Plant Physiol. (1984) 75, 947–950
0032-0889/84/75/0947/04/$01.00/0

Salt Stimulation of Phosphate Uptake in Maize Root Tips Studied by ^{31}P Nuclear Magnetic Resonance[1]

Received for publication December 7, 1983 and in revised form April 9, 1984

JUSTIN K. M. ROBERTS*, CAREY S. LINKER, ANTHONY G. BENOIT, OLEG JARDETZKY, AND RICHARD H. NIEMAN
Stanford Magnetic Resonance Laboratory, Stanford University, Stanford, California 94305 (J. K. M. R., C. S. L., A. G. B., O. J.,); and United States Salinity Laboratory, United States Department of Agriculture, 4500 Glenwood Drive, Riverside, California 92502 (R. H. N.)

ABSTRACT

The effects of external salt and inorganic phosphate (Pi) on the concentrations of vacuolar Pi, and cytoplasmic Pi, ATP, glucose-6-phosphate and UDP-glucose in maize root tips were examined using ^{31}P nuclear magnetic resonance spectroscopy. We observed a more than two-fold stimulation of Pi uptake from 10 millimolar KH_2PO_4 solutions when root tips were exposed to 100 millimolar NaCl + $CaCl_2$. This stimulation of Pi uptake was associated with an increase in the concentration of cytoplasmic Pi in root tip cells. Thus, the molar ratio of cytoplasmic Pi to Pi + ATP + glucose-6-phosphate + UDP-glucose increased greatly in root tips exposed to salt and Pi. We speculate that it is this disturbance in relative concentrations of cytoplasmic phosphates (which we show are normally tightly regulated) that is responsible for both the greater rate of uptake of Pi by vacuoles of excised maize root tips, and the previously documented stimulation of Pi translocation from root to shoot in whole maize plants exposed to salt and Pi.

A significant interactive effect of salinity and nutrient Pi on yield in maize has been described by Bernstein *et al.* (1). High concentrations of Pi (2 mM) caused lower yields in salt-treated plants than low concentrations of Pi (0.05 mM). This lower yield appears to result from excessive uptake of Pi, with translocation to the leaves, leading to symptoms of phosphorus toxicity (1, 9). Thus, some aspect of uptake and/or translocation is disturbed by the combination of salt and high Pi. An inability to regulate Pi transport has also been observed in phosphorus-deficient barley plants when they were supplied with Pi (4, 6).

The study of Pi transport and metabolism in plants is complicated by the existence of metabolically distinct pools of Pi, in the vacuolar and cytoplasmic intracellular compartments (2), and by the rapid exchange of P between Pi and organic phosphates in the cytoplasm (8). ^{31}P-NMR[2] spectroscopy permits observation of both these aspects of Pi metabolism. The method permits simultaneous monitoring of the cytoplasmic and vacuolar Pi pools in plant tissues (11, 15) and also can be used to estimate rates of exchanges between cytoplasmic Pi and ATP in maize root tips (14). Recently, ^{31}P-NMR has been used to measure cytoplasmic and vacuolar Pi pool sizes in cell suspension cultures (10) and pea root tips (7) exposed to Pi. In both studies uptake of Pi resulted in large increases in the concentration of vacuolar Pi, while the concentration of cytoplasmic Pi was essentially unchanged; this result was observed over external concentrations of Pi ranging from 0.2 to 45 mM. In this paper we describe the time course for changes in cytoplasmic and vacuolar Pi pool sizes in maize root tips exposed to solutions of salt and Pi. We show that salt decreases the ability of maize root tips to regulate cytoplasmic Pi levels when the root tips are exposed to Pi.

