Physiological and Molecular Responses of Aeroponically Grown Tomato Plants to Phosphorus Deficiency

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ABSTRACT. Phosphorus is one of the essential but limiting nutrients in nature. In this study, we link the physiological changes occurring under phosphate (Pi) starvation to gene expression. Roots of aeroponically grown tomato (*Lycopersicon esculentum* L.) plants were sprayed intermittently with nutrient solutions containing varying concentrations of P. Decreasing the concentration of Pi in the nutrient solution resulted in reduced biomass production and altered the tissue concentration of nutrients in roots and shoots. Phosphorus starvation increased the root:shoot biomass ratio and decreased net CO₂ assimilation and stomatal conductance. Phosphorus concentrations in roots and shoots decreased with decreasing concentration of Pi in the nutrient solution. Pi-deficient plants had a higher concentration of Ca in roots and Mg in shoots. Expression of the Pi starvation-induced gene, *TPSII*, persisted even after 3 weeks of Pi starvation. The transcript accumulation in leaves was found to be a specific response to Pi starvation and not to the indirect effects of altered N, K, Fe, Mg, or Ca status. Accumulation of transcripts was also observed in stem and petioles, suggesting a global role for *TPSII* during Pi starvation response of tomatoes.

Aeroponics is a convenient way to study effects of nutrient deficiency on physiological, biochemical, and molecular processes in plant roots (Waisel, 1996; Zobel, 1989). This water and nutrient delivery system helps evaluate the effects of starvation for a particular nutrient, without interference from changes in concentration of other nutrients, temperature, and pH. Aeroponics has been used effectively to study plant growth, root development, root exudates, and plant responses to salt (Clayton and Lamberton, 1964; Waisel, 1996; Zobel, 1989). Although aeroponic systems have been used commercially, their application in studies of nutrient deficiency and uptake has been limited (Barak et al., 1996; Weathers and Zobel, 1992). Peterson and Krueger's (1988) intermittent spray aeroponic system, which provides plants with fresh nutrient solution, is well suited for studying effects of nutrient starvation on root growth. We used this system to study the effect of phosphate (Pi) starvation on tomato plant physiology and changes in gene expression (Liu et al., 1997).

Phosphorus is one of the least available of all essential nutrients in the soil (Barber, 1980). Plants grown under Pi deficiency conditions exhibit multiple responses (Marschner, 1995), including enhanced root growth, altered root morphology (Lynch, 1995), enhanced uptake of Pi (Clarkson and Scattergood, 1982), production of phosphatases and RNases (Hewitt and Tatham 1960; Löffler et al., 1992), acidification of growth media, and synthesis of new proteins (Goldstein et al., 1989). Phosphorus deficiency also leads to altered nutrient accumulation in tomatoes (Besford, 1979; Biddinger et al., 1995; Cerda et al., 1979; Rufty et al., 1990). In addition, excessive Pi sometimes results in Zn and Fe deficiency, as well as increased uptake of Cl in leaves (Cerda et al., 1979).

Altered gene expression is a component of plant response to Pi starvation (Liu and Raghothama, 1995, 1996; Liu et al., 1997; Malboobi and Lefebvre, 1995; Muchhal et al., 1996). We have examined the expression of a tomato Pi starvation induced gene (*TPSII*) during the early periods of Pi starvation (Liu et al., 1997). *TPSII* transcript accumulates in tomato as a specific response to Pi starvation and is reversible following resupply of Pi.

In this study, we link the physiological response of plants to gene expression during Pi starvation in tomato plants. Expression of the *TPSII* gene persisted with extended periods (3 weeks) of Pi starvation, indicating a correlation between gene expression and Pi deficiency.

