Differential Tolerance of Tomato Strains to Maintained and Deficient Levels of Phosphorus

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Abstract. Seven strains of tomato (Lycopersicon esculentum Mill.), with similar growth rates under adequate P availability, differed in dry matter accumulation by up to 73% when grown under P deficiency in a sand-alumina medium. The rate of P uptake per unit of root weight or length was a primary factor in strain capacity to acquire P under low-P stress. Variation in efficiency of internal P use also contributed to differential tolerance to P deficiency. The factors indicated to be responsible for tolerance to P-deficiency stress were present to different degrees in the different strains. Uptake rates per unit root were equally well correlated with total P absorption whether based on root length or root weight, suggesting that it may be most practical to screen for superior uptake capacities on the basis of root weight.

Low-input agricultural strategies are currently being formulated for lesser developed countries, particularly in the tropics, where the low availability of P is a major constraint in many soils (19). The identification of genetic variation in the ability of crops to grow acceptably on P-deficient soils is necessary if breeding for low-P tolerance is to proceed. Differential responses among strains of corn, beans, corn, rice, wheat, and white clover to P deficiency in soil have been reported (2, 6, 13, 18).

Intraspecific differences in response to artificially induced P deficiency in horticultural crops were associated with specific physiological processes, such as the kinetics of P absorption (15) and the efficiency of internal P use (26). However, ecological studies of wild plants have suggested to some workers that P uptake capacity per unit root and internal P utilization capabilities may not function adaptively in plants growing on P-deficient soils (3, 4). Consequently, the suitability of these parameters as selection criteria in breeding programs has been questioned (4). It would be helpful to demonstrate directly intraspecific differences in agricultural species for tolerance of P deficiency in soil, and then to determine the contribution of specific traits, such as P uptake capacities and utilization efficiencies, to those differences (11).

The heterogeneity and complexity of soils make the identification and study of genetically superior plants in that medium difficult (26). The sand-alumina culture technique is an alternative that mimics the restricted P diffusion and limiting P concentrations that contribute to low P availability in soil (10). Plant germplasm thus can be grown in a medium likely to allow effective expression of mechanisms conferring adaptive value in soil.

Seventy-five tomato strains of diverse geographical origin previously were screened for differential growth in P-deficient sand-alumina cultures (9). Differences in total dry weight accumulation of 70% to 80% were observed at low P among strains that grew similarly when P was not limiting. Comparable growth of strains relative to each other in sand-alumina and soil experiments tended to support the presupposition that similar adaptive mechanisms were functional in both media. The purpose of this investigation was 1) to confirm the differential ability of 7 tomato strains to grow in P-deficient sand-alumina, 2) to determine the mechanistic basis of that differential ability, particularly in terms of the roles of P uptake and internal P utilization efficiency, and 3) to consider the potential of using these parameters in breeding low-P tolerant crops.

Methods and Materials

Cultural conditions. The sand-alumina culture technique (10) was used to provide growth-limiting (low P) concentrations in Expts. 1, 2, and 3 as well as nongrowth-limiting (high P) concentrations of P in Expts. 1 and 3. High and low concentrations in the sand-alumina cultures were about 1 mM and 10 μM, respectively. In earlier experiments, dry matter accumulation at low P under these conditions was about 35% to 45% of growth at high P.

Low-P alumina was prepared by allowing 505 g of activated alumina to absorb phosphate from 3 liters of 0.01 M NaCl containing a loading concentration (LC) of 150 mM P. Alumina to supply high P concns was loaded at LC 500 millimolal. Neither the high-P nor the low-P alumina was "predesorbed" (10).

Low-P and high-P sand-alumina cultures with 50 g of alumina per pot were connected to P-free and P-containing (1 mM) watering systems, respectively, in the Univ. of Wisconsin Biotron. Environmental conditions, nutrient solution compositions, seed germination, and transplanting procedures were as described previously (10).

