Equivalent Stress Comparisons Among Tomato Strains Differentially Tolerant to Phosphorus Deficiency

R.R. Colman¹
Department of Horticulture, University of Hawaii, Honolulu, HI 96822

G.C. Gerloff² and W.H. Gabelman³
Department of Botany, University of Wisconsin, Madison, WI 53706

Additional index words. Lycopersicon esculentum, sand–alumina, phosphate, stress physiology, P deficiency

Abstract. Physiological and morphological features of tomato (Lycopersicon esculentum Mill.) strains differentially adapted to phosphorus (P) deficiency differed depending on whether the strains had been grown under a single level of low P in screening experiments or under specific levels of P that reduced growth of each of the strains to about 50% of maximum, i.e., at “equivalent stress”. These differences indicated that stress compensation contributed significantly to the physiological and morphological phenotypes of strains under the specific P level used for screening for low-P tolerance. Consequently, the true extent of genetic differences for these traits was not directly measurable. Differences between strains for root:shoot ratios, internal-P-use ratios, and kinetic parameters of P uptake still were observed at equivalent stress. Thus, the genetic bases of these differences seem secure. The P concentration in lower leaves of tolerant strains was lower than in intolerant strains, and the maximum rates of 32P by uptake excised roots were increased in the tolerant strains.

A common approach in screening for tolerance to nutrient stress has been to grow strains at a single level of nutrient supply that is less than adequate for optimum growth. This approach has shortcomings for identifying physiological and morphological factors responsible for differences in stress tolerance, because 2 types of influences contribute to the level of expression of those factors under stress. First, plants respond generally to increasing nutrient deficiency with increased root:shoot ratios, increased nutrient uptake capacity, and decreased tissue concentration of the nutrient. Of greater interest to plant breeders, however, is the 2nd type of influence, namely strain-specific variations in response to nutrient stress that are under genetic control. If screened at a single nutrient level and harvested at the same time, differentially adapted strains are concurrently under differing degrees of stress, and it is difficult to distinguish the degree to which observed physiological and morphological variations are due to general stress as opposed to specific strain influences. For example, in a previous study on response to P deficiency (6), tomato strain 214 appeared to have a less efficient P-uptake mechanism, but a uniquely high root:shoot ratio compared to other strains. Because strain 214 was under greater deficiency stress at harvest (probably as a result of depressed P uptake), it was difficult to determine the degree to which the high root:shoot ratio reflected a characteristic of the strain or if it was merely a response to increased deficiency stress.

It is important to be able to distinguish general stress compensation responses from true physiological or morphological strain differences, because only the latter areheritable. This distinction may be realized by evaluating strains at an equal degree of nutrient deficiency stress, i.e., at what we refer to as “equivalent stress”. We consider 2 strains under equivalent stress if P deficiency reduces their growth similarly when compared to controls adequately supplied with P. In this study the responses of 6 strains of tomato, previously established as tolerant or intolerant to a specific low concentration of P in sand–alumina cultures, were measured under P culture conditions that provided equivalent P-deficiency stress. Certain physiological and morphological traits associated with low-P tolerance had been expressed at varying degrees of efficiency in the different strains in the previous screening experiments (6). Similar comparisons among strains were made at equivalent stress to expose true strain differences.

Materials and Methods

Expt. 1. The sand–alumina culture technique was used to provide a range of P concentrations (1 – 25 μM) in solution by loading activated alumina in the presence of 50, 100, 200, or 400 mM P (“loading concentrations”) as previously described (5). Sand–alumina cultures with 50 g of alumina per 2-liter pot and P-sufficient sand-only cultures were connected to P-free and P-containing (+P) watering systems, respectively, in the Univ. of Wisconsin Biotron. Salt concentrations in the nutrient solution for +P treatments were 2.5 mM KNO₃, 0.5 mM KH₂PO₄, 1.0 mM MgSO₄·7H₂O, 0.5 mM NaCl, 0.5 mM NaH₂PO₄·H₂O, 1.3 mM Ca(NO₃)₂·4H₂O, 23 μM H₃BO₃, 4.5 μM MnSO₄·H₂O, 0.38 μM ZnSO₄·7H₂O, 0.16 μM CuSO₄·5H₂O, 0.0073 μM (NH₄)₂MoO₄·4H₂O, and 40 μM FeEDTA. In the P-free nutrient solutions, concentrations of the salts providing the macroelements were 2.5 mM KNO₃, 1.0 mM MgSO₄·7H₂O, 0.5 mM NaCl, 0.5 mM KCl, and 1.3 mM Ca(NO₃)₂·4H₂O. The micronutrient composition was as presented previously. Seedlings of tomato strains 55 (PI 102886), 127 (PI 118406), 212 (PI 126407), 214 (PI 126409), and 479 (PI 367966) were produced by germinating seed in perlite with +P nutrient solution for 14 days. They were transplanted to and grown in the sand—
alumina and sand-only cultures under 16-hr photoperiods with 550 μmol·s⁻¹·m⁻² at 27°C (days) and 20°C (nights) and 70% relative humidity for 3 weeks. The cultures were arranged in randomized complete blocks with 4 replicates.