Materials and Methods

PLANT MATERIAL. Tomato plants were grown in an aeroponic growth system similar to that described by Peterson and Krueger (1988). The system is sufficient to grow 24 plants (8 treatments and 3 replications) under desired nutrient and temperature conditions. Tomato seeds of the variety OS4 were germinated in seedling trays filled with Scotts ready earth plug mix (The Scotts Co. Marysville, Ohio). When seedlings reached the fourth leaf stage (20 d after sowing), they were removed from the growth medium and roots were carefully washed with water. The base of the stem was then placed inside a circular foam plug with a 2-cm slit cut along its diameter, and seedlings were positioned in the aeroponics system. Vacuum grease (Dow Corning silicone) was applied to the bottom surface of the plug to prevent salt accumulation around the stem. Roots were sprayed with a fine mist of half-strength Hoagland II solution (Jones, 1982) for 3 s every 10 min. One week after transferring the plants to the aeroponics system, Pi deficiency treatments were initiated by reducing the initial concentration of Pi (250 μ M) to 100, 50, 10, or 0 μ M P. The concentration of Pi was adjusted by decreasing the amount of potassium Pi added to the nutrient solution. Required amounts of potassium sulfate were added to replace potassium removed as a result of Pi treatments. Calcium nitrate was replaced with ammonium nitrate in the Ca minus treatments. Similarly, MgSO₄ was replaced with CaSO₄ to test the effect of Mg depletion on gene expression. Experiments were conducted under greenhouse conditions with 12 h of supple-

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mental light from 400-W high-pressure sodium lamps. The experiments were repeated under similar conditions. Plants were harvested 3 weeks after initiating treatments, and fresh mass of roots and shoots was recorded. The tissues were dried in a forced-air oven at 70°C for 72 h, and dry mass was recorded. Plants were also harvested at weekly intervals, after initiating Pi starvation treatments, for RNA isolation.

DETERMINATION OF NUTRIENTS IN ROOTS AND SHOOTS. Dried root and shoot samples were ground in a Wiley mill to pass a 20-mesh screen. One hundred milligrams of ground tissue sample was digested in 2.5 mL of H₂O₂ and 1 mL HClO₄ (Adler and Wilcox, 1985). The resulting solutions were diluted to 50 mL with distilled water. Samples were analyzed for NH₄-N using Nessler's procedure (Jackson, 1958). Phosphorus was determined by the modified Murphy-Riley procedure (Murphy and Riley, 1962). Potassium was determined by flame emission using a spectrometer (SpectrAA-10; Varian Techtron Pty, Mulgrave, Victoria, Australia). Calcium and Mg also were determined by absorption with the addition of LaCl₃ to overcome P interferences. Nonlinear regressions were performed using SAS PROC NLIN (SAS Institute, Cary, N.C.) to describe the effect of Pi level on growth and nutrient ion concentrations in shoot and root tissues.

MEASUREMENT OF LEAF GAS EXCHANGE AND STOMATAL CONDUCTANCE. Net CO₂ assimilation rate (A) and stomatal conductance (g_s) were measured between 1000 and 1200 HR on the first and second fully expanded leaves using a portable photosynthesis system (LI-6200; LI-COR, Lincoln, Nebr.) with a tripod-mounted 1-L leaf chamber (Premachandra et al., 1994).

RNA ISOLATION. Roots, leaves, petioles, and stems of tomato plants grown in the presence (250 μM) and absence of Pi were harvested 1, 2, or 3 weeks after withholding Pi and frozen in liquid N. Total RNA was isolated from the roots, leaves, petioles, and stems by hot phenol extraction and lithium chloride precipitation (Pawlowski et al., 1994).

NORTHERN ANALYSIS. Ten micrograms of total RNA was electrophoretically separated on 1% denaturing formaldehyde agarose gels and blotted onto a BA-S nitrocellulose membrane (S&S, NH) according to standard protocols (Sambrook et al., 1989). The nitrocellulose filter was prehybridized for 4 h in a solution containing 50% (v/v) formamide, 5× Denhart's, 0.1% (w/v) SDS, 6× SSPE, and 100 μg·mL⁻¹ denatured salmon sperm DNA at 42 °C. The DNA probe was labeled with ³²P-dCTP using a DNA labeling kit (DECAprime II; Ambion, Texas). Hybridizations were carried out with 10⁶ cpm of probe/mL in a buffer identical to prehybridization buffer at 42 °C for 16 h. Filters were washed twice in 2× SSC, 0.2% SDS, at room temperature for 5 min, twice in 1× SSC, 0.1% SDS, at 50 °C for 15 min, and twice in 0.1× SSC, 0.1% SDS, at 60 °C for 20 min before autoradiography.

