Plant Physiol. (1992) 98, 137–142
0032-0889/92/98/0137/06/$01.00/0

Received for publication May 17, 1991
Accepted August 17, 1991

Enhanced Sensitivity to Ethylene in Nitrogen- or Phosphate-Starved Roots of *Zea mays* L. during Aerenchyma Formation[1]

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ABSTRACT

Adventitious roots of maize (*Zea mays* L. cv TX 5855), grown in a well-oxygenated nutrient solution, were induced to form cortical gas spaces (aerenchyma) by temporarily omitting nitrate and ammonium (−N), or phosphate (−P), from the solution. Previously this response was shown (MC Drew, CJ He, PW Morgan [1989] Plant Physiology 91: 266–271) to be associated with a slower rate of ethylene biosynthesis, contrasting with the induction of aerenchyma by hypoxia during which ethylene production is strongly stimulated. In the present paper, we show that aerenchyma formation induced by nutrient starvation was blocked, under noninjurious conditions, by addition of low concentrations of Ag^+, an inhibitor of ethylene action, or of aminoethoxyvinyl glycine, an inhibitor of ethylene biosynthesis. When extending roots were exposed to low concentrations of ethylene in air sparged through the nutrient solution, N or P starvation enhanced the sensitivity to exogenous ethylene at concentrations as low as 0.05 microliters ethylene per liter air, promoting a more rapid and extensive formation of aerenchyma than in unstarved roots. We conclude that temporary deprivation of N or P enhances the sensitivity of ethylene-responsive cells of the root cortex, leading to cell lysis and aerenchyma.

The roots of many plant species, especially those well adapted to wetland conditions, respond to inadequate oxygenation of their environment by formation of aerenchyma (16, 17). In maize, lysigenous aerenchyma can form in young seminal (18) as well as nodal (adventitious) roots (11, 12) when they extend under conditions of hypoxia into nutrient solution that is partially oxygen deficient. Aerenchyma, comprising an interconnected series of gas-filled cavities or lacunae (11), improves the internal aeration of the roots (13). At an early stage in aerenchyma formation, cells of the root cortex, located about 10 mm behind the apex, undergo premature lysis and disintegration to form lacunae (7). Hypoxia stimulates ethylene biosynthesis, and the increase in internal ethylene concentration is closely associated with induction of cell lysis and aerenchyma formation (1, 11). However, aerenchyma can also be induced in maize roots under fully aerobic conditions by temporary deprivation of certain nutrient ions (NH_4^+ and NO_3^- or $H_2PO_4^-$) but not K^+ (10, 19). In an earlier paper, we examined cell lysis and ethylene biosynthesis in the root tip of maize during temporary deficiency of N or P and compared the responses to those induced by hypoxia (10). We found that temporary shortage of these nutrients consistently inhibited the rate of ethylene production of the excised tips to only one-half to one-third that of unstarved controls. The activity of ACC[3] synthase and the ACC concentration were likewise depressed by N or P starvation. Induction of cell lysis by nutrient starvation, unlike hypoxia, was thus not related to a greater endogenous concentration of ethylene.

In the present study, we used inhibitors of ethylene biosynthesis and ethylene action to determine whether ethylene is associated with the onset of cell lysis in nutrient-deprived roots. We also examined the response of such roots to exogenous ethylene. We show that nutrient deficiency induces cell lysis by markedly increasing the sensitivity of root cortical cells to ethylene while slowing its rate of production.

MATERIALS AND METHODS

Plant Growth Conditions

Caryopses of maize (*Zea mays* L. cv TX 5855) were germinated and grown in a controlled environment room, essentially as described earlier (10). Briefly, the environmental conditions were day/night temperatures of 25/20°C, RH 75/65%, and a 14-h light period of 650 μmol photons m^{-2} s^{-1} PAR. Plants were grown in complete nutrient solution and kept fully oxygenated by bubbling vigorously with air. Experimental treatments began at 10 to 11 d from the start of imbibition, when the initial whorl of adventitious roots (always four per plant) had emerged about 50 mm from the stem base into the nutrient solution.

Experimental Treatments

For N starvation, plants were transferred from the complete nutrient solution to a solution of similar composition but lacking both NH_4^+ and NO_3^-, as described before (10). Control plants were always maintained with the complete nutrient

[1] Research supported in part by U.S. Department of Agriculture Competetive grants No. 88-37264-399 and 90-37264-5523. Texas Agriculture Experiment Station paper No. 20962.

