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조직 배양을 이용한 안토시아닌 결핍 돌연변이 토마토의 저인 내성 평가

이동희[1] · 배공영[2] · 박소홍[2]
[1]서울시립대학교 문리과대학 생명과학과, [2]서울시립대학교 문리과 대학 환경원예학과

Characterization of Low-phosphorus Tolerance in an Anthocyanin-deficient *Lycopersicon esculenturn* by tissue culture

Dong-Hee Lee[1], Gong-Young Bae[2] and So-Hong Park[2] *(Department of Life Science, The University of Seoul, 90 Jeonnong-Dong Dongdaemun-Gu, Seoul, Korea)*

ABSTRACT : An anthocyanin-deficient tomato (*Lycopersicon esculentum* Mill.) strain, H957, shows an unusual tolerance to low phosphorus (P). To investigate whether the tolerance originates from a tissue/cellular strength, plant tissue culture procedure was employed which facilitate to characterize the tolerance independent of morphological features. The tolerance was analyzed by comparing H957 against H883, its maternal wild type, while each explant was co-cultured on minimal P media. Comparisons were made in fresh weight, dry weight, callus and shoot formation, mineral contents, and P utilization ratios at 0-400 •ÏM P. Growth of the two strains was severely impaired at 0 and 12.5 •ÏM P. At 25-200 •ÏM P, however, H957 consistently showed a greater fresh and dry weight than H883. Shoot onset of H957 was less delayed than H883 compared to optimal P conditions. H957 tissue contains an overall lower P concentration than H883. These observations indicate that H957 may tolerate to low P by its tissue or cellular strength in P utilization aside from its morphology.

Key words : low-P tolerance, P-deficiency, anthocyanin-deficient tomato

Introduction

Crops require frequent application of phosphorus (P)-containing fertilizers in soil. Otherwise, P-deficient plants show retarded leaf expansion, premature leaf fall, and purplish (anthocyanin) pigmentation[1,2,3,22]. P-deficient plants also exhibit delayed maturity[4,5]. Following supplementation of P fertilizer in soil, however, excess phosphates are readily leached from soil and eventually contaminate water resources. Indeed, high phosphate levels cause a major disturbance of eutrophication in the aquatic ecological balance[6,12]. Increasing price of commercial fertilizer deteriorates farmers' benefit per cost ratio.

To cope with problems stemming from frequent application of P fertilizer, a principal strategy might be to develop low-P tolerant (LPT) strains which has strength in acquiring P from the environment or using internal P more efficiently[7,10,11]. These LPT strains 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-deficient tomato strain, Heinz variety 957 (H957), sustains a normal morphology under low P supply[10,11]. Unlike its maternal wild type, Heinz variety 883 (H883), H957 does not develop any necrotic areas on leaves, nor other typical P-deficiency symptoms under low P conditions.

To characterize whether the LPT traits in H957 stems from a tissue/cellular strength, this study utilize plant tissue culture procedures. This procedure helps characterizing H957's potential LPT traits irrespective of the plant morphology such as root and shoot configurations[13,14,15]. At low media P concentrations, explants of H957 were co-cultured with those of H883. Their performances were compared in terms of fresh weight, dry weight, callus and shoot formation, and tissue P concentration[16,17].

Materials and Methods

Plant material

Seeds of H957 and H883 were planted in a 1:1 mixture of vermiculite and perlite, and watered as necessary with deionized water. Explants were excised from leaves of seedlings at 4-5 leaf stage. Leaf tissue was sterilized in 10% sodium hypochlorite solution for 10 minutes and soaked in 95% ethanol for 30 seconds. The sterilized tissue was rinsed with deionized water.

Media preparation and Culture condition

The culture media were prepared according to Murashige and Skoog (MS) media[19]. To fix seven levels of P concentration, potassium phosphate (KH_2PO_4) was added to the media at 400, 200, 100, 50, 25, 12.5 and 0 μM. The original MS medium contains KH_2PO_4 at 1300 μM; however, points between 1300 and 400 μM P were omitted since preliminary data indicated no difference in overall tissue growth. The shortage of potassium due to less KH_2PO_4 was compensated by adding KCl in the culture media.