Results and Discussion

Phosphorus deficiency resulted in progressive reduction in plant growth and biomass production by shoots (Fig. 1A) and roots (Fig. 2A) of aeroponically grown tomato plants. Plants supplied with 250 μM P produced maximum root and shoot biomass. The root:shoot ratio doubled in Pi-starved plants (0 μM Pi) relative to that of Pi-sufficient (250 μM) plants. This was not surprising considering that Pi deficiency often results in reduced shoot growth and increased root proliferation (Anghioni and Barber, 1980). Enhanced root growth under Pi deficiency is considered to be an adaptive mechanism to cope with the deficiency (Lynch, 1995). We also have observed a proliferation of short basal roots with profuse root hairs in Pi-starved plants (data not shown). Bates

and Lynch (1996) found that Pi deficiency stimulated root hair elongation in *Arabidopsis thaliana* plants nearly 3-fold.

Phosphorus deficiency also decreased CO₂ assimilation and stomatal conductance (Table 1). The net C assimilation of plants provided with 250 μM Pi was nearly 2.5 times greater than that of plants receiving 0 μM Pi. The impaired metabolism of Pi-deficient plants was clearly reflected in their severely reduced growth, particularly for above-ground parts. Shoots and roots had signifi-

Fig. 1. Relationship between P concentration in an aeroponics culture and the dry mass and percentages of K, Mg, and P of tomato shoot tissues. Dry mass and percentages of K and Mg were each fitted by nonlinear regression to an asymptotic exponential function of the form $Y = a(1 - be^{-cX})$; r^2 values were 0.77 and 0.84, respectively. Percentage Mg was fitted to a asymptotic exponential function of the form $Y = a(1 - e^{-bX}) + c$; r^2 value = 0.92. The relationship between shoot percentage P and P concentration is described by $Y = 0.1128 + 0.0009X$, $r^2 = 0.86$, $P < 0.001$.

cantly higher concentration of Pi in response to increasing concentrations of Pi in the nutrient solution (Figs. 1 and 2). The higher tissue content in plants supplied with increasing amounts of P may reflect their ability to accumulate the nutrient in cells. Plant cells store excess Pi in vacuoles and remobilize it during periods of Pi deficiency (Lee et al., 1990). Severe reduction in plant growth correlates with low tissue P content (Hogue et al., 1970).

Pi-deficient plants contained significantly more Ca in roots compared to that of Pi-sufficient plants (Fig. 2). Decreased content of Ca in Pi-sufficient plants is likely due to nutrient dilution effects. The dilution effect could be significant in determining tissue contents, particularly in the leaves of Pi-sufficient plants, which were large and succulent relative to the small, narrow leaves of Pi-deficient plants. In contrast to the high Ca content in roots of Pi-deficient plants, the Mg concentration increased preferentially in shoots of plants exposed to P deficiency (Fig. 1). The increase was particularly evident when P concentration in the nutrient solution was reduced to $<100 \mu\text{M}$. Plants grown in the absence of externally supplied Pi had nearly 3.8 times more Mg in shoots compared to

that of Pi-sufficient ($250 \mu\text{M}$) plants. Increased content of Mg in leaves of Pi-deficient plants could not be attributed either to lack of its availability or to pH effects of the nutrient sprays. Because plants were supplied with fresh nutrient solution at regular intervals, the differential uptake of nutrients, especially Mg and Ca, by Pi-deficient plants could be due to interactions between Pi in the nutrient solution and uptake and translocation of the ions. In addition, leaves of Pi-deficient plants had significantly less K compared to that of Pi-sufficient plants (Fig. 1). The imbalance in K concentration could be one factor contributing to increased Mg content in the leaves of Pi-deficient plants. High levels of K have been shown to decrease Mg and Ca uptake (Claassen and Wilcox, 1974) as well as their translocation (Ohno and Grunes, 1985) from roots to shoots. Another important factor contributing to different levels of nutrients in plants supplied with different amounts of Pi could be altered stomatal function. Pi starvation decreased transpiration and resulted in altered levels of nutrients in roots and shoots.