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[3] Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; AVG, aminoethoxyvinyl glycine.

solution. In some experiments, inhibitors of ethylene metabolism were included in the complete and −N solutions at the start of the treatment. Ag^+, used to inhibit ethylene action, was in the range 0.05 to 0.6 μM, by addition of $AgNO_3$ (addition of NO_3^- was negligible). To inhibit ethylene synthesis, AVG was used at concentrations of 1, 5, and 10 μM. For both Ag^+ and AVG, tests were conducted to determine any associated inhibitory effects on root growth (see "Results"). Root extension was measured by marking the epidermis with particles of carbon (charcoal slurry) exactly 10 mm behind the tip. Further extension was measured from this reference mark (11).

In treatments involving exogenous ethylene, the attached roots of each plant were transferred to a polyethylene container (0.5-L capacity) containing either complete or −N or −P nutrient solution. Each plant was held around the base of the stem by a rubber stopper and made airtight by sealing with silicone rubber (Dow Corning). Ethylene was prepared to the required concentration by mixing from gas cylinders ethylene-free air with 100 μL ethylene L^{-1} air, using electronic flow controllers. The resulting concentration of ethylene was checked by GC. The nutrient solution and roots were sparged with ethylene in air, passed in at the bottom of the polyethylene container by a capillary tube. The gas mixture escaped through a wide-bore hypodermic needle connected to 10-mm-bore plastic tubing leading outside the building. Air in the growth room was tested for ethylene contamination by GC. Except where there was an obvious leak, ethylene was below the limits of detection (0.01 μL L^{-1}).

Root Structure

Transverse, hand-cut sections were made close to the tip of the first or second whorl of nodal (adventitious) roots, 10 replicate roots per treatment, in zones between 3 and 4 d old, age being estimated from the measured extension rate beyond the carbon mark made on the root surface as described before. Quantification of areas of lacunae (gas-filled space), collapsed lysing cells, and intact cells was conducted from camera lucida drawings. For scanning EM, freshly cut sections were fixed in formalin, ethyl alcohol, acetic acid, 5:95:5 by volume), dehydrated in a graded acetone series, and critical point dried before sputter-coating under vacuum.

N Analysis

Plant material sampled after various periods of N starvation was dried at 70°C, ground, and analyzed for total N using a Perkin-Elmer model 2400 CHN analyzer.

RESULTS

Inhibition of Aerenchyma Formation with Ag^+ and with AVG

Omission of N or P from the nutrient solution induced formation of aerenchyma in adventitious roots (Fig. 1), despite vigorous oxygenation of the medium. When low concentrations of Ag^+ were included in the solution, aerenchyma formation was effectively blocked (Fig. 1D). There was a partial inhibition of gas space formation in the presence of 0.05 μM Ag^+, with further inhibitions at 0.1 μM and complete inhibition at 0.6 μM (Fig. 2). The small amount of aerenchyma in controls receiving the complete nutrient solution was also suppressed by additions of Ag^+. At the concentrations of Ag^+ we used, there were no obvious symptoms of toxicity, and root growth continued but with some retardation at 0.1 and 0.6 μM Ag^+ (Table I). Aerenchyma also failed to develop when AVG was present in the nutrient solution during the nutrient starvation period (Fig. 3). Both the collapse of cells and the formation of prominent lacunae were inhibited by AVG, and the small amount of cortical cell breakdown usually observed in control roots was also suppressed. The greatest extent of cell breakdown found in our experiments consistently was with the −N treatment, in which concentrations of up to 10 μM AVG were necessary to suppress aerenchyma formation. The presence of AVG caused only a small reduction in root extension rates (Table II) so that its effects, like that of Ag^+, were not associated with toxicity.