Twenty-one days after transplanting, the plant shoots were cut at sand level and immediately dried at 65°C. Analysis of variance (ANOVA) was conducted on logarithmically transformed shoot dry weights (SDWs) of the 5 strains at the 5 levels of P availability. An examination of residuals confirmed the satisfactory fit of the ANOVA model.

Expt. 2. Data from Expt. 1 were used to select, for each strain, a single loading concentration of P that would produce a sand-alumina culture P concentration capable of reducing the growth of the strain by 50% compared to a P-sufficient control. Activated alumina for sand-alumina cultures was prepared accordingly at loading concentrations of 90, 100, 115, 130, and 140 mM P for strains 55, 479, 212, 127, and 214, respectively. An additional strain 134 (PI 118782) was included in Expt. 2. This strain responded similarly to P-deficiency stress in previous experiments as did strains 55, 479, and 212 (6). Therefore, to produce a 50% growth reduction, activated alumina prepared with a 100 mM P-loading concentration served as a P source for strain 134.

Plants of each strain were grown for 21 days in a 5-replicate randomized complete block design in the same Biotron growth room under the same environmental conditions as in Expt. 1. Alumina-free sand cultures watered with +P nutrient solution provided P-sufficient controls. All sowing, transplanting, and harvesting procedures were staggered by 24 hr between adjacent blocks of plants to facilitate detailed examination of physiological parameters of the plants on each harvest day.

At harvest, the shoots of all plants were excised at the sand level. Shoots of P-sufficient plants were dried intact at 65°C. Shoots of P-stressed plants were divided into upper leaves (the uppermost one-third), lower leaves (the lower two-thirds), and stem before drying. Contents of shoot fractions of P-stressed plants were subsequently determined with a vanado-molybdate procedure.

Roots of P-stressed plants were carefully recovered, washed free of adhering alumina, and placed in 0.5 mM CaCl₂ at 4°C to preserve the integrity of the root cell membranes. Total root lengths for each root system were subsequently determined in random order, using a modified line-intercept method (16). Root length determinations required about 30 min each to complete. The roots were continuously bathed in 0.5 mM CaCl₂ at room temperature (23°C) during root length determinations. For strains 55, 134, 127, and 214, ten samples with about 40 mg fresh weight were selected for 32P absorption studies using methods originally described by Epstein et al. (9). The time the roots of these strains were held in cold storage varied between 30 min and 3 hr, depending on the order of length determinations. The 10 samples form each strain’s root system were placed in stainless steel tea balls and resubmerged in 0.5 mM CaCl₂. To begin the absorption study, the tea balls were briefly drained and then immersed in one of 10 32P-labeled, vigorously aerated sodium phosphate solutions containing 0.5 mM CaCl₂. The P concentrations in these absorption solutions were 1, 2, 4, 6, 8, 12, 16, 32, 64, and 128 μM. The specific activity of 32P ranged from 1.2 to 24.0 μCi·liter⁻¹ (1Ci = 37 CBq) at the lowest and highest P concentrations, respectively. Preliminary testing revealed no detectable effects of variation in specific activity over this range.

Solutions were maintained at about 22°C and were adjusted prior to absorption to pH 6.0 ± 0.2. After 10 min of P absorption, the tea balls were rinsed in 2 separated one-liter volumes of 0.5 mM CaCl₂ and then allowed to desorb for 20 min in 250 ml of label-free absorption solutions at about 4°C. The roots from each tea ball were subsequently transferred to preweighed scintillation vials and placed in a drying oven at 65°C overnight. Ten milliliters of distilled water were added to the dried roots in the vials, and the vials were vigorously agitated periodically for 1 hr. Radioactivity then was determined using Cerenkov scintillation counting (3). Counting efficiency did not vary significantly over the range of root dry weights used in the absorption experiments.