MATERIALS AND METHODS

Maize (*Zea mays* L.) hybrid WW × Br38 (Customaize Research, Decatur, IL) were grown for 2 d in the dark at 25°C. Root tips, 1 mm long, were excised, rinsed with, and stored in 50 mM glucose plus 0.1 mM $CaSO_4$. Root tip samples, weighing approximately 3 g, were perfused as described previously (13) at 40 to 50 ml/min, with media described in "Results"; all perfusion media were saturated with O_2. All solutions contained 50 mM glucose plus 0.1 mM $CaSO_4$, and were adjusted to pH 4.4 (so that no insoluble forms of calcium phosphates existed). All 'salt' solutions consisted of a constant 10:1 molar ratio of NaCl:$CaCl_2$. ^{31}P-NMR spectra were obtained using a modified Bruker HXS-360 spectrometer, operating at 145.7 MHz in the Fourier transform mode. Sequential spectra were taken over 30-min periods. The interval between pulses was 10 s, allowing essentially complete relaxation of intracellular ^{31}P spins (all resonances have longitudinal relaxation times less the 2 s, except the Glc-6-P resonance, for which the value is less than 3 s). Hence, the relative areas of peaks in these ^{31}P-NMR spectra of the root tips correspond to the relative tissue concentrations of the various ^{31}P compounds observable by NMR (12). Relative areas of spectral peaks were determined by cutting and weighing the peaks. Changes in the relative concentrations of Glc-6-P, cytoplasmic Pi, vacuolar Pi, ATP, and UDP-Glc were determined by comparing peak areas to the area of a reference peak from methylene diphosphonate (pH 8.9 in Tris), included in the sample volume in a glass capillary. Absolute tissue concentrations of phsophates visible by NMR were determined by comparing the areas of peaks in root tip spectra with peak areas of standard phosphate solutions; the comparison was made using the peak from methylene diphosphonate as a standard in both samples. Our ^{31}P-NMR spectra show resonances between the Glc-6-P and the cytoplasmic Pi peaks (Fig. 4). These have not been assigned (14), although they most likely are due to several monophosphate esters. No significant changes in the intensities of these peaks were observed in response to the various treat-

[1] Supported by grants from the National Institutes of Health (RR00711), the National Science Foundation (PCM 82-04877 and GP 23633), and a United States Department of Agriculture cooperative agreement (AGRIC 58 9AZ-2-665).

[2] Abbreviation: NMR, nuclear magnetic resonance.

ments described here; however, an inability to adequately resolve and so accurately quantitate these signals renders this result somewhat tentative.

Perfusion of root tips with salt resulted in no detectable decrease in the spectral signal-to-noise ratio observed, in the probe used for these experiments. Such a decrease in spectrometer sensitivity, often encountered (5), was observed in preliminary experiments performed using a modified Varian XL-100 spectrometer. The addition of 10 mM Pi to the perfusion medium resulted in a strong NMR spectral peak that coincided with the vacuolar Pi resonance, expected because both solutions are at a pH well below the pK_a of $H_2PO_4^-$. The contribution of external Pi to the vacuolar Pi NMR signal was estimated, on completion of the experiment, by perfusing the root tips with phosphate-free medium for 5 min, and then acquiring a spectrum for 5 min immediately afterward. The difference in intensity of the 'vacuolar' Pi resonance before and after perfusion with phosphate-free medium was assumed to be due to removal of external Pi. This difference in area was then subtracted from all the vacuolar peak areas of spectra obtained after addition of external Pi. Washing of phosphate-treated root tips with phsophate-free medium for periods longer than 5 min resulted in a much slower rate of decline of the vacuolar Pi resonance (data not shown), indicative of leakage of intracellular Pi.

The growth of maize root tips was determined by following the increase in fresh weight of root tip samples over 24-h periods. The root tips were either perfused, as described above, or were placed in Petri dishes, on filter paper moistened with the appropriate solution. Both methods gave similar results.

RESULTS AND DISCUSSION

The inhibitory effect of salt on the growth of 1-mm long excised maize root tips over 24 h is shown in Figure 1. The salt concentration at which root tip growth tip is inhibited 50% (Fig. 1) is similar in magnitude to the salt concentration giving 50% reduction in yield of field-grown maize (3). Because of the results in Figure 1, in subsequent experiments salt treatments all involved exposure of root tips to 100 mM NaCl plus 10 mM $CaCl_2$.