Experimental design. Seven tomato strains from previous screenings (Table 1) were grown in Expt. 1, 2, and 3 to establish firmly their low-P tolerance. Experiment 3, in addition, was designed to investigate whether plants originating from seedlots of different ages responded differently to P deficiency. All experiments employed complete block designs with 4 (Expt. 1) or 5 (Expt. 2 and 3) replicates. The 7 strains of tomato grown in Expt. 1 and 2 were grown in Expt. 3 in factorial fashion with 2 levels of P (high and low) and 2 groups of seedlots ("seedgroups"). Two seedlots produced in 1976 and 1977 formed Seedgroup I; 3 seedlots from 1981 and 1982 formed Seedgroup II. All seed had been stored in air-conditioned room at about 20°C.
Harvest procedures. Shoots of low-P plants were harvested and dried at 65°C after 26, 24, and 21 days of growth in Expt. 1, 2, and 3, respectively. High-P plants were harvested earlier than low-P plants to distribute the labor requirements for root recovery more evenly. The low-P plants consequently were larger than the high-P plants at harvest. Roots from low-P cultures were recovered by agitating decapitated root systems gently in 18.5 liter containers of tap water followed by meticulous washing in distilled water. Lateral roots were severed at the central root cylinder, and the root cylinders were immediately dried at 65°C. Lateral roots were stored in formalin acetic acid until subsequent analysis for weight, diameter, length, and P content. Total root lengths were measured by a line-intercept method (25). Root diameters were measured under a dissecting microscope equipped with an ocular micrometer. Root and shoot P concentrations were determined with a vanadiumolylbdate procedure.

Data on dry weights, tissue P content, and total root length were used to derive the following additional parameters related to the efficiency of P use and absorption: total mg P uptake (TPU), P utilization ratio (PUR = mg total dry weight per mg P absorbed), and root extension ratio (REL = mg root length per g shoot dry weight). REL was chosen to quantify root:shoot allocation patterns in preference to root weight ratios, because root extension and root length probably are related more functionally to P uptake than is root weight (16).

Net P assimilation rates per day per g dry weight (PARG) and net P assimilation rates per day per meter (m) of root (PARM) were calculated after Williams (27) for the period between transplant and harvest. The P content of seedlings at transplant was negligible relative to total content at harvest. Seedling transplants were selected visually at 14 days from imbibition to be uniform in root and shoot size. Seedling root dry weights and lengths were estimated from earlier experiments to be about 3 mg and 0.1 m, respectively. Computer simulation studies showed that errors of 50% in estimated initial root weight or length resulted in PARG and PARM values only 7% to 10% different from those calculations using the estimated average root weight and length. Visual selection for uniformity, therefore, provided adequate precision for the comparative purposes of these experiments.

Microscopic examination of roots in Expt. 1, 2, and 3 revealed that all roots had root hairs of varying lengths and at varying but usually very high densities along the entire length of root. A high coefficient of variation for root hair length in tomato (40%) also has been noted recently by other workers (14). Sampling requirements for discriminating differences in root hair length or density were prohibitive for detecting intraspecific variation in our studies. Root hair properties were not noticeably different among strains.

Statistical analyses. Separate analyses of variance (ANOVA's) were conducted for total dry weight (TDW) accumulation of the 7 strains grown with low P in Expt. 1 and in Expt. 2. In Expt. 3, logarithmically transformed shoot dry weights of high- and low-P plants were examined for evidence of differential strain and seedgroup performance (Table 2).

In order to assess the overall performance of the 7 strains, low-P TDW data from Expt. 1, 2, and 3 were combined for ANOVA with strain and experiment as factors (Table 3). Because of the significant strain x seedgroup and P level x seedgroup interactions within Expt. 3 (Table 2), data from the 2 seedgroups were considered as from separate experiments (3-I and 3-II) in the overall ANOVA.

Emergence of strain 479 in Seedgroup II, Expt. 3 was retarded, apparently due to poor seed quality, and growth was much reduced at both high and low P. The low-P data alone were clearly unrepresentative of the relative growth potential of this strain and were omitted in the overall ANOVA. The degrees of freedom for the strain x experiment interaction were thus reduced to 17 (Table 3). A plot of residuals vs. expected values confirmed the homogeneity of variance and satisfactory linear fit of the overall ANOVA model.

Similar statistical procedures were used for data on physiological and morphological traits. To facilitate comparisons, "standardized" overall means were calculated by dividing, for each strain, the overall (i.e., 3-experiment) mean for each trait by the average overall mean of all strains for that trait.