Nanomoles of P absorbed per gram of root dry weight per hour were calculated, considering the specific radioactivity of each concentration used. Values of Vmax and Km (as measures of the capacity and affinity, respectively, of the P absorption mechanism in the roots) were calculated using 2 computer programs (SEQUEN and PING-PONG) kindly provided by W. Cleland (4). Values of Vmax, Km, total SDW, percentage of P in fractions and total shoots, and percentage of growth reduction (%Red) were subjected to 2-way ANOVA with strains as factors and days as blocks. Root extension ratio (RER) and %Red were calculated as follows: a) RER = m total root length· SDW⁻¹; and b) %Red = [1-(P-stressed SDW-P-sufficient SDW⁻¹)] x 100.

Results and Discussion

Expt. 1. Consistent with previous studies, the 5 strains in Expt. 1 produced similar SDWs when grown with sufficient P (+P”), but dissimilar SDWs when low-P concentrations in solution were limiting growth (Fig. 1). The greatest difference between strains at 11.2 μM P was only 30% (highest mean SDW as a percentage of the lowest), but increased to 330% when the P concentration was reduced to 3.7 μM. Likewise, the average reduction from maximum growth for all strains due to P deficiency stress was 27% at 11.2 μM P and 57% at 3.7 μM P. Growth reductions caused by a further reduction in culture P levels to about 1.6 μM increased the maximum percentage difference between strains to 385% (strain 214 vs. strain 479), but
reduced the absolute differences. These observations, combined with the practical concern of producing adequate amounts of various plant tissues for further analysis, led to the choice of a 50% growth-reduction target for equivalently P-stressed plants in Expt. 2. Appropriate loading concentrations were estimated for each strain by hand-fitting curves through the strain means across P levels.

The data in Fig. 1 suggest that comparisons of growth and development among strains are likely to be most discriminatory when P deficiency stress is sufficient to reduce growth at least 50%. Yield reductions of this magnitude required P concentrations in soil solution below 0.02 ppm (0.6 μM) for tomato and several other vegetable crops (15). Soils with P levels this low are rare in many areas of the continental U.S. (8), although they are widespread in the tropics. This finding underscores the need for reliable laboratory and controlled-environment techniques for identifying differential tolerance to low P. The sand–alumina technique provided simple, effective, and predictable control of P concentrations in these experiments.

Expt. 2. All strains produced comparable SDW in P-sufficient cultures in Expt. 2. Shoot dry weight reductions due to P-stress averaged 47% and were comparable between all strains except 212, which was significantly less stressed than the most-stressed strains (strains 214 and 479) (Table 1). Strains 55, 127, 134, 214, and 479 were considered under equivalent stress for physiological and morphological comparisons. Some caution may be appropriate when comparing P stress-dependent parameters of strain 212 with those of 214 and 479.

Root extension ratios differed between strains when compared at equivalent stress as they had when compared at different levels of growth reduction in the previous screening studies (Table 1). However, the relationships between strain means for this trait changed significantly when the strains were compared at equivalent stress. In the screening studies only strain 214 produced a more extensive root system in proportion to its shoot than other strains. At equivalent stress, 2 low-P tolerant strains (55 and 134) produced root systems as extensive as that of strain 214. Therefore, preferential root growth in response to P deficiency was not a discriminating feature between these low-P tolerant and low-P intolerant strains. It may, however, contribute to the tolerance of specific strains such as 55 and 134.

Strain comparisons of P-use ratio (PUR) were also quite different depending on whether strains were grown to different degrees of P deficiency or to a similar degree of deficiency. Whereas in the screening studies strain 55 was one of the 2 most inefficient strains at internal use of P; when grown at equivalent stress, it produced significantly more dry matter per milligram of absorbed P than all other strains (Table 1). In constrast, strain 214 showed the best internal P use in the screening studies, but was unimpressive at equivalent stress.