The effect of 10 mM KH_2PO_4 on maize root tip growth in the presence or absence of salt is shown in Figure 2. Very little effect of external Pi is apparent. These results differ from similar experiments examining long-term growth in salt- and Pi-treated maize plants (9). First, in those long-term experiments, a Pi-induced stimulation of shoot growth in plants not treated with salt was observed (9), a result attributed to Pi deficiency in plants not treated with high concentrations of Pi (9). Such a mechanism

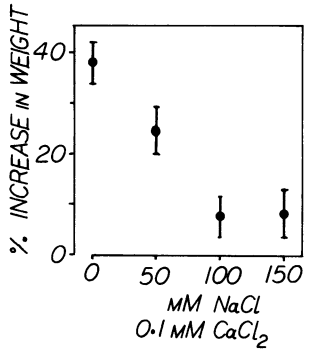

FIG. 1. Inhibition of maize root tip growth by salt. Root tip samples were perfused with oxygenated 50 mM Glc/0.1 mM $CaSO_4$ for 2 h, after which the indicated amount of NaCl and $CaCl_2$ was added. After 24 h the increase in fresh weight was determined. The error bars indicate SE for 10 replicates at each salt concentration (from several separate experiments).

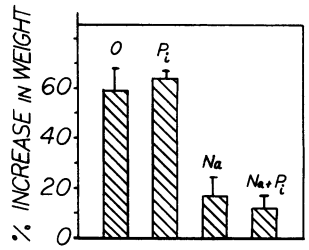

FIG. 2. Effect of 10 mM KH_2PO_4 on maize root tip growth. Root tip samples were placed in Petri dishes on filter paper wetted with 50 mM Glc/0.1 mM $CaSO_4$ plus: 0 (nothing); Pi (10 mM KH_2PO_4); Na (100 mM NaCl + 10 mM $CaCl_2$); Na + Pi (100 mM NaCl + 10 mM $CaCl_2$ + 10 mM KH_2PO_4). After 24 h the increase in fresh weight was determined. The error bars indicate SD for 4 replicates of each treatment (two separate experiments).

would explain why we see no such growth stimulation in excised maize root tips (Fig. 2), for the large amounts of vacuolar Pi observed by ^{31}P-NMR in root tips not fed Pi (11, 14), indicates that there is no phosphorus deficiency, at least over periods as short as 24 h after excision. Second, the long-term experiments indicated a large Pi-induced inhibition of shoot growth in salt-treated maize plants (1, 9), a result attributed to the accumulation of toxic levels of phosphorus in the leaves (1, 9). The absence of such a large effect of Pi on short-term growth of salt-treated root tips (Fig. 2) may, therefore, result from a failure of the tissue to accumulate phosphate to toxic levels in this short time period.

The effect of external salt and Pi on the concentrations of phosphates in maize root tips observable by ^{31}P-NMR is shown in Figure 3. Figure 3 is derived from ^{31}P-NMR spectra such as those shown in Figure 4, changes in relative concentrations being estimated from the changes in the areas of particular spectral peaks. The intensities of the Glc-6-P, cytoplasmic Pi, and vacuolar Pi peaks were sufficiently strong in spectra accumulated over periods of 30 min (180 scans) for quantitation, whereas it was necessary to combine four successive 30-min spectra to improve the signal-to-noise ratio for the weaker ATP and UDP-Glc peaks. Thus, concentrations of ATP and UDP-Glc were determined at 2-h intervals.

Perhaps the most striking result shown in Figure 3 is the more than 2-fold stimulation in the rate of increase in vacuolar Pi induced by salt, in root tips exposed to 10 mM KPi. This greater rate of Pi uptake by the vacuoles is maintained throughout the 12-h treatment period. In contrast, both increases and decreases in the concentrations of cytoplasmic phosphates are apparent during this treatment period (Fig. 3). Most notable are the large changes in cytoplasmic Pi levels in root tips exposed to phosphate. In both Pi treatments, the concentration of cytoplasmic Pi dramatically increases following addition of the Pi (or salt plus Pi), after which cytoplasmic Pi levels return slowly to near normal levels (Fig. 3). Changes in the concentrations of Glc-6-P, UDP-Glc, and ATP with time do not qualitatively differ from treatment to treatment; the only differences apparent in Figure 3 are the consistently lower concentrations of these phosphates in the salt-treated tissue, particularly in the presence of Pi. No simple explanation of this phenomenon is apparent to us.