Different interpretations of the results of the ANOVA's were required for the separate purposes of this investigation (24). The experiments described herein were viewed as a random sample of all possible similar experiments where the purpose of the analysis was to assign tolerance ratings to the strains. These tolerance ratings would estimate the expected performance of the strains in subsequent studies. Strain and block factors were considered fixed, and experiment was considered a random factor. The appropriate basic error term for drawing conclusions about strain performance was the strain x experiment mean square (Table 3) with such a "mixed effects" model. Where the purpose of the analysis was to determine the differences between strains in growth and associated candidate causal factors in these 3 specific experiments, the appropriate basic error term for comparisons was the error mean square (Table 3).

Results and Discussion

Variations in strain tolerance to P deficiency. Dry matter accumulation of the 7 strains at high P was similar (Table 4). Overall means for total dry weight (TDW) at low P separated into 2 distinct groups. Each of the 7 strains was classified as either tolerant (t) or intolerant (i) of P deficiency in sand-alu-

Table 2. Analysis of variance of logarithmically transformed shoot dry weights in Expt. 3.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean square</th>
<th>F value²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>6</td>
<td>0.346</td>
<td>17.28**</td>
</tr>
<tr>
<td>P Level</td>
<td>1</td>
<td>7.037</td>
<td>351.84**</td>
</tr>
<tr>
<td>Seedgroup</td>
<td>1</td>
<td>0.009</td>
<td>0.46</td>
</tr>
<tr>
<td>Block</td>
<td>4</td>
<td>0.065</td>
<td>3.24*</td>
</tr>
<tr>
<td>Strain x P Level</td>
<td>6</td>
<td>0.273</td>
<td>13.64**</td>
</tr>
<tr>
<td>Strain x Seedgroup</td>
<td>6</td>
<td>0.086</td>
<td>4.32**</td>
</tr>
<tr>
<td>P Level x Seedgroup</td>
<td>1</td>
<td>0.089</td>
<td>4.43*</td>
</tr>
<tr>
<td>Strain x P Level x Seedgroup</td>
<td>6</td>
<td>0.025</td>
<td>1.24</td>
</tr>
<tr>
<td>Error</td>
<td>108</td>
<td>0.020</td>
<td>---</td>
</tr>
<tr>
<td>Total</td>
<td>139</td>
<td>---</td>
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</tr>
</tbody>
</table>

²:**Significant at 5%, or 1% level.
The strain with the highest average TDW across experiments (strain 214) produced 73% more than the strain with the lowest average TDW across experiments (strain 214). There was no relationship between growth at high P and tolerance rating at low P. Differential growth of these strains at low P must be controlled specifically by differential capacities for P uptake and/or utilization. This control cannot be assumed when strains differ in growth under conditions of nonlimiting P.

The significant strain x experiment interaction (Table 3) indicated that relative strain performance varied somewhat in separate experiments. The data from Expt. 3 indicated that the relative performance of strains under P deficiency was related to the seedgroup from which the plants originated. Lettuce cultivar responses to salt stress have been found to vary with differences in seed age and source (21).

Regardless of the cause, interactions between genotype and environment complicate studies of crop tolerance to P deficiency. Differential strain expression of P tolerance seemed to be caused by subtle environmental influences, because interactions were noted in a well-controlled and reproduced environment. Difficulties also have been encountered in studies with maize inbreds (R. Yost, Univ. Hawaii, personal communication). Such variability in response would contribute to poor correlations between greenhouse and field studies of low-P tolerance (11) and stress the need to insure that tolerance ratings are reproducible.

**Bases of low-P tolerance.** The differential growth of the 7 tomato strains in these studies was associated with significant differences in both total P uptake (TPU) by roots and P utilization (PUR) within plants after absorption (Table 5). Strain differences in TPU were associated with significant differences in both the total amount of root (root length) and the P uptake rate per unit of root (PARG and PARM). A high capacity for P uptake per unit of root apparently was an important adaptation to P deficiency in sand-alumina, because PARG values were significantly increased for all tolerant strains. PARM values showed similar though less clear cut association with tolerance. High uptake rates explain why strains 134 and 159 accumulated significantly more P than strain 214, despite the similar total root lengths of all 3 strains. Even though strain 127 produced significantly shorter root systems than strain 214, it still accumulated as much P overall as 214 because of a decreased absorption of P per unit root length.

