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Phosphorus Use Efficiency in Anthocyanin-free Tomato (*Lycopersicon esculentum* Mill.)

Lee, Dong-Hee

Department of Life Science, The University of Seoul, 90 Jeonnong-Dong, Dongdaemun-Gu, Seoul, Korea

An anthocyanin-free tomato plant, H957, and its parental wild type, H883, were hydroponically grown to test for tolerance to a low phosphorus (P) in H957. The tolerance was evaluated by comparing growth and metabolism of H957 vs. H883 at different P concentrations ranging 25~400 μM. Fresh weights were measured weekly. Dry weight, mineral contents, photosynthetic rate, and P utilization ratios of the plants were measured after five weeks of growth in the hydroponic culture. Although the growth of both varieties was severely impaired at 25 μM P, H957 showed a greater fresh weight and dry weight at 50~400 μM P. H957 showed a higher net photosynthetic rate on older leaves while both varieties showed similar photosynthetic rate on young leaves. H957 tissue contains an overall lower P concentration in its tissue than H883. These observations together indicate that the anthocyaninless mutant H957 tolerate to lower P concentration. It does so by utilizing internal P with better efficiency rather than by absorbing external P better.

Keywords: P utilization efficiency, P-deficiency, anthocyanin-free, tomato, hydroponics

INTRODUCTION

Phosphorus (P) is often a widespread limiting factor in soils. Under P-deficiency, plants show retarded leaf expansion, premature leaf fall, and purplish (anthocyanin) pigmentation (Bot *et al.*, 1994; Mimura *et al.*, 1990; Lee *et al.*, 1990). These symptoms resemble those of nitrogen deficiency; however, P-deficient plants exhibit a characteristic dark green coloration on the leaves and necrotic areas on the leaves, petioles, or fruits; often, overall maturity is significantly delayed (Gerloff, 1987; Marschner, 1995). Due to a higher mobility of P through phloem, the P-deficient symptoms develop first in older leaves (Salisbury and Ross, 1994; Qiu and Israel, 1992; Marschner, 1995).

Plants require frequent supplementation of phosphate. When not absorbed by plants, phosphates eventually contaminate lakes and streams. High phosphate levels cause major disturbances (eutrophication) in the aquatic ecological balance (Larcher, 1995). Furthermore, the increasing price of commercial fertilizer deteriorates farmers' benefit per cost ratio. To cope with such problems, a principal strategy might be to develop P-efficient cultivars which acquire P from the environment or utilize internal P more efficiently (Blum, 1988; Coltman, 1987; Theodorou and Plaxton, 1993). These potential P-efficient cultivars may require less application of P-containing fertilizers, thus reducing chances for P contamination in the environment and helping crop growers keep costs down.

An anthocyanin-free mutant of tomato, Heinz variety 957 (H957), sustains a normal morphology under low P supply. Unlike its parental wild type, Heinz variety 883 (H883), H957 does not develop any purplish coloration, necrotic areas on leaves, nor other typical P deficiency symptoms under low P conditions. This visual tolerance may be an indication for unusual P utilization efficiency in H957.

To test for the potential high P utilization efficiency in H957, two cultivars (H883 and H957) were hydroponically grown at different levels of P concentration. Their performances were compared in terms of visual appearance, fresh weight, dry weight, photosynthetic rate, tissue P concentration, and P utilization ratio.

*Corresponding author: Fax +82-2-210-2950

MATERIALS AND METHODS

Seeds of H957 and H883 were planted in a 1:1 mixture of vermiculite and perlite, and watered as necessary with deionized water. Early in the four-leaf stage, the seedlings were transplanted to hydroponic pots. The culture media were prepared according to Meyer *et al.* (1973). Six levels of P concentrations were fixed by loading KH_2PO_4 at 400 μM, 200 μM, 100 μM, 50 μM, 25 μM, and 0 μM. The shortage of K due to less KH_2PO_4 was compensated by adding KCl in the culture media. The nutrient solution was changed weekly.

The hydroponic culture systems were maintained in constant environment growth chambers. The growth chambers were set at 32°C (day) and 24°C (night) under 16 hour photoperiods with light intensity of 2400 μE m^{-2}. Aeration of the hydroponic solution was omitted since preliminary investigations revealed that it was not necessary. Water lost from the hydroponic pots, due to transpiration and natural evaporation, was replaced as necessary to insure that reduction in water volume did not exceed 10%.