High internal-P-use efficiency in strain 55 was associated with low P concentrations in all shoot components (Table 2). Concentrations of P in lower leaves of all strains were always considerably lower than concentrations of P in upper leaves, because P is redistributed preferentially to growing points when plants become P deficient (1). All strains other than 55 had similar P concentrations in upper leaves. However, on the average, the P concentration in the lower leaves of low-P tolerant strains was lower than in low-P intolerant strains (1 df comparisons, P ≤ 0.05). Low-P intolerant strains may require higher P concentrations in lower leaves than low-P tolerant strains to maintain photosynthesis or other critical metabolic processes. Net photosynthesis per unit of P was higher in a P-efficient strain of snapbean than in a P-inefficient strain (17). Similar evidences of superior metabolic effectiveness under nutrient stress have been apparent with K-efficient snapbeans, and N-efficient tomatoes (10).

Maximum depletion of P from the absorption solutions during the absorption period was less than 1%. The average maximum rate of P absorption by excised roots in Expt. 2 was 1.7 mg·g dry weight⁻¹·day⁻¹ (Table 3). This rate is equivalent to 2.7 μmol·g fresh weight⁻¹·day⁻¹, assuming a fresh weight:dry weight ratio for root tissue of 20 ± 3:1 (unpublished data), and is close to the 2.4 μmol P·g fresh weight⁻¹·day⁻¹Vmax rate reported previously for excised tomato roots (7). Net P assimilation rates in the screening experiments were considerably higher than Vmax rates in the equivalent stress experiments. High P-absorption rates also have been observed for intact plants of tomato and several other species (12, 13). The reason for higher uptake into roots of intact plants, compared with excised roots, is unclear. Uptake into excised roots proceeds linearly for hours; thus, it seems unlikely that Vmax rates are low because of regulation by rising internal P concentrations, nor do sugars appear to be limiting in excised roots (2). The 4°C storage temperature prior to uptake conceivably could have damaged the roots. However,
the close agreement with uptake rates in the previously cited tomato study (7), which did not involve cold storage, suggests this is an unlikely explanation of the discrepancy.

Low-P tolerant strains showed higher absorption rates on the average (1 df comparisons, *P < 0.05*) than the low-P intolerant strains (Table 3). These data suggest that kinetic analysis of short-term P uptake rates may be of some value for predicting P accumulation rates in intact plants, even when the latter are compared at dissimilar states of P deficiency. This suggestion contrasts with earlier conclusions comparing P uptake into excised roots and intact plants (14). However, in the equivalent stress experiment, *V* \_\text{max} \text{ for low-P intolerant strain 214 was indistinguishable from *V* \_\text{max} values for low-P tolerant strains 55 and 134, whereas in the screening studies the average P uptake rate for strain 214 was significantly lower than the uptake rate for low-P tolerant strains. This inconsistency could be due simply to the considerably smaller number of replicates available for statistical purposes in the equivalent stress experiment than in the screening experiments.

Differences in ion affinity as represented by *K* \_m values were not important in explaining P absorption differences between tolerant and intolerant strains in the equivalent stress experiment (Table 3). Increased affinity for phosphate may aid mycorrhizal tomato roots in obtaining P from solutions containing too little P for absorption by roots not infected by mycorrhizal fungi (7).

Phosphate uptake mechanisms may not be solely responsible for differences in long-term P absorption between low-P tolerant and low-P intolerant strains. Plant-induced changes in the rhizosphere, such as changes in pH, can alter P availability significantly (11). The limited ability of strain 214 to accumulate P from sand–alumina cultures could be related to a mechanism of this kind. Nevertheless, the higher average *V* \_\text{max} values for low-P tolerant vs. intolerant strains support the previous suggestion (6) that differences in uptake capabilities contributed significantly to differences in low-P tolerance.

Strains of tomato differed in physiological and morphological traits thought to be involved in tolerance to low P availability, but the relative differences varied when strains were compared at a single low-P level vs. a common level of P-deficiency stress. Thus, stress compensation responses apparently were contributing significantly to the phenotypic expression of these traits when screening was at a specific P level, and the observed variations in RER, PUR, and P-absorption rates under a single level of P stress might not have a genetic basis. With the data from the equivalent stress experiments, we are more confident that genetic differences between strains for RER, PUR, and P-absorption rates could be measured with appropriately designed genetic experiments. Such experiments would seem to require evaluation of genetic family structures across a range of P deficiency to permit comparisons of physiological and morphological features free from stress compensation influences.

### Literature Cited

and translocation in strains of tomato. PhD Diss., Univ. of Wisconsin, Madison.