The large changes in the concentration of cytoplasmic Pi seen when root tips are exposed to 10 mM KPi (Figs. 3 and 4) contrasts with results of previous ^{31}P-NMR studies using cell suspension cultures (10) and pea root tips (7). In these studies, no significant changes in the concentration of cytoplasmic Pi were observed, except when cell suspension cultures that had been depleted of intracellular Pi were fed Pi (10). Nevertheless, both our results here, and these earlier NMR studies (7, 10), show that the rate of uptake of Pi from external solutions is not dependent in some simple fashion on the concentration of cytplasmic Pi. However,

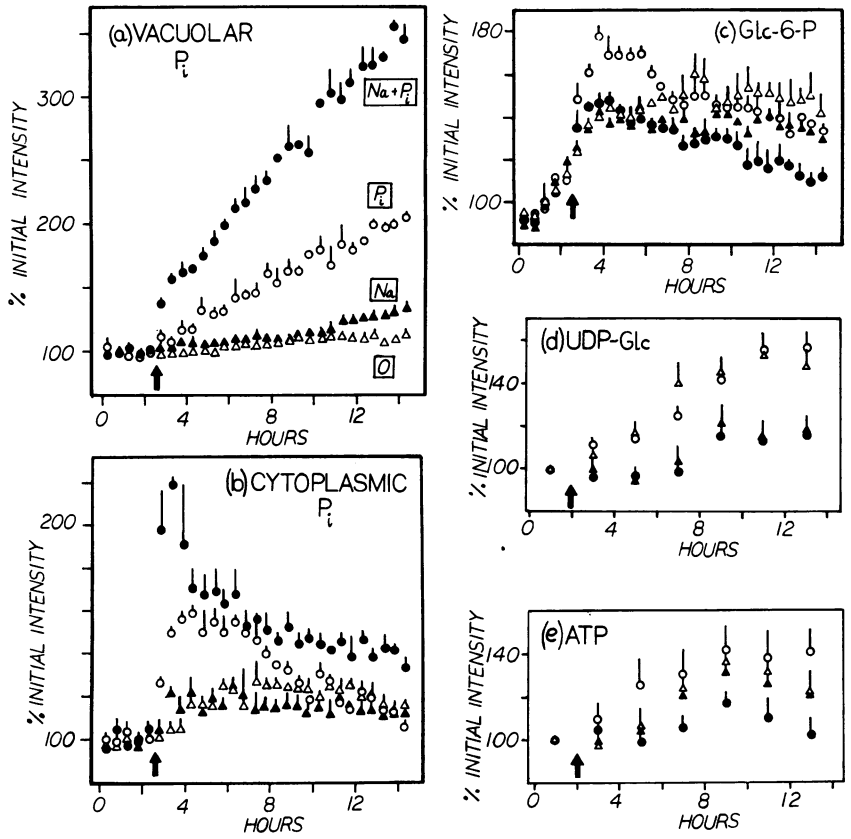

FIG. 3. Effect of external salt and Pi on the concentrations of phosphates in maize root tips. The graphs show changes in intensities (areas) of peaks in ^{31}P-NMR root tip spectra obtained every 30 (A–C) or 120 (D, E) min. Root tips were initially perfused with oxygenated 50 mM Glc + 0.1 mM $CaSO_4$; after 2.5 h, at the time indicated by the solid arrow, either nothing (△), or 10 mM KH_2PO_4 (○), or 100 mM NaCl + 10 mM $CaCl_2$ (▲), or 100 mM NaCl + 10 mM $CaCl_2$ + 10 mM KH_2PO_4 (●) were added. Each point is the mean from two experiments for each treatment; the vertical lines coming from data points are the ranges (only upper values are shown). Initial tissue concentrations of the phosphates were: vacuolar Pi, 9 ± 1.5 mM; cytoplasmic Pi, 1.05 ± 0.1mM; Glc-6-P, 2.2 mM ± 0.1 mM; UDP-Glc, 0.51 ± 0.05 mM; ATP, 0.54 ± 0.05 mM.

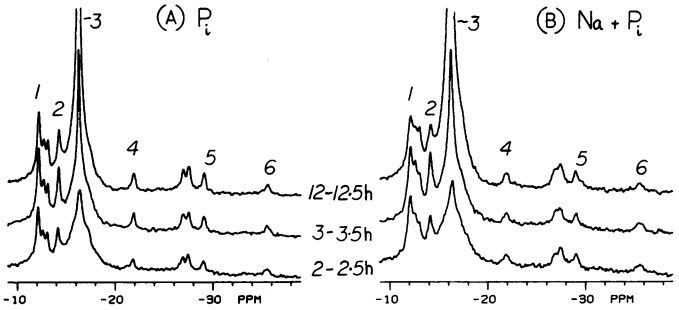

FIG. 4. ^{31}P-NMR spectra of maize root tips. Samples were perfused with Glc plus (A) 10 mM KH_2PO_4 or (B) 10 mM KH_2PO_4 + 100 mM NaCl + 10 mM $CaCl_2$ (the bottom spectra were obtained just before salt and/or Pi were added). The spectra shown are transforms of 180 free induction decays obtained over 30 min. The peak assignments are: 1, Glc,6-P; 2, cytoplasmic Pi; 3, vacuolar Pi; 4, ATP; 5, UDP-Glc; 6, ATP.