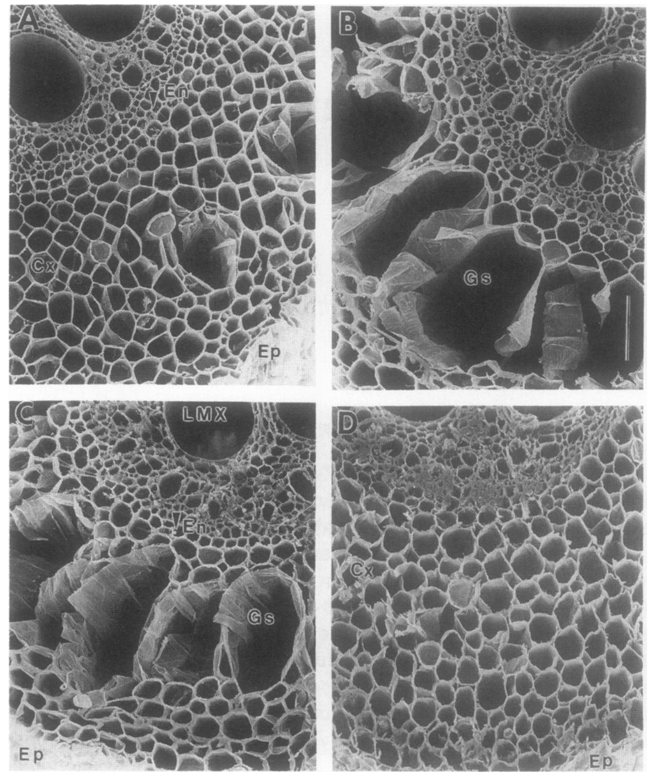

Figure 1. Scanning electron micrographs of transverse sections of adventitious roots of *Z. mays* showing induction or inhibition of aerenchyma. A, Complete nutrient solution (control); B, −N solution; C, −P solution; D, −N solution with 0.6 μM Ag^+. Bar, 100 μm. Cx, cortex; En, endodermis; Ep, epidermis; Gs, gas space; LMX, late maturing metaxylem.

Increased Sensitivity of N- or P-Starved Roots to Exogenous Ethylene

When roots were exposed to nutrient solution sparged with low concentrations of ethylene in air, the influence on aerenchyma formation was dependent on the concentration of

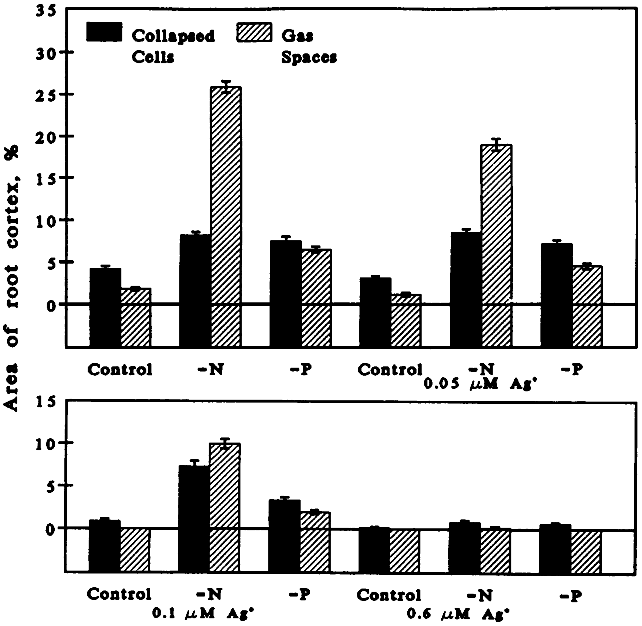

Figure 2. Inhibition of cortical cell lysis by Ag^+ in roots of *Z. mays*. Roots received a complete nutrient solution (control), −N solution, and −P solution. Collapsed cells refer to the total area (in transverse section) showing distinct lysis of cell walls, without formation of lacunae; gas spaces refer to the total area occupied by lacunae. Treatments (nutrient starvation and Ag^+) began 4 d before roots were sampled for sectioning, and transverse sections for estimation of aerenchyma in the root cortex were made in the zone that was approximately 4 d old.