Mineral Elements and Organic Acids in Branch and Root Xylem Sap of Healthy and Blight-affected Sweet Orange Trees

H.K. Wutscher and R.E. McDonald
ARS/USDA, 2120 Camden Road, Orlando, FL 32803

Additional index words. nitrogen pressure extraction, tissue-sap correlations, Citrus sinensis

Abstract. Sap extracted by nitrogen gas pressure from branches and lateral roots of healthy and citrus blight-affected ‘Hamlin’ and ‘Valencia’ orange, Citrus sinensis (L.) Osbeck, trees on rough lemon (C. limon Burm. f.) rootstock in 2 commercial groves was analyzed for N, K, Ca, Mg, S, Na, Fe, Mn, Zn, Cu, Cl, Si, and organic acids. The branch and root wood (from which the sap was extracted), leaves, and feeder roots were also analyzed. Sap extracted from branches of blight-affected ‘Hamlin’ trees in the spring had higher Zn, Cu, Cl, and Si concentrations than sap of healthy trees. Nitrogen was increased twofold and Fe slightly increased with blight in the root sap. Branch sap collected from ‘Valencia’ trees in the fall showed no differences. Branch sap contained more organic acids than root sap and there was no difference between blighted and healthy trees. Citric and malic were the principal acids.

Xylem sap of tree crops is usually difficult to extract, but its composition in apple and pear roots has been studied in some detail (2–4, 13, 18, 19). There are peaks in mineral content of deciduous crop sap in the spring and the fall (4). In citrus, most of the work done has been with nitrogenous compounds (which also vary with season) in sap extracted by various methods (10–12). There is little information on the levels of mineral elements and organic acids in xylem sap of citrus or other evergreen crops.

Citrus blight, a tree decline of unknown cause, has been described as a "water transport dysfunction" (27). Resistance to water uptake in injection tests and accumulation of Zn in the outer trunk wood are diagnostic criteria for the disorder (22). Various nutritional aspects of blight have been reported (1, 16, 21–23), and measurements on wood slurry's showed that the internal pH of blight-affected citrus trees is slightly higher than that of healthy trees (25, 26). Internal pH of plants is influenced by nutrient uptake (9); therefore, we simultaneously studied the pH and the composition of sap, the concentration of the same elements in the tissue from which sap was extracted and in the leaves and feeder roots as well, because surrounding tissues sometimes influence the concentration of materials in extracted sap (12, 14).

Materials and Methods

Samples of branches and lateral roots (1 to 2 cm in diameter, feeder roots, and leaves were collected during 3 weeks in Mar. 1983 in a 35-year-old commercial grove of ‘Hamlin’ sweet orange on rough lemon rootstock when the trees were in full bloom. The trees were planted 8 × 8 m on slightly sloping Astaturula fine sand soil (hyperthermic, uncoated typic quartzipsamment). They were not irrigated and received standard grove care. The trees had been fertilized with 120 kg N and 100 kg K/ha 4 months before the start of the experiment. Minor elements were applied in April as a leaf spray. Citrus blight had been a problem for some years, and more than 50% of the original trees had been removed. Eight trees showing moderate symptoms of blight and 8 healthy trees were selected in pairs and their status confirmed by Zn analysis of the outer trunk wood (22) 3 months before sampling. A similar set of 8 trees each was selected in Sept. 1983 by the same method in a 12-year-old block of ‘Valencia’ sweet orange on rough lemon. 65 km from the ‘Hamlin’ block. The trees were planted 4.5 × 6 m on similar soil, but this grove was irrigated with overhead sprinklers. Only 23% of the trees had blight, mostly in early stages.

About 2 kg of branches and roots were taken from each tree between 0700 and 0900 HR from each of 4 trees on a single day. Samples had to be collected before 0900 HR, as essentially no sap could be extracted from material taken later, similar to results reported previously (11). This requirement was especially true for branch pieces, which, in general, yielded less sap than root pieces. The samples were cut to 45 cm in length and brought to the laboratory wrapped in wet burlap to prevent drying. The pressure bomb used for extraction has been described (20). The distal ends of the shoot and the proximal ends of the root pieces were pared to fit into a well in a metal plate and inserted into a metal cylinder. The cylinder was filled with nitrogen gas to a pressure of 343 kPa, and the emerging sap was collected from the well with a syringe and stored in glass vials at −10°C.

Received for publication 5 Aug. 1985. We are grateful to Mr. Andy Rose, Florida Citrus Groves Corporation, for providing the trees for the experiment and to Mr. Victor Chew, Agricultural Research Service/U.S. Department of Agriculture, for statistical analysis of the data. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.