The fresh weight was measured weekly. The initiation of flower buds or flowers was recorded daily. Flower buds were counted at the termination of each experiment. Photosynthetic rate was measured using a LI-6200 Potable Photosynthetic System. For these measurements, three plants each from the two varieties and the different P concentrations were randomly chosen. Sampling was made on a younger leaf (the first leaf from apex) and an older leaf (one at the fifth nodes from the base). Also, measurement of the rates of dark respiration and transpiration were made on the same leaves. Leaf areas, necessary for calculating the photosynthetic rate, was obtained by employing a Bausch & Lomb Optical Area Meter.

For anthocyanin quantification, two plants whose fresh weight ranked near the median were selected from each group. Samples were prepared according to Rengel and Kordan (1987). Leaf tissue was homogenized in Corex tubes containing acidified methanol and placed in the dark at 4°C for 30 hours. They were centrifuged at 1200 g for 20 minutes. Absorbancy (A) was calculated based on the formula ($A=A_{530}-0.25\times A_{657}$).

Dry weight of leaves and root-stem complex were individually measured and summed for whole-plant weight. After the measurement, mineral content per root-stem complex and leaves was determined by employing a SOLAAR 969 Atomic Absorption Spectrometer.

RESULTS

When both varieties were subjected to low P conditions, H957 appeared to perform better than H883, its parental wild type. H957 maintained a healthier appearance than H883 at low P levels. When grown at 50 μM P, H883 showed dark green or purplish pigmentation on the upper or the lower leaf surfaces (Figure 1A). Most of H883's leaves underwent significant curling or wrinkling at the lower P concentrations. On lower leaves of H883, severe necrosis first appeared along the margin and, later, spread over all leaflets. At 25 μM P where both varieties showed a stunted growth, H957 still sustained a healthier appearance than H883 (Figure 1B). Anthocyanin concentration increased in H883 as the P supply was reduced in the culture solution; in H957, no change was observed from that of 400 μM (Figure 1C). The P-deficiency appeared to delay the maturity of the two tomato plants. The onset of flower bud setting and anthesis, however, was less affected for H957 (Figure 1D). These data suggest that H957 could better maintain its normal metabolism under low P availability.

H957's Growth Tolerant to Minimal P-supply

The P concentration was shown to have a distinctive impact on the rate of fresh weight increase of both varieties. As the P concentration decreased in the nutrient solution, each variety showed a consistent reduction in fresh weight (FW) (Figure 2A). Significant differences between the two varieties were observed in FW at concentrations ranging from 25 to 400 μM after 4 weeks of hydroponic culture: H957 demonstrated greater FW than H883. It was noted that H957's FW at 25 μM was comparable to H883's FW at 50 μM P. Data analysis on FW at P-free condition was omitted due to insufficiency of measurements because most seedlings died early during the study.

The pattern characteristic in FW comparison was also observed when the dry weight (DW) was compared. Throughout the P concentrations range of 25 to 400 μM P, H957 exceeded H883 in DW at the equivalent P-supply in the culture media. The greatest DW difference was observed at 400 μM P, where H957 had an increase 175.2% more DW than H883. The smallest difference was at 25 μM P when H957 had 13.1% greater DW than H883. DW comparison at 0 μM P was eliminated due to insufficiency of the measurements.

