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Tolerance of Tomato Strains to Phosphorus Deficiency in Root Culture

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Abstract. **Root culture was adapted for screening a diverse collection of tomato (*Lycopersicon esculentum* Mill.) strains for tolerance to low supplies of P. Fifty-one tomato strains were screened in 10 consecutive experiments for root fresh weight (RFW) production with high non-growth-limiting (25 μm) and low growth-limiting (7 μm) concentrations of P in a sterile, liquid medium. Twenty strains showing the most and least restricted root growth at low P in the initial 10 experiments were grown simultaneously in three final screening experiments. Restrictions in RFW at low P averaged 51% for four strains, consequently classed as low-P "intolerant", and averaged 27% for three strains, consequently considered low-P "tolerant". At high P, RFW of tolerant strains averaged 17% less than RFW of intolerant strains. At low P, RFW of tolerant strains averaged 23% greater than RFW of intolerant strains. The greater RFW production of intolerant strains at high P was due to higher internal P use ratios (IPUR = mg root dry weight (RDW) per mg P adsorbed). Differences in growth at low P were due primarily to differences in P uptake. However, the relative contributions of P use and P uptake efficiencies to low-P tolerance were different among strains. Root hairs of tolerant strains at low P were longer and covered a greater proportion of the root length than root hairs of intolerant strains. The pH of the culture medium of one tolerant strain was significantly lower than the medium pHs of the other strains.**

A principal component for improving agricultural productivity on low-P soils is the development of crop cultivars improved in their capacity to acquire and make use of limited soil P (13). Adequate genetic variation exists within agricultural species to breed successfully for improved P acquisition and use under conditions of limited P availability (8, 13). However, improved laboratory or greenhouse techniques for evaluating genetic variation in tolerance to P stress are needed to accelerate the development of cultivars for low-P soils.

Root culture may be useful for screening crop germplasm for low-P tolerance. Plant acquisition of P from soil is related closely to the branching and extension of roots and to the development of root hairs (2). These characteristics of root growth are observed easily in root culture (14). Plant roots can increase P availability in soil by lowering rhizosphere pH (9), and root culture pH can be monitored readily. The nutritional requirements for root culture of a number of important agricultural species are known, and the cultures may be grown under a wide range of controlled environmental conditions (14).

The purposes of these experiments were to evaluate the extent of variation among tomato strains in tolerance to low P supplies in root culture and to examine the physiological and morphological bases for low-P tolerance. As in previous work (5, 6), "tolerance" is used to describe strains with the least restricted growth with limited P availability, and "efficiency" is used to describe comparative performance in physiological or morphological characteristics that contribute to the overall tolerance to low-P stress, e.g., total P uptake or RDW per milligram of P in the root tissue.

General procedures described by Butcher and Ingram (3) were used to establish root cultures. A P-free basal medium was prepared by eliminating the P salt from the modified White's medium prescribed (3). Preliminary experiments indicated that maximum RFW in root culture of a number of tomato strains was obtained with 25 μM P provided from an aqueous solution of NaH_2PO_4. A 7-μM P level in culture restricted growth by ≈50%. These two P levels were chosen and referred to in subsequent screening experiments as "high P" and "low P".

An initial screening of 51 tomato strains for growth at high P and low P was conducted in 10 experiments. Each strain was evaluated in at least two experiments. Root cultures were established by transferring three sterile, 6-day-old root tips, 1-cm-long, to 125 ml wide-mouth Erlenmeyer flasks containing 50 ml of sterile nutrient media adjusted with NaOH and HCl to pH 4.9 ± 0.1. Culture vessels were sealed after root tip transfer with aluminum foil and arranged on a laboratory bench top in a completely randomized design. The cultures were hand-swirled every 2 or 3 days. Average daily maximum and minimum temperatures in the naturally lighted air-conditioned laboratory during these experiments were 24° ± 1°C and 20° ± 1°, respectively. RFW was determined after 19 days in culture after blotting the roots in paper towels to remove free moisture. The percent restriction in root growth due to low-P stress was calculated for each strain as follows: $\%Res_{RFW} = [1 - (RFW_{low\text{-}P}/RFW_{high\text{-}P})] \times 100$.