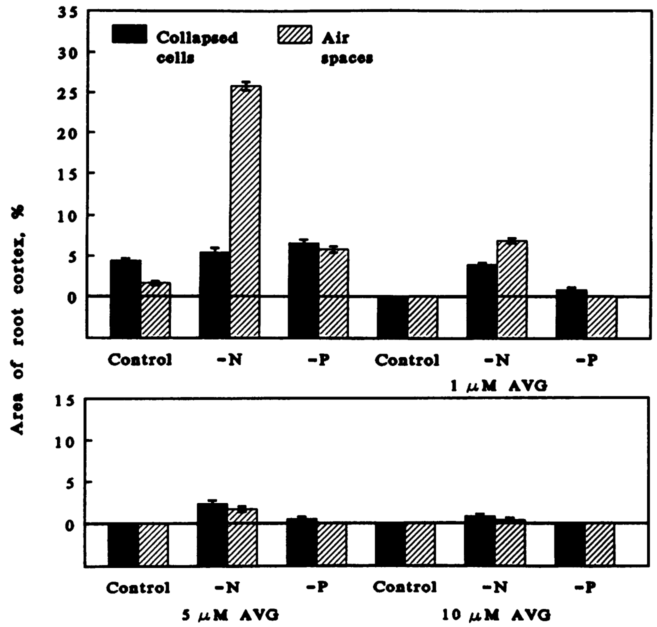

Figure 3. Inhibition of cortical cell lysis by AVG in roots of *Z. mays*. Conditions were as described in Figure 2.

ethylene as well as on nutrient deficiency (Fig. 4). There was a small production of aerenchyma with −N or −P treatments at 3 d from the start of nutrient starvation. The lowest addition of ethylene (0.05 $\mu L\ L^{-1}$) induced a greater formation of gas spaces at 3 d from the start of treatment with −N solution, whereas the roots in the complete solution (control) or in −P solution were relatively insensitive to this low concentration of ethylene. At 1.0 $\mu L\ L^{-1}$ ethylene concentration, roots in complete solution began to show cortical breakdown, whereas aerenchyma formation was more strongly promoted in −N and −P roots. However, at 5 $\mu L\ L^{-1}$ ethylene, it was not possible to distinguish among controls, −N, and −P roots, because the promotion of cortical breakdown by this higher concentration was uniformly maximal, with gas spaces occupying about 40% of the cross-sectional area of the root cortex, this being the greatest extent to which aerenchyma has been observed to form in maize roots.

Similar differences in sensitivity to exogenous ethylene were found in other experiments comparing aerenchyma formation in complete, −N, and −P roots at 4, 5, and 6 d of nutrient starvation treatment for nodal roots originating from either the coleoptile or first leaf nodes (data not shown).

Tissue N Concentrations during Starvation

In the roots of plants maintained in the complete nutrient solution, the N concentration was greatest in the apical 5 mm (Table III), with a small decline with distance from the tip, a gradient that remained stable through the 12-d experiment. With N starvation, the concentration of N declined most rapidly in the older root zones (>60 mm), and this effect

Table I. *Effect of Ag^+ Concentration and Nutrient Solution Composition on Root Extension*

All values are the mean of 10 replicate roots, measured during a 4-d period. LSD ($P < 0.05$) = 3.5 (between different levels of Ag^+) and 3.0 (between different nutrient solutions).

Concentration of Ag^+	Nutrient Solution Composition		
	Complete	−N	−P
μM		*mm*	
0	98	107	95
0.05	99	105	96
0.10	86	87	86
0.60	56	57	54

Table II. *Effect of AVG Concentration and Nutrient Solution Composition on Root Extension*

All values are the mean of 10 replicate roots, measured during a 4-d period. LSD values ($P < 0.05$) = 3.5 (between different AVG levels) and 3.0 (between different nutrient solutions).

Concentration of AVG	Nutrient Solution Composition		
	Complete	−N	−P
μM		*mm*	
0	100	105	94
1	95	93	92
5	77	74	77
10	60	60	63