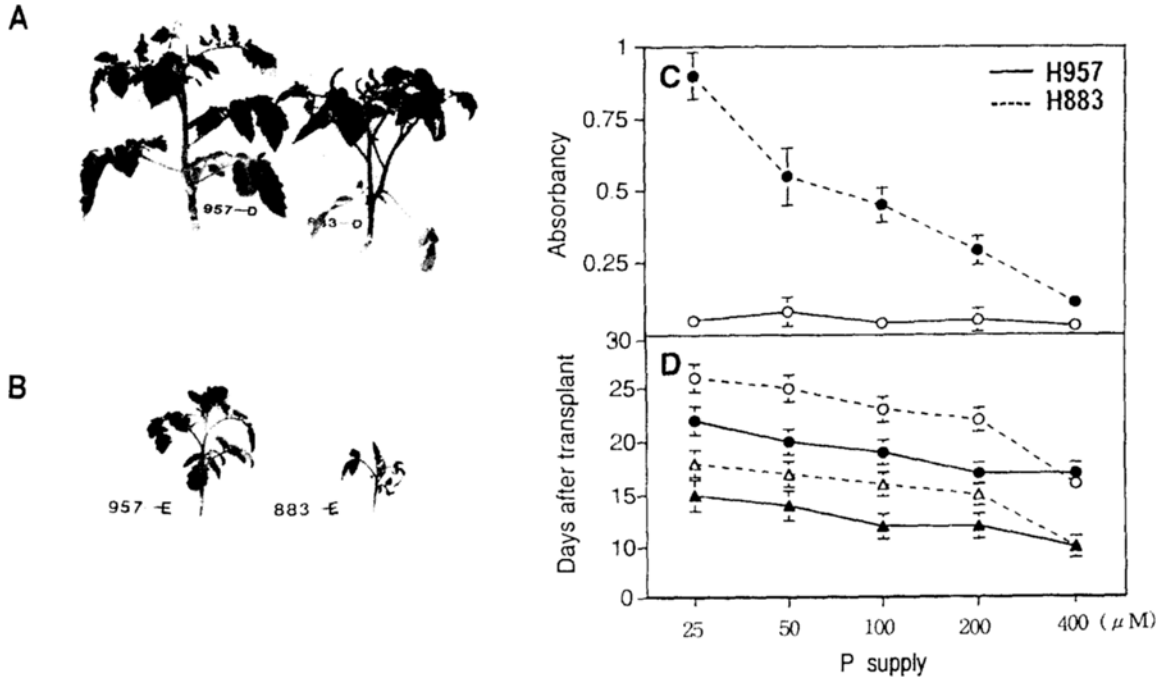

Fig. 1. Visual comparison was made between H883 and H957 grown for 4 weeks. At 50 μM P, H883 showed typical P-deficiency symptoms: dark green or purplish pigmentation on the upper leaves, severe necroses along the margin of old leaves. H957 showed a relatively milder P-deficiency symptom. -D refers to the growth at 50 μM P. (B) At 25 μM P, both varieties underwent a significant reduction in size; however, H957 showed a more normal appearance. -E refers to the growth at 25 μM P. (C) Anthocyanins were measured on two plants whose fresh weight ranked near the median for their group. Leaf tissue was sampled by area. Samples were homogenized in 10 ml of methanol acidified with 2N HCl and the absorbance of anthocyanins was calculated: $A = A_{530} - 0.25 \times A_{657}$). (D) The two varieties were compared for the days taken until budding (circles) and anthesis (triangles). Days after transplant refer to the periods of hydroponic culture spent until budding or anthesis.

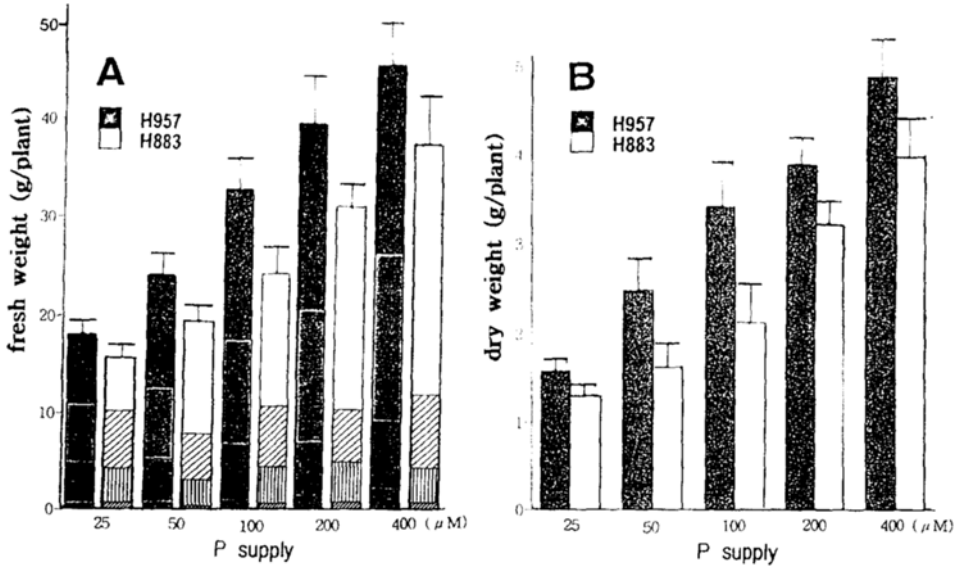

Fig. 2. FW and DW comparison. (A) Throughout 4 week hydroponics culture, fresh weight was measured from 10 plants weekly. Whole bar represent total FW gained during the hydroponic culture. Each block refers to weekly FW increase. Error bars represent standard error (SE) of means for the total fresh weight increase. (B) At the termination of culture, dry weight was measured following an overnight dehydration in a forced circulation chamber. Error bars represent SE of means for the dry weight.