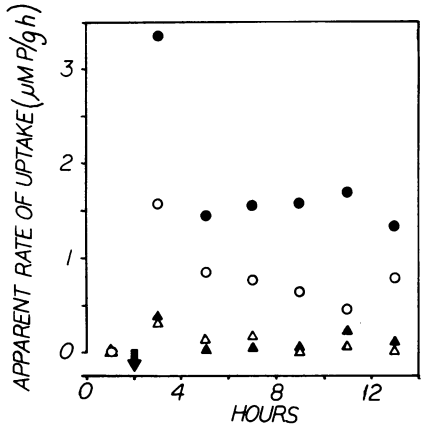

FIG. 5. Effect of external salt on the rate of Pi uptake by maize root tips. The data shown are derived from Figure 3. Apparent uptake was determined by calculating the change in tissue vacuolar and cytoplasmic phosphate (including P-ester) concentrations every 2 h. Symbols as in Figure 3.

if one compares the rate of Pi uptake (Fig. 5), with the molar ratio of cytoplasmic Pi-to-total cytoplasmic phosphate (Pi + Glc-6-P + UDP-Glc + ATP) (Fig. 6), it is apparent that higher rates of uptake are associated with higher ratios of cytoplasmic Pi to total cytoplasmic phosphate. The higher ratios are particularly apparent in root tips treated with both salt and Pi (Fig. 6), the treatment resulting in the greatest rate of phosphate uptake (Fig. 5).

CONCLUSIONS

We have demonstrated that, with respect to salt stress and the effects of salt on Pi metabolism, excised maize root tips possess certain characteristics observed in whole maize plants. First, the concentration dependence of growth inhibition of root tips (Fig. 1) is similar to the dependence of yield from maize plants (3). Second, salt results in a much greater rate of uptake of Pi by root tips from solutions of 10 mM KH_2PO_4 (Figs. 3 and 5), a phenomenon observed in whole plants of maize (9). It is this greater rate of uptake of Pi that appears to be primarily responsible for the deleterious effects of the combined salt-Pi treatment (9).

The enhancement of Pi uptake by salt appears to result in an increase in the concentration of Pi in the cytoplasm of root tips (Figs. 3 and 6). While this increase in cytoplasmic Pi might be due to stimulation of an active transport process, it is reasonable to speculate that salt simply increases the permeability of the plasmalemma to Pi, resulting in a larger influx of Pi from the external solution. This view is consistent with the fact that the salt-induced stimulation of Pi uptake is not seen at low external Pi concentrations (0.1 mM *versus* 2 mM KPi which, in combi-

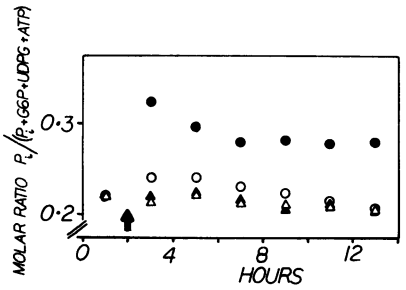

FIG. 6. Effect of external salt and Pi on the molar ratio of cytoplasmic Pi to total cytoplasmic phosphate (Pi + Glc-6-P + ATP + UDP-Glc) in maize root tips. The data shown are derived from Figure 3, tissue concentrations being determined by multiplying the intensity of the appropriate peak at a particular time (or average value over 2 h) by the initial concentration given in the legend to Figure 3. Symbols as in Figure 3.

nation with salt, results in toxic accumulation of P [9]). We have determined that the tissue concentrations of cytoplasmic Pi is just over 1 mM (Fig. 3 legend, and [14]). Assuming a cytoplasmic volume of 70% (see [7] for discussion), passive influx of Pi would be possible at external concentrations of 2 mM.

