nitrogenous compounds and proteases during germination of *Vigna sinensis* seeds. *Physiol Plant* 36: 317-329
- STARCK Z, R KARWOWSKA, E KRASZEWSKA 1975 The effect of several stress conditions and growth regulators on photosynthesis and translocation of assimilates in the bean plant. *Acta Soc Bot Pol* 44: 567-588
- WARDLAW IF 1974 Phloem transport: physical chemical or impossible. *Annu Rev Plant Physiol* 25: 515-539
- WESTE JE, KS ROWAN, DJ CHALMERS 1974 The distribution of phosphorus-containing compounds in tomato plants during development of phosphorus deficiency. In RL Bielecki, AR Ferguson, MM Cresswell, eds, *Mechanism of Regulation of Plant Growth*. Bull 12, Royal Soc New Zealand, Wellington, pp 151-157