Explants were cultured for 2 weeks on an Evans media containing 3% sucrose, 0.4 mg thiamine HCl, 100 mg myo-inosiol, 0.5 mg kinetin, and 2.0 mg naphtaleneacetic acid (NAA) in 1 L of MS solution before transfer to a shoot inducing media including 0.1 mg NAA and 0.3 mg zeatin in 1 L of Evans media. Following one week culture on the zeatin media, the explants were placed on the basal media. The culture plates were placed at 25℃ under 16 hour photoperiods with light intensity of 1000 μE $m^{-2}s^{-1}$.

Measurements of explants

The initiation of callus and shoot formation was recorded daily during tissue culture. The number of shoot was counted under a colony counting magnifier (10X). The total number of shoot was summed at the termination of the culture. For each tissue type at the different P concentrations, fresh weight per explant was obtained from explants whose excess agar and moisture were removed against toweling. The fifty disks were dried in a forced circulation oven for five days to determine dry weight per disk. After the dry weight measurement, mineral content was determined by employing a SOLAAR 969 Atomic Absorption Spectrometer at the A&L Great Lake Laboratories (Fortwayne, Indiana, USA). The P utilization ratio was obtained by calculating the reciprocal of the tissue P concentration percentage according to Gerloff[9].

Results

The present study utilize plant tissue culture procedures to characterize the LPT traits in H957. The plant tissue culture provide a unique opportunity to evaluate the LPT traits regardless of whole plant features which affect P acquisition from the environment (root structure), P movement across root to xylem, and P distribution and mobilization in shoot[4]. Using tissue culture techniques, the LPT traits of H957 were assessed exclusively at the tissue or cellular level.

H957 surpassed H883 in FW and DW at minimal P supply

The P concentration was shown to have a distinctive impact on

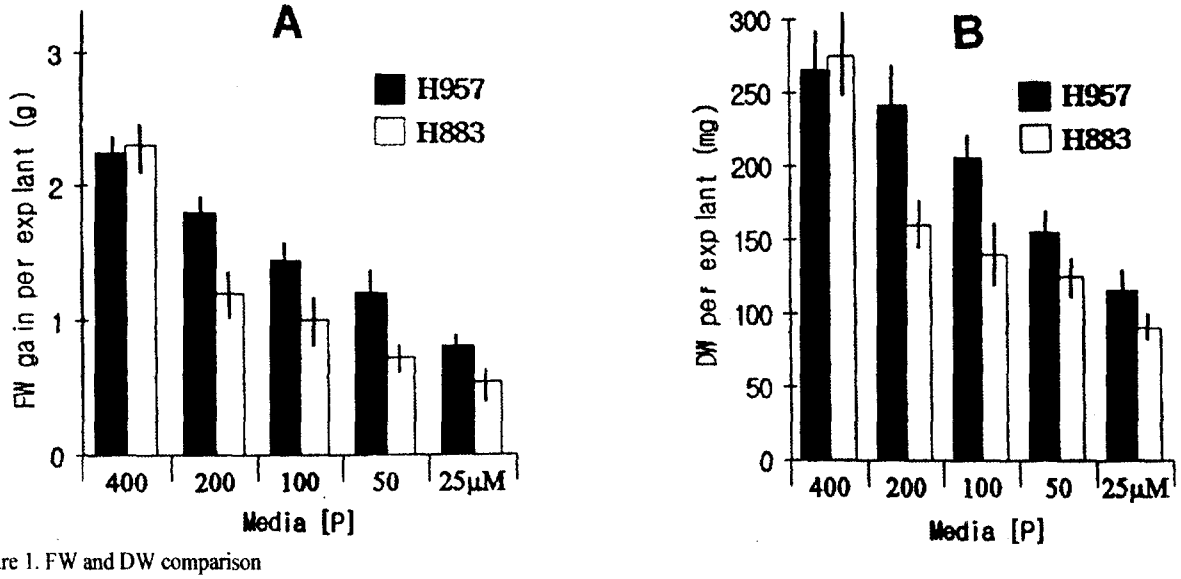

Figure 1. FW and DW comparison

(A) Fresh weight (FW) was measured at the end of tissue culture (N=100). Media [P] indicates the original P concentration in the culture media. Dark/light bars represent H957/H883. Error bars refer to the standard error. (B) Dry weight was measured from the same explants following a dehydration in a forced circulation chamber.