Following the influx of Pi into the cytoplasm of the root tip cells, transfer of phosphate from cytoplasm to vacuole occurs (Fig. 3). The rate of uptake of Pi by the root tip vacuoles appears to be completely unrelated to the concentration of Pi in the cytoplasm (Fig. 3). However, the rate of Pi uptake by vacuoles is correlated with the ratio of Pi to total phosphate in the cytoplasm (Figs. 3 and 6). Thus, the much higher ratio of Pi to total phosphate in the cytoplasm of root tips treated with salt and Pi may account for the salt-induced stimulation of Pi uptake by the root tip vacuoles. This result may also account for the stimulation of Pi translocation from root to shoot observed when whole maize plants are exposed to salt and Pi (1, 9). Both these phenomena can be interpreted as an attempt by the root cells to decrease the molar ratio of Pi to other phosphates in the cytoplasm. It is possible that a similar mechanism accounts for the deleteriously high rates of Pi uptake and translocation observed when phosphorus-deficient plants are provided with Pi (4, 6). Certainly, our results show that the movement of P between cytoplasm and vacuole is integrated to the metabolism of phosphate in the cytoplasm.

Acknowledgment—We thank Dr. A. N. Lane for helpful discussions.

LITERATURE CITED

1. BERSTEIN L, LE FRANCOIS, RA CLARK 1974 Interactive effects of salinity and fertility on yields of grains and vegetables. Agron J 66: 412–421
2. BIELESKI RL 1973 Phosphate pools, phosphate transport, and phosphate availability. Annu Rev Plant Physiol 24: 225–252
3. CARTER DL 1975 Problems of salinity in agriculture. *In* A Poljakoff-Mayber, J Gale, eds, Plants in Saline Environments. Springer-Verlag, New York, pp 25–35
4. CLARKSON DT, J SANDERSON, CB SCATTERGOOD 1978 Influence of phosphate-stress on phosphate absorption and translocation by various parts of the root system of *Hordeum vulgare* L. (Barley). Planta 139: 47–53
5. GADIAN DG 1982 Nuclear Magnetic Resonance and its Application to Living Systems. Oxford University Press, Oxford.
6. GREEN DG, WS FERGUSON, FG WARDER 1973 Accumulation of toxic levels of phosphorus in the leaves of phosphorus-deficient barley. Can J Plant Sci 53: 241–246
7. LEE RB, RG RATCLIFFE 1983 Phosphorus nutrition and the intracellular distribution of inorganic phosphate in pea root tips: a quantitative study using ^{31}P-NMR. J Exp Bot 34: 1222–1244
8. LOUGHMAN BC 1976 Metabolic processes in roots related to absorption and transport of phosphate. *In* N Sunderland, ed, Perspectives in Experimental Biology, Vol 2. Pergamon Press, Oxford, pp 423–431
9. NIEMAN RH, RA CLARK 1976 Interactive effects of salinity and phosphorus nutrition on the concentrations of phosphate and phosphate esters in mature photosynthesizing corn leaves. Plant Physiol 57: 157–61.
10. REBEILLE F, R BLIGNY, J-P MARTIN, R DOUCE 1983 Relationship between the cytoplasm and the vacuole phosphate pool in *Acer pseudoplatanus* cells. Arch Biochem Biophys 225: 143–148
11. ROBERTS JKM, PM RAY, NG WADE-JARDETSKY, O JARDETSKY 1980 Estimation of cytoplasmic and vacuolar pH in higher plant cells by ^{31}P-NMR. Nature 283: 870–872
12. ROBERTS JKM, O JARDETZKY 1981 Monitoring of cellular metabolism by NMR. Biochim Biophys Acta 639: 53–76
13. ROBERTS JKM, D WEMMER, PM RAY, O JARDETZKY 1982 Regulation of cytoplasmic and vacuolar pH in maize root tips under different experimental conditions. Plant Physiol 69: 1344–1347
14. ROBERTS JKM, D WEMMER, O JARDETZKY 1984 Measurement of mitochondrial ATPase activity in maize root tips by saturation transfer ^{31}P nuclear magnetic resonance. Plant Physiol 74: 632–639
15. ROBERTS JKM 1984 Study of plant metabolism in vivo using NMR spectroscopy. Annu Rev Plant Physiol 35: 375–386