Twenty of the original 51 strains showing the largest and smallest percent restrictions in RFW at low P in the 10 initial screening experiments were selected and cultured simultaneously in a series of three final screening experiments with P levels, culture conditions, and experimental design as in the initial screening experiments. After 19 days in culture, roots were retrieved from the culture flasks and rinsed in 0.5 mM $CaCl_2$ to remove residual external P. RFW, RDW after drying overnight at 65°C, and final culture medium pH were recorded. Tissue P concentrations in the dried roots were determined after dry-ashing using a Mo blue procedure (12). The efficiency of internal P use was measured with the internal P use ratio (IPUR = mg RDW per mg P in the tissue). In the second and third final screening experiments, visual ratings of root hair distribution (RHD) and comparative root hair length (RHL) were assigned to each culture 1 day prior to harvest according to the following scales: RHD = 1, 2, or 3 if root hairs were present on 0% to 33%, 34% to 66%, or 67% to 100% of root length, respectively; and RHL = 1, 2, or 3 for comparatively short, medium, and long root hairs, respectively, based on the range of root hair length apparent among strains. Although crude, these empirical ratings allowed meaningful distinctions to be made among strains,

Table 1. Identification and origin of tomato strains.

Strain	Plant Introduction no.	Other identification	Origin
2		'Kewalo'	Hawaii
8		CL 5915-455-D_4-3-3	AVRDC[z]
11	092859		China
12	092863		Manchuria
35	304228	'Tidling Bush'	New York
37	309666	'Epoch'	Indiana
43	338492	'Balkan'	Bulgaria

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Table 2. Root fresh weight (RFW), P uptake, internal P use ratio, final medium pH, root hair distribution, and comparative root hair length of seven tomato strains grown with high P and low P in root culture.

P level	Low-P tolerance rating	Strain	Root responses						
			Root fresh wt (RFW) (mg)	Restriction in RFW at low P (%)	P uptake (mg)	Internal P use ratio[z]	Final medium pH	Root hair distribution[y]	Comparative root hair length[x]
High	Intolerant	8	181 a[w]		29 a	747 a	6.2 ab	2.7 ab	1.5 b
		2	163 ab		28 a	731 a	6.2 ab	2.3 ab	2.0 ab
		12	157 ab		28 a	705 a	6.0 b	2.2 b	1.3 b
		11	154 abc		29 a	630 a	6.2 ab	3.0 a	2.0 ab
	Tolerant	43	147 bc		29 a	552 b	6.2 ab	3.0 a	3.0 a
		35	135 bc		29 a	556 b	6.2 ab	2.8 ab	2.5 a
		37	127 c		29 a	577 b	6.3 a	2.7 ab	1.7 b
	I vs. T[v]		**		NS	**	NS	NS	**
Low	Intolerant	8	86 bc	53 a	9.0 bc	1129 ab	5.9 ab	2.8 a	1.2 c
		2	83 c	48 a	8.1 e	1196 a	5.8 b	2.2 bc	1.0 c
		12	69 d	55 a	8.5 de	975 c	5.9 ab	1.8 c	1.2 c
		11	78 cd	49 a	8.7 cd	1012 bc	6.1 a	2.2 bc	1.0 c
	Tolerant	43	104 a	29 b	9.9 a	1206 a	5.5 c	3.0 a	2.8 a
		35	91 bc	32 b	9.2 b	1084 abc	5.9 ab	2.5 ab	2.0 b
		37	98 ab	20 b	9.4 b	1199 a	6.1 a	2.7 ab	1.2 c
	I vs. T[v]		**	**	**	*	NS	**	**

[z]Milligrams of root dry weight per mg P in root tissue.
[y]Scale: 1, 2, and 3 = 1% to 33%, 34% to 67%, and 68% to 100%, respectively, of root length with root hairs.
[x]Scale: 1, 2, and 3 = comparatively short, medium, or long, respectively, based on range of root hair length observed among all strains.
[w]Mean separation at each P level by LSD, $P = 5\%$.
[v]Single degree of freedom contrast for intolerant (I) vs. tolerant (T) strains. Nonsignificant (NS) or significant at the 5% (*) or 1% (**) levels.

because consistent differences in root hair distribution and length were conspicuous. Data from the three experiments were considered as replicates in an overall analysis of variance with strains as treatments and experiments as blocks using SAS (1).