The response was expressed as the absolute slope from the plot of fresh (dry) weight vs. logarithmically transformed P concentration between 50 μM and 400 μM P. H957 had a greater response to the increase in P supply than H883: 3.2 vs. 1.1 for FW and 1.6 vs. 0.5 for DW. The 25 μM P point was not included to determine the P-responsiveness of either variety since H883's growth was severely impaired at the P concentration. When FW and DW were further analyzed among all P concentrations, the need for less P in H957 was clearly shown. H957 grown at 100 μM P showed a fresh weight gain equivalent to that of H883 grown in a solution with 400 μM P. In terms of dry matter accumulation, H 957's performance at 100 μM surpassed any of H 883's at 100~400 μM P. This shift suggests that H 957 has greater dry matter content per unit of fresh weight compared to H883.

Higher Photosynthesis in H957's Old Leaf Under Minimal P Supply

When net photosynthetic rate (NPR) was determined on single leaflets, the diminishing P-supply was shown to give negative impacts on photosynthesis. With decreasing P concentration in culture media, the NPR decline commonly for H957 and H883. Figure 3 shows that the extent of decrease, however, varies according to the leaf age and variety. In young leaves, the diminishing P-supply reduce the photosynthesis both for H957 and H883; no statistical difference was apparent between the two varieties. In old leaves, photosynthetic activity declines more rapidly than young leaves at the two minimal P-supply (25 and 50 μM P). The response to the P-deficiency was greatly varied between H957 and H883. Comparing to P-sufficient old leaves, pho-

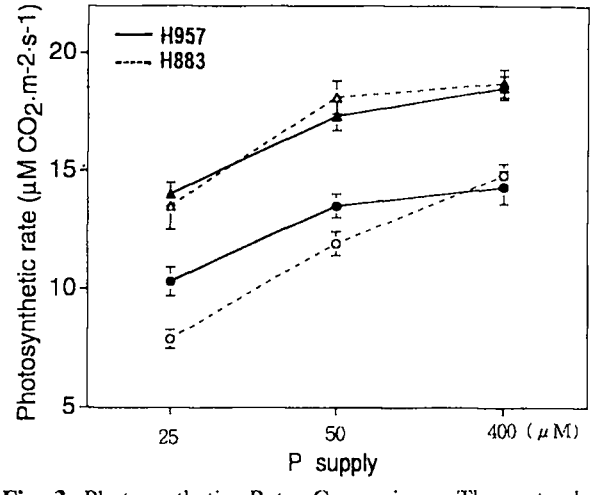

Fig. 3. Photosynthetic Rate Comparison. The net photosynthetic rate (NPR) were determined by measuring net CO_2 exchange rate with a Li-Cor photosynthesis measuring system (Model LI-6200) at the end of culture. Three plants were randomly chosen each for 25 μM, 50 μM, and 400 μM P. NPR was measured on the first leaf from the apex for young leaf (triangles). Older leaf's NPR was measured on one at the second node from the base (circles). For standardization of the NPR, leaf area was measured by a Bausch & Lomb Optical Area Meter.