The effect of 3 weeks of Pi deficiency on gene expression was examined by Northern analysis of RNA isolated from tomato plants. A 0.5-kb *TPSII* transcript was detected in roots and leaves of Pi-starved plants (Fig. 3). The transcript accumulation was also observed in petioles and stems of Pi-starved plants (Fig. 4). Expression of the *TPSII* gene in different parts of plant suggest a global role for this gene under Pi starvation. The level of transcript remained relatively high even after 3 weeks of Pi starvation. This data indicated that expression of *TPSII* is associated with P deficiency. Phosphorus concentration in Pi-starved ($0 \mu\text{M}$) roots and leaves was ≈ 4 times less than that of Pi-sufficient ($250 \mu\text{M}$) plants.

The level of *TPSII* message in old leaves was relatively less than that observed in young leaves of Pi-starved plants (Fig. 5). Young and old leaves of Pi-sufficient plants had no detectable *TPSII* transcripts. The lack of *TPSII* message in old leaves of Pi-sufficient plants may be a result of growing these plants in Pi-rich ($250 \mu\text{M}$) conditions. The specificity of *TPSII* expression in leaves by Pi deficiency was further confirmed by starvation experiments involving other essential nutrients (Fig. 6). The transcript level of the *TPSII* increased in the leaves of Pi-starved plants, whereas the starvation of K, N, Fe, Mg, and Ca did not lead to an increase in the transcript accumulation. These data indicate that expression of *TPSII* gene in roots (Liu et al., 1997) and leaves may be regulated by the same factors induced under Pi starvation. Our earlier studies suggested that changes in gene expression is one of the most rapid responses to Pi starvation by plants (Liu et al., 1997). The present study shows that expression of the *TPSII* gene is not only induced early, but also persists over extended periods of Pi starvation. It is presumed that altered gene expression in response to Pi starvation may lead to adaptive changes in plants. These adaptive changes could help plants to survive better under Pi-limiting conditions. Although the function of *TPSII* is not known, a association between the gene expression and Pi deficiency suggests a role for the gene product in adaptation of plants to Pi starvation.

Fig. 2. Relationship between phosphorus concentration in an aeroponics culture and the dry mass and percentages of Ca and P of tomato root tissues. Root dry mass was fitted by nonlinear regression to an asymptotic exponential function of the form $Y = a(1 - be^{-cX})$; $r^2 = 0.75$. Percentage Ca was fitted to an asymptotic exponential function of the form $Y = a(1 - e^{(-bX)}) + c$; r^2 value = 0.69. The relationship between root percentage P and P concentration is described by $Y = 0.1362 + 0.0014X$, $r^2 = 0.84$, $P < 0.001$.

Table 1. Effect of phosphorus starvation on rate of photosynthesis and stomatal conductance of aeroponically grown tomato plants.

Phosphate concn (μM)	Net CO_2 assimilation rate ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	Stomatal conductance ($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)
0	5.67 ± 2.03	0.27 ± 0.052
5	8.14 ± 1.48	0.33 ± 0.019
50	13.29 ± 0.65	0.46 ± 0.016
250	14.02 ± 0.94^a	0.42 ± 0.02

^aValues are means ± 1 SD ($n = 3$).

Fig. 3. Expression of *TPSII* transcript during extended periods of phosphate (Pi) starvation. Northern blot of total RNA (10 µg/lane) isolated from leaves (top) and roots (bottom) of tomato plants grown in aeroponics. Plants were sprayed with nutrient solutions containing either 250 µM Pi(+) or no Pi(-) for indicated periods of time.

Fig. 4. Expression of *TPSII* gene in tomato plants. Plants were sprayed with nutrient solutions containing either 250 µM phosphate(Pi)(+) or no Pi(-) for 1 week. Total RNA isolated from roots, leaves, petioles, and stems was subjected to northern blot analysis. The labeled probe hybridizes to a 0.5-kb message in P-starved tissues.

Fig. 5. Expression of *TPSII* gene in old and young leaves of tomato. Plants were sprayed with nutrient solutions containing either 250 µM phosphate(Pi)(+) or no Pi(-) for 1 week. Total RNA was isolated from leaves and subjected to northern blot analysis. The size of the transcript is shown by an arrow.

Fig. 6. Effect of different nutrient starvation on the expression of *TPSII* gene in tomato leaves. Tomato plants grown in aeroponics were sprayed with nutrient solutions deficient in phosphate (Pi)(-), K(-), N(-), Fe(-), Ca(-), or Mg(-) for 5 d. Total RNA isolated from leaves of nutrient starved plants was analyzed by northern blots.

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