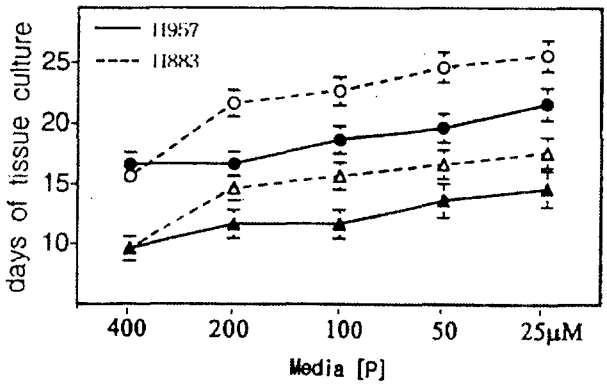

Figure 2. Comparison on callus and shoot onset period. The two strains were compared in terms of period until callus and shoot formed on the explants. The duration up to the onset was expressed as in days of tissue culture until callus (triangle) and shoot (circle) formed. Error bar represents the standard error.

the rate of fresh weight increase of both strain. As the P concentration decreased in the culture medium, each strain showed a consistent reduction in fresh weight (FW) (Figure 1-A). Significant differences between the two strains were observed in FW at concentrations ranging from 50 to 400 μM after 4 weeks of tissue culture: H957 demonstrated greater FW than H883. It was noted that H957's FW at 50 μM was comparable to H883's FW at 100 μM P. Data analysis on FW at P-free condition was omitted due to insufficiency of measurements because most explants lost viability early during the study.

When the dry weight (DW) was compared, the pattern characteristic in FW comparison was also observed. H957 exceeded H883 in DW at the equivalent P-supply in the culture media, throughout the P concentration range of 25-200μM P. The greatest DW difference was observed at 200μM P, where H957 had an increase 45% more DW than H883. The smallest difference was at 50μM P when H957 had 15.1% greater DW than H883. The DW comparison at 0μM P was eliminated due to insufficiency of the measurements.

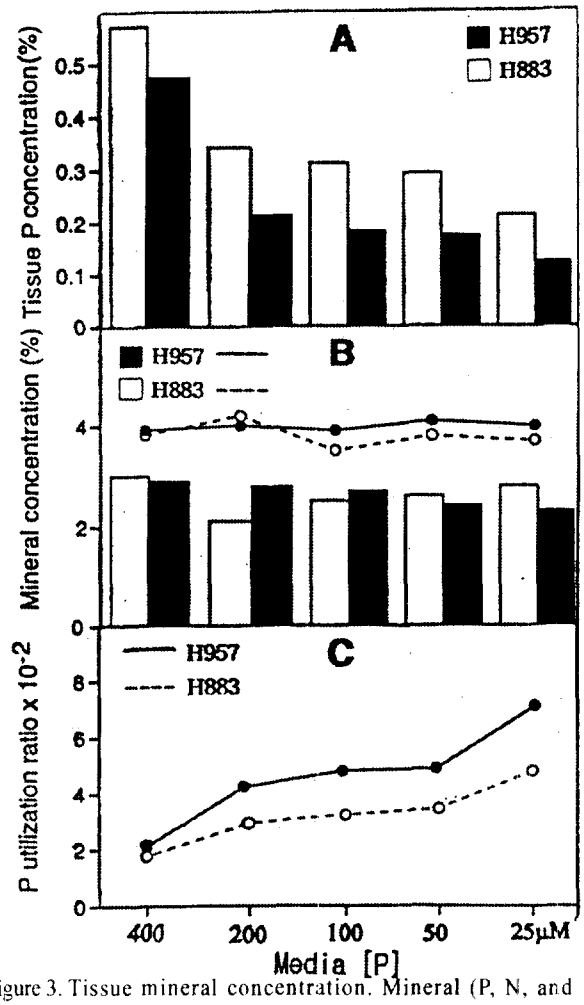

Figure 3. Tissue mineral concentration. Mineral (P, N, and K) concentration was determined by ash analysis on the pool of five leaf disks per each P concentration. (A) Tissue P concentration was compared between the two strains. H957's tissue showed substantially lower P concentration than H883. (B) Different from tissue P concentration, N and K concentration did not show significant difference between the two strains. N concentration was compared in lines while K concentration was in bars. (C) PUR (P utilization ratio) was compared when the tissue P concentration percentage was transformed into the reciprocals as described in Materials and Methods.

H957's less sensitivity to decreasing P supply

The response was expressed as the absolute slope from the plot of fresh (dry) weight vs. logarithmically transformed P concentration between 50μM and 400μM P. H957 was less sensitive to the decrease in P supply than H883: 1.1 vs. 3.0 for FW and 1.6 vs. 4.5 for DW. The 12.5μM P point was not included to determine the P-responsiveness of either strain since H883's growth was severely impaired at the P concentration.