Ratings of low-P tolerant or low-P intolerant were assigned to strains in two groups that differed significantly in the average percent RFW restriction at low P in the three final screening experiments. The number of strains retained in the tolerant and intolerant groups was reduced further to three and four, respectively, by eliminating strains that grew poorly at high P. Strains with high growth potential were considered of more interest as breeding material than strains that grew poorly with unlimited P. Mean separations of growth and physiological and morphological parameters of all strains were performed with a 5% LSD, and specific comparisons of the intolerant and tolerant groups of strains were done using single degree of freedom contrasts.

Phosphorus deficiency restricted the average RFW production of the 51 strains in the 10 initial screening experiments from 16% for the most tolerant strains to 56% for the most intolerant strains. Four intolerant strains (2, 8, 11, and 12) and three tolerant strains (35, 37, and 43) were identified in the three final screening experiments (Table 1). Root fresh weight restrictions due to P deficiency averaged 51% for the four intolerant strains, but only 27% for the three tolerant strains (Table 2). At low P, the average RFW of tolerant strains was 23% greater than that of intolerant strains, with strain 43 producing 32% more RFW than the intolerant average. However, at high P, the average RFW of tolerant strains was 17% less than that of intolerant strains. This evidence complements other findings (7) in supporting the hypothesis that nutritionally efficient genotypes may be at a selective disadvantage when breeding proceeds under nutritionally nonlimiting conditions (10).

The greater RFWs of intolerant strains at high P were due to more efficient internal use of P (Table 2). Internal P use ratios were consistently about 25% higher for intolerant strains than for tolerant strains at high P. Total P uptake of tolerant and intolerant strains did not differ at high P.

Differences in RFW production between intolerant and tolerant strains at low P were due primarily to differences in P absorption. At low P, tolerant strains acquired 10% more P on the average than intolerant strains. Differences in IPURs were less consistent between tolerant and intolerant strains at low P than at high P.

The relative contributions of P use and P uptake efficiencies to low-P tolerance were different among strains, as reported previously (5). At low P, the most low-P intolerant strain 12 exhibited inefficient P uptake and inefficient internal P use (Table 2). In contrast, the relatively poor growth of strain 2 at low P was due entirely to poor P uptake, as the internal use efficiency of strain 2 at low P was among the highest of all strains. The superior performance of strain 43 at low P was due to an uniquely high efficiency in P uptake and to an excellent P use efficiency relative to that of the other strains.

The high efficiency in P uptake of strain 43 may be related to several other of its characteristics. The average pH of the culture solutions of strain 43 was significantly lower than that of any other strain (Table 2). Acidification of the rhizosphere has been shown to increase P availability greatly in soil by solubilizing soil P (9). It is questionable whether this mechanism would be useful in root culture, because all the P in the medium is added in soluble form. Phosphorus deficiency stress generally reduced root hair length of all strains, but strain 43 was among those strains least affected, producing roots densely covered with comparatively long root hairs at high and low P. The tolerant strains had significantly denser and longer root hairs overall at low P than the intolerant strains.

Recent efforts to recombine superior P uptake and P use efficiencies genetically in tomato strains differentially tolerant to P deficiency in silica sand–alumina culture (4) resulted in some progeny that grew as well with greatly limited P as did their parental strains with nonlimiting P (7). The present studies have shown that strain differences in P uptake, P use, root hairs, and medium pH modification can be identified in root cultures. However, comparisons of strain responses to P deficiency in root culture and low-P soils still are needed to determine whether the kinds of differences revealed in root culture are related to adaptation to low P tolerance in soil. A previous study suggested that kinetic analysis of short-term P uptake rates with excised roots may predict long-term P accumulation rates in intact plants reasonably well (6), although poor correlations of P uptake between excised roots and intact plants also have been found (11).

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Stimulating Productivity of Hydroponic Lettuce in Controlled Environments with Triacontanol

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Abstract. **Triacontanol (1-triacontanol) applied as a foliar spray at 10^{-7} M to 4-day-old, hydroponically grown leaf lettuce (*Lactuca sativa* L.) seedlings in a controlled environment increased leaf fresh and dry weight 13% to 20% and root fresh and dry weight 13% to 24% 6 days after application, relative to plants sprayed with water. When applied at 8 as well as 4 days after seeding, triacontanol increased plant fresh and dry weight, leaf area, and mean relative growth rate 12% to 37%. There was no benefit of repeating application of triacontanol in terms of leaf dry weight gain.**

Triacontanol (TRIA), a naturally occurring 30-carbon primary alcohol, has been found to increase yield of some crops (4, 8–11). However, effects of TRIA often have been inconsistent. For example, yield increases were not reported for maize (3), wheat (2), and some other crops (6). One reason for this inconsistency may involve formulation (9, 12). Since TRIA is almost insoluble in water, researchers have used detergents or surfactants to emulsify TRIA in effort to keep it in suspension (3, 10). Recently, an aqueous, colloidally dispersed formulation was developed that gives more consistent results than suspensions in chloroform–Tween 20 or acetone–NAA (9).