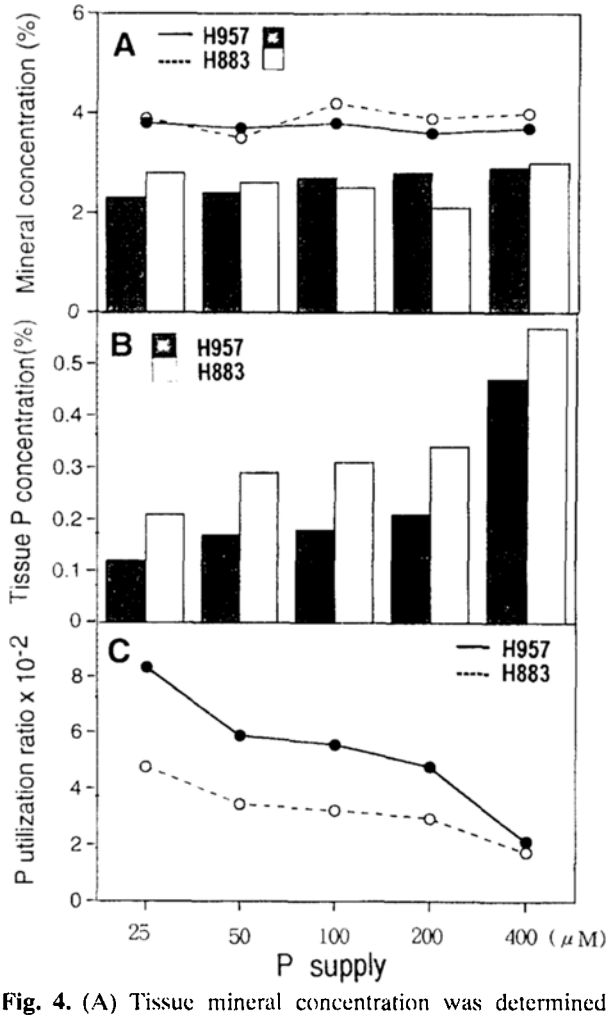

Fig. 4. (A) Tissue mineral concentration was determined by ash analysis on the pool of three plants whose dry weight reside at or near the median. Tissue N concentration was compared with lines and K concentration was compared with bars. (B) Tissue P concentration was determined as in 4-A. Throughout the six different solution P concentration, H957's tissue P concentration was considerably lower than H883. (C) P utilization ratio (PUR) represents the ratio of mg dry weight per mg tissue P. PUR was obtained by calculating reciprocals of tissue P concentration.

tosynthesis is seriously impaired in H883's old leaves under the P-deficient condition. Yet, H957's old leaves exhibited a significant NPR in the presence of minimal P-supply. These results indicate that H957 can better tolerate to a reduced tissue P concentration in old leaves, due to rapid mobilization of internal P to young leaves, than H883 (Goldstein *et al.*, 1988; Smith *et al.*, 1990). With regard to the rates of transpiration and stomatal conductance, neither variety showed statistically significant differences (data not shown).

Lower Tissue P Content in H957

Determination of tissue P content by ash analysis revealed that tissue P concentration was closely correlated with the loading P concentration: For both varieties, correlation coefficients ranged from 0.96 to 0.99 between tissue P concentration percentage (w/w) and logarithmically transformed culture solution P concentration. At each loading P concentration, both varieties showed similar N or K tissue contents (Figure 4A). At each nutrient solution P concentration, H957 had approximately one third lower P content than H883 (Figure 4B). The lower tissue P concentration observed in H957 showed that its better performance at equal exogenous P concentration was not the simple expression of its higher tissue P concentration. Rather, the lower tissue P concentration of H957 is indicative of its tolerance to lower internal P concentrations compared to H883. In terms of phosphorus utilization ratio (PUR), H957 exceeded H883 at the all loading P concentrations in this study (Figure 4C).

DISCUSSION

This study demonstrates that increased phosphorus utilization efficiency exists in the anthocyaninless *Lycopersicon esculentum* Mill. H957. When the phosphorus supply was reduced to deficient levels in the hydroponic solution, H957 performed better than H883, its wild type counterpart. H957 maintained healthier appearance than H883 at deficient P levels. The onsets of flower bud setting and flowering were less delayed for H957. The numbers of the flower buds and subsequent flowers were also less affected. These visual evidences suggest that H957 could better maintain its normal metabolism under low P availability.

The need for less P in H957 was clearly shown when comparing fresh weight increase and dry matter accumulation. At equal solution P concentration, H957 exceeded H883. Furthermore, H957 grown at 100 μM showed a fresh weight gain equivalent to that of H883 grown in a solution with 400 μM P. In terms of dry weight, H957 accumulated approximately the same dry matter at 50 μM P as H883 did at 400 μM P. This shift suggests that H957 has greater dry content per unit of fresh weight compared to H883.

Two different types of response were evident when the FW or DW changes were compared at increasingly higher nutrient solution P concentrations. H957 gained much more fresh and dry weight than H883 with an incremental P increase starting at 50 μM P. Based upon its responsiveness, H957 can be considered as a "Responder" to changes in P concentration in the hydroponic culture solution. "Responder" is a term used for a line of tomato varieties which show higher response in dry weight increase to the unit of nutrient added (O'Sullivan *et al.*, 1974).