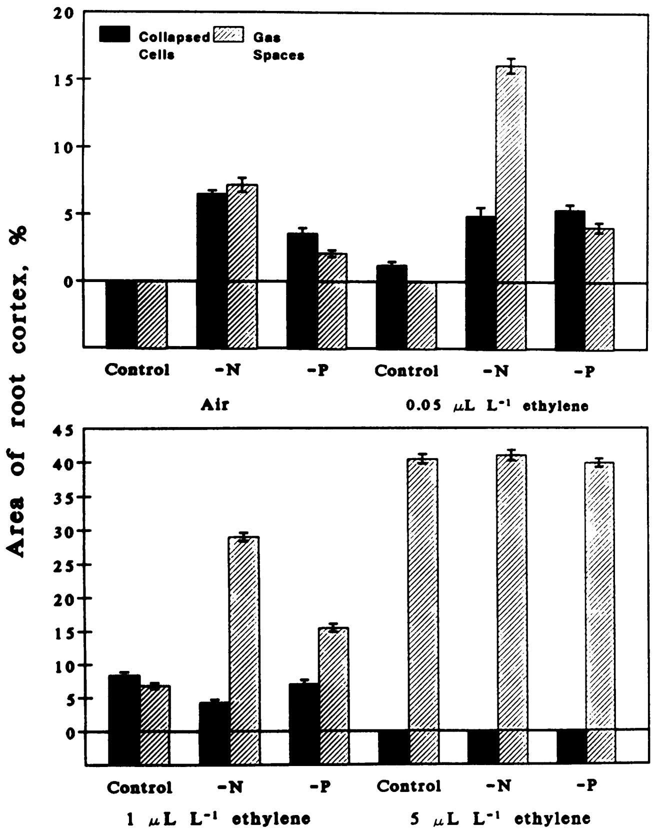

Figure 4. Changes in induction of aerenchyma in adventitious roots of *Z. mays* by exogenous ethylene in response to nutrient starvation. Transverse sections for estimation of aerenchyma in the root cortex were made in a zone behind the root tip that was approximately 3 d old.

continued to intensify and extend toward the tip region with time. Although the apical 0- to 5-mm zone showed the smallest decline with N starvation, values were clearly lower after 4 d starvation. The roots continued to extend during the starvation treatment, so that the apical 0- to 60-mm segments were composed of new cells that had been laid down since the start of the treatment.

DISCUSSION

Exogenous ethylene stimulated formation of aerenchyma in unstarved control roots (Fig. 4, 1 $\mu L\ L^{-1}$), in agreement with earlier reports (10, 11). Aerenchyma formation in −N or −P roots was associated with a slowing of ethylene production (10), unlike hypoxia which stimulated ethylene biosynthesis (1, 11, 15). However, the present results show that the mechanism of induction with nutrient deficiency must ultimately be dependent on the response of root cortical cells to endogenous ethylene. During nutrient deficiency, roots continued to elongate without formation of aerenchyma in the presence of low concentrations of Ag^+ (Figs. 1 and 2), which acts as an inhibitor of ethylene action (2, 8). An inhibitor of ethylene biosynthesis, AVG (33), also blocked aerenchyma formation in nutrient-deficient roots (Figs. 1 and 3), indicating that ethylene production, although somewhat reduced by nutrient starvation (10), is still necessary for the response. The continued rapid elongation of roots in the presence of AVG suggests that inhibition of aerenchyma formation was not associated with toxicity, which might occur if AVG at these concentrations affected other enzymes requiring pyridoxal phosphate. These responses in nutrient-deficient roots are reminiscent of those previously found in hypoxic ones, in which aerenchyma formation under nontoxic conditions could be blocked by application to intact elongating roots of Ag^+ (12) or of AVG (15), the latter effect being reversible by sparging simultaneously with ethylene.

A transient deficiency of N or P in the rooting medium clearly enhanced the sensitivity of cortical cells to exogenous ethylene (Fig. 4). An alternative hypothesis, that nutrient starvation lowers the resistance of the tissue to the diffusion

Table III. *Concentration of N in Root Tip Segments of Nodal Roots and Shoots of Young Maize Plants during N Starvation*
Plants were 10 d old at the start of N starvation. The nodal roots were from the first whorl (coleoptile node).

Tissue Analyzed	0 Complete	Duration of N Starvation: 4 d Complete	4 d −N	8 d Complete	8 d −N	12 d Complete	12 d −N
	mmol g^{-1} dry wt						
Nodal root (mm from tip)							
0–5	5.41	5.26	3.70	5.16	3.60	5.42	3.11
5–10	4.61	5.73	2.04	4.39	1.76	4.26	1.23
20–30	4.23	4.40	1.59	4.21	1.51	3.87	0.88
50–60	4.10	4.31	1.29	4.19	1.24	3.79	0.72
>60	3.79	4.08	1.19	3.93	1.10	3.58	0.50
Shoots	3.59	3.58	1.79	3.46	1.09	3.39	0.71