The biomass production group of the National Aeronautics and Space Administration Controlled Ecological Life Support System (CELSS) program seeks to define optimum growth requirements for crops to satisfy human nutritional requirements, waste recycling, and air revitalization for long-term space habitation (13). Research in our laboratory emphasizes optimizing productivity of high-yielding leaf lettuce cultivars as a model crop to supply minerals, vitamins, and fiber for a CELSS vegetarian diet in the shortest time and smallest growing space possible. The present study was conducted to investigate the potential of TRIA to enhance lettuce yield in an optimizing environment of CO_2 enrichment and radiation enhancement.

Seeds of 'Waldmann's Green', a high-yielding lettuce cultivar, were germinated and grown in nutriculture systems located within two enlarged and improved chambers of the Minitron system (1). Seeds were sown onto a cloth-lined trough wetted with half-strength modified Hoagland's No. 1 nutrient solution (5). Four days after sowing, seedlings were transplanted to individual holders within Minitron chambers. Plants were grown for the first 4 days in half-strength nutrient solution at pH 6.0, followed by a shift to single-strength solution containing double-strength N as 5 mM NH_4^+ + 25 mM NO_3^-. Photosynthetic photon flux (PPF) was 430 ± 5 $\mu mol \cdot s^{-1} \cdot m^{-2}$ (LI-COR, model LI-1800) at the top of the leaf canopy until 11 days from seeding, when it was increased to 900 ± 10 $\mu mol \cdot s^{-1} \cdot m^{-2}$. The lower PPF was achieved by 84% input wattage from cool-white fluorescent lamps and 16% from frosted incandescent lamps in the environmental room housing the transparent Minitron chambers. High PPF was provided by a bank of nine 150-W parabolic reflector flood lamps mounted above each chamber, in addition to the fluorescent + incandescent lamps on the ceiling of the growth room. A water/plexiglas barrier was placed between the lamp banks and each Minitron chamber. Photoperiod was 20 hr on a 24-hr cycle. Relative humidity was 75% ± 5% during the light cycle and 85% ± 5% during darkness. Shoot and rhizosphere temperatures were maintained at 25° ± 0.5°C day and night. Air flow rate through root ($4.9 liter \cdot min^{-1}$) and shoot compartments ($8.2 liters \cdot min^{-1}$), as well as CO_2 injection into the shoot atmosphere mixing chamber, were maintained with rotameters (Matheson, models 603, 604, and 610, respectively). Atmosphere flowing through the chambers contained ambient levels of CO_2 until 11 days from seeding. Atmospheric CO_2 was maintained at 69 ± 2 $mmol \cdot m^{-3}$ ($1500 \mu l \cdot liter^{-1}$) from 11 to 19 days of growth. Carbon dioxide in the flowing atmosphere was monitored with a non-dispersive infrared gas analyzer (Horiba, model PIR-2000).

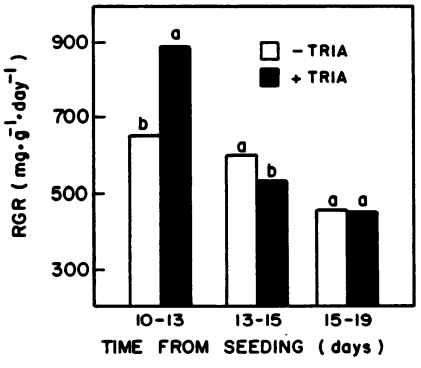

Fig. 1. Effect of 10^{-7} M triacontanol (TRIA) sprayed at days 4 and 8 after seeding on relative growth rate (RGR) of lettuce from 10 to 19 days of growth. Plants were grown at a PPF of 900 ± 10 $\mu mol \cdot s^{-1} \cdot m^{-2}$ and 69 ± 2 $mmol \cdot m^{-3}$ CO_2 from 10 to 19 days after seeding.

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