The lower tissue P concentration observed in H957 showed that its better performance at equal exogenous P concentration was not the simple expression of its higher tissue P concentration. Instead, the lower tissue P concentration strongly suggests that H957 could grow and function more efficiently per unit of P absorbed from the nutrient medium and could do so even at lower internal P concentration. Also, H 957 required a lower external P concentration for normal growth than did H883. This was similarly concluded by O'Sullivan *et al.* (1974) pertaining to the relationship between N efficient tomato lines and their lower N tissue content. Consistent with O'Sullivan's observations, H957 showed less severe P deficiency symptoms.

The P utilization efficiency, as shown by analyzing the tissue P concentration data, in H957, was also demonstrated in a companion study (data not shown). Three-week old seedlings from both varieties, developed under the same growth condition, were subjected to P starvation by transferring those seedlings to a P-free nutrient solution. When this occurred, H883 developed severe necrosis over the lower leaves within four days, whereas these symptoms did not appear in H957 until 10 days after the transfer. Recognizing that P may be readily mobilized and translocated from older leaves to younger leaves during P stress, this observation implies that H957 is more tolerant than H883 to the dilution, due to growth, of its internal P in the absence of an external P supply (Clarkson, 1984). From observing H957's healthier appearance even at 25 μM P, it can

be concluded that H957 has the capacity for normal metabolism at reduced tissue P concentrations.

When seedlings similar to those used in the P starvation experiment were cultured in unaerated hydroponic pots with elevated P concentrations of 1.6, 3.2, and 4.8 mM P, all seedlings of H883's maintained normal appearance and growth rate to maturity. Seedlings from H957, however, showed reduced growth compared to H883 at the same level of P concentration. Moreover, all of H957's seedlings at 4.8 mM P withered and died within three days. This observation suggests that H957's P utilization efficiency may play a negative role when P levels are very high. No work is known to have been published on the relationship between low P-efficiency and tolerance to high P levels.

Statistical analysis on the photosynthetic data did not show a significant difference in actively growing leaves between the two varieties. This suggests that the high mobility of P in the plants enabled the leaves to maintain photosynthetic activity at low P levels. A negative correlation was shown between the photosynthetic rate and leaf area both in P-deficient and sufficient plants (Chapin and Wardlaw, 1988; Fredeen *et al.*, 1990; Heldt *et al.*, 1991). Accordingly, the overall negative correlation between the photosynthetic rate and nutrient P concentration can be explained in line with Chapin and Wardlaw's assertion, seeing that leaf area was considerably reduced in this study for both varieties at deficient P levels.

Little is known about a low-mineral-tolerant (P or otherwise) cultivar's performance at its nonstress concentration (Blum, 1988). By analyzing a wide range of P concentrations (optimum, nonstress, and stress), this study suggests that a low-P tolerant variety (H 957) can also perform better at a nonstress P concentration than a non-tolerant variety.

The terminology of mineral efficiency is confusingly used: one for tolerance to low nutrient supply, and another for input-output productivity (Blum, 1988; Larcher, 1995). The consistent results observed, in this study, for both aspects (tolerance and high productivity) implies the concepts of tolerance and productivity in P-nutrition can be unified under a term P-effectiveness for plant growth. H957 showed sufficiently greater performance, even with lower tissue P concentration; in addition, its tolerance continued until its growth was impaired by extremely low P-levels. Therefore, H957 can be referred to as P-effective strain.

H957 and H883 likely share identical genetic backgrounds except for anthocyanin biosynthesis; the elevated P utilization efficiency might be an outcome of attenuated anthocyanin biosynthesis in H 957. Altered regulatory apparatus controlling anthocyanin biosynthesis may exert pleiotropic effects on P utilization in H957. Further, a novel gene locus might exist to regulate P-utilization efficiency in tomato plants and have been altered along with the anthocyanin mutation in H957. In any case, biochemical and molecular studies are necessary to investigate how the attenuation of anthocyanin biosynthesis coincides with the enhancement of P utilization efficiency in H957 which was clearly shown in this study. Currently, H957's tolerance to low P is being characterized on minimal P culture media by plant tissue culture procedures. Its performance will be very crucial to determine whether its potential low P tolerance results from cellular strength in the presence of low P supply.

Cultivars that can grow in low P conditions will be of great use to farmers that want to use less fertilizer for environmental and commercial reasons. Although this present study is mainly focused on vegetative growth, H957 did show sufficiently greater performance, even with lower tissue P concentration. Its tolerance continued until its growth was impaired by extremely low P-levels. It does so by utilizing internal P with better efficiency rather than by absorbing external P better.

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