The low P conditions interfered with the callus and shoot formations of the two strains. H957, however, was less affected than H883 compared to 400μM P in the callus onset (Figure 2). With decreasing media P concentration, H957 spent less time (days) until the onset of callus formation than H883. Significant difference was also observed between the two strains with respect to the onset of shoot formation. The extent of difference between the strains is widened in the shoot formation and H957's shoot formation was less affected than H883. These data together suggest that H957 could better form callus and shoot under the low P availability than H883, its parental wild type control.

Lower P tissue concentration 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 strains, correlation coefficients ranged from 0.85 to 0.91 between tissue P concentration and logarithmically transformed culture media P concentration. At each media P concentration, H957 had approximately one third lower P content than H883 (Figure 3-A). In terms of N or K tissue contents, however, both strains showed similar values at each media P concentration (Figure 3-B). When the tissue P concentration percentage was presented as P utilization ratio, H957's better performance is clearly shown compared to H883 (Figure 3-C). Together, the lower tissue P concentration observed in H957 showed that its better performance at equivalent media P concentration was not the simple expression of its higher tissue P concentration. The lower tissue P concentration rather suggests that H957 could tolerate to lower internal P concentrations by means of better P utilization efficiency residing in the tissue or cell.

Discussion

This study demonstrates that H957's tissue can tolerate to limited P supply during tissue culture. When the P supply was reduced to deficient levels in the tissue culture media, H957 performed better than H883, its wild type counterpart. H957 maintained healthier appearance than H883 at deficient P levels. The onsets of shoot formation were less delayed for H957. The numbers of the multiple shoot were also less affected. These evidences suggest that H957 was less affected by low P availability.

The need for less P in H957 was clearly shown when comparing fresh weight increase and dry matter accumulation. At equal media P concentration, H957 exceeded H883. Furthermore, H957 grown at 100 μM showed a fresh weight gain equivalent to that of H883 grown in a media 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. Two different types of response were evident when the FW or DW changes were compared at diminishing media P concentrations. H957 lost much less fresh and dry weight than H883 with diminishing P concentration.

The lower tissue P concentration observed in H957 showed that its better performance at equal external P concentration was not the simple expression of its higher tissue P concentration. Instead, the lower tissue P concentration strongly suggests that H957 required a lower external P concentration for normal growth than did H883. Furthermore, H957 could tolerate to the minimal P supply by using internal P more efficiently at the tissue or cellular level.

H957 and H883 might share identical genetic backgrounds except for anthocyanin biosynthesis; the LPT trait might have been affected by attenuated anthocyanin biosynthesis in H957. Altered regulatory apparatus controlling anthocyanin biosynthesis may exert pleiotropic effects on P utilization in H957. Further, a novel gene locus might exist to govern low-P tolerance in tomato plants and have been altered along with the anthocyanin mutation in H957.

Cultivars that can grow under 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 or cellular P with better efficiency.

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요 약

안토시아닌 결핍 돌연변이 토마토 변종인 H957은 낮은 인산 공급에 대하여 耐性을 보인다. 본 低燐酸 耐性을 연구하기 위해 미량의 인산을 포함한 조직배양 배지 위에서 H957의 생장도를 측정하였다. 이를 위하여 야생형의 모계 품종 (H883)과 공동 배양을 실시하여 생/건물질의 중량, callus/shoot 형성도, 무기질함량을 검사하였다. 본 연구에 사용된 배지의 인산 농도는 0-400μM 사이의 일곱 가지이다. 0과 12.5μM의 인 농도에서는 두 변종의 조직은 거의 생존하지 않았으나 50-200μM에서는 H957이 H883을 앞선 것으로 나타났다. H957은 생/건물질 중량 부문에서 매우 앞선 것으로 나타났으며, callus 및 shoot 형성도에 있어서도 미량의 인산 농도에 대해 H883보다 덜 민감한 것으로 나타났다. 이는 세포 및 조직 차원에 있어 H957의 높은 抗低燐酸 특성을 입증하는 것이다. 아울러, 인의 함량을 측정한 결과, H957 조직은 H883보다 낮은 농도의 인을 함유하고 있는 것으로 나타났다. 이 결과는 H957의 抗 低燐酸 특성이 이미 흡수된 인의 세포 및 조직 차원에서의 높은 활용성에 기인함이라고 판단된다.

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