of exogenous ethylene, thus enhancing the internal concentration more readily, is untenable. Its corollary must be that, in the absence of an exogenous ethylene source, endogenously produced ethylene could escape more readily, thus lowering the internal concentration still further. A differential response was detected when the concentration of ethylene in air was as little as 0.05 μL L^{-1}; in fact, experiments using higher concentrations (5 μL L^{-1}) alone would have failed to detect a differential response. In this study of aerenchyma initiation, as in preceding ones, we compared the response of root tissues of similar age rather than tissues at similar distances from the root apex because of the possible effect of the root environment on elongation rates. With time, cells located in the midcortex in roots of maize and other graminaceous species maintained under optimal conditions commonly show degeneration and lysis (9, 26), but these changes become extensive in maize in root zones located approximately 200 mm or more behind the root apex and about 7 d or more after the cells were initiated. The enhanced sensitivity to ethylene therefore accelerates a process that would otherwise be destined to take place over longer times. An approximate estimate of the change of sensitivity is obtained by comparing the ethylene concentrations required to "saturate" the response, *i.e.* give maximal lysis of responsive cells. If we compare the response to exogenous ethylene in 4-d-old root zones, cell lysis (which includes both collapsed cells and gas spaces) in unstarved control roots was saturated at 5 μL L^{-1}, whereas −P roots were half-saturated at 1 μL L^{-1} and −N saturated at 0.05 μL L^{-1}. Thus, although −N treatment inhibited ethylene biosynthesis to 30 to 50% of unstarved controls, it apparently increased the sensitivity of cortical cells by a factor of 100 and that of cells in the −P treatment by a factor of 2 to 3. Results of other studies (3, 23, 25) suggest that a rate of ethylene production of 2 to 3 nl g^{-1} h^{-1} (the postwounding rate observed in −N roots, ref. 10) would result in endogenous concentrations of 0.1 to 1.0 μL L^{-1}. This would readily saturate aerenchyma formation in the highly responsive −N roots, and the somewhat greater rate of ethylene production by −P roots would likewise be adequate to signal cell lysis.

This is the first report of an environmentally signaled enhancement in sensitivity to ethylene in root tissues, to the best of our knowledge. However, instances of changes in sensitivity to ethylene are known to occur in other plant organs in response to developmental stage, particularly during senescence of fruits (6, 21, 32) and flower petals (20, 31), abscission of leaves (24, 30), and dehiscence of fruits (22). Interestingly, in some cases, younger leaves and fruits are more sensitive to ethylene-induced separation phenomena than are those in middevelopment, whereas increased sensitivity can occur again with maturity or senescence (22, 24, 30). It is tempting to suggest that such changes in sensitivity, including the present results, indicate either a change in the concentration or affinity of ethylene-binding sites or that the signal transduction sequence for ethylene action is up-regulated by N- or P-starvation. Correlation between changes in apparent binding with changes in sensitivity could not be found in studies of fruit ripening and flower senescence (4) or leaf senescence (14). However, evidence of ethylene-binding sites, as distinct from ethylene metabolism, is becoming increasingly reliable. The properties of the binding sites in seedlings of pea and rice, examined under conditions that limited ethylene metabolism, were consistent with those expected of an ethylene receptor (27, 28). The extent to which the simple alkenes ("ethylene homologues") 1-butene, *cis*-2-butene, and *trans*-2-butene and the cyclic olefin, 2,5-norbornadiene, inhibited ethylene binding demonstrated a connection between ethylene binding and physiological action (29). In the ethylene-insensitive mutant *etr* of *Arabidopsis thaliana*, ethylene binding was reduced to one-fifth, and several physiological responses to ethylene responses were modified, suggesting that a receptor mutation was involved (5). To explain the present results, one can hypothesize that N or P starvation increases receptor number or affinity in roots, so that they respond to ethylene at concentrations below those present in nonstressed roots. The mechanism by which N or P starvation might bring about such changes is unknown, but it is evident that short periods of N starvation were sufficient to produce a sharp decline in the concentration of total N (Table III) in the 5- to 10-mm zone behind the tip where the earliest stages in cell lysis are detectable in the cortex (7).

Presumably, it is this decline, as well as equivalent changes in the P status of P-starved roots, that triggers the process leading to cell lysis.

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