A greater range and number of significant differences were found when uptake rates were expressed per m of root than when expressed per g of root (Table 5). The measurement of root length is time consuming, however, and because of the relative ease of measurement, uptake parameters based on root weight rather than root length may be the choice in experiments of this type. Because TPU and shoot P content were very highly correlated ($r = 0.98$), uptake rates calculated on the basis of root weights and shoot P content are probably sufficiently informative in use for large or otherwise time-intensive investigations, such as screening experiments.

The diversion of plant growth from shoot to root in response to P deficiency is well-documented. Preferential root growth in response to P deficiency is thought to help stressed plants obtain more P. However, differences in root:shoot allocation did not appear to function adaptively in these studies. Only 1 strain significantly increased root system per g of shoot (Table 5), and that strain (214) was 1 of the least low-P tolerant. Its higher root:shoot ratio might have been a unique response to severe P-deficiency stress. In contrast to these results, higher root:shoot ratios did appear to contribute to the low-P tolerance of corn genotypes (22).

**Implications for the development of low-P tolerant crops.** If mechanisms conditioning tolerance to diffusion-limited P availability in sand-alumina also are of adaptive value in soil (9), these results support the use of efficiency in P uptake per unit of root and internal utilization as selection criteria in developing agricultural crops for P-deficient soils. More information on the similarity of plant responses to P deficiency in sand-alumina and soil would be useful in assessing the strength of that support.

Other factors could influence the effectiveness of using root uptake and internal utilization parameters as criteria for developing low-P tolerant crops. For example, mycorrhizal associations often improve the P uptake of plants significantly in P-deficient soils. It is conceivable that strains, such as 214 in these studies might compensate for poor uptake capacity by deriving proportionally greater benefit in the field from mycorrhizal uptake. In contrast, mycorrhizal associations might reinforce the adaptive potential of strains selected for high efficiency of internal P utilization (low tissue concentrations), because the degree of mycorrhizal infection and P uptake is high in plants with reduced tissue concentrations (20).

The impact of mycorrhizal associations on plant-based tolerance mechanisms has not been explored systematically. However, data from 1 study (13) showed that the P-deficiency tolerance of 1 corn cultivar in the absence of mycorrhiza was superior to the tolerance of 2 other corn cultivars, even when the latter were
mycorrhizal. Differences between cultivars were minimal with optimal fertilization. Superior growth under P deficiency may be obtained without mycorrhiza in cultivars of some crop species. The most efficient approach in developing low-P tolerant genotypes may be to select for mycorrhizal responsiveness among genotypes already superior for plant-based P-deficiency tolerance mechanism.

Ecological studies of wild plants adapted to high- and low-P sites recently have been cited to support the assertion that adaptation to P deficiency is not accompanied by improved capacities for P uptake or internal utilization, but rather by selection for reduced growth rate, which, in turn, reduces the degree of metabolic stress encountered by plants (3, 4). Consequently, it is argued, “breeding of crops for successful growth on infertile soils can be accomplished only by accepting reduced yield, and [that] breeding programs selecting for high capacity for nutrient uptake (g \(^{-1}\) root) or high efficiency of nutrient utilization may have limited success” (4).

Review of the literature on the adaptation of wild plants to P deficiency may not necessarily lead to the above conclusions (8). Several examples indicate that species adapted to low-P soils can absorb P at higher rates per weight of root than unadapted species under moderate P-deficiency stress (1, 4, 5, 23). Similarly, high P-utilization efficiency has been noted in studies of low-P adapted plants (1, 12, 28).

Finally, adaptive strategies most appropriate for agricultural species on P-deficient soils may differ in principle from strategies most appropriate for wild plants. The slow growth typical of wild species may reduce the depletion of external P supplies to levels sustainable by natural replenishment processes (7), or alternatively, may allow survival on internal P reserves during periods between nutrient flushes (3, 17). However, in a developing agriculture based on low-input management of low-P soils, P must be returned to the soil to compensate for P removal by crops (19). The P absorption rate and subsequent growth of crop species need not be dependent on natural rates of P replenishment. High tissue P concentrations likewise may not be critical for agricultural species unlikely to be exposed to fluctuating P levels.
supply. As a result, less restricted growth rate, aggressive P absorption, and low internal P concentration all may be more appropriate for agricultural species than for wild species growing on infertile soils.

**Literature Cited**


