Effect of Putrescine Pretreatment to Roots on Growth and Lactate Metabolism in the Root of Tomato (*Lycopersicon esculentum* Mill.) under Root-zone Hypoxia

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**Summary**

Root–zone–hypoxia–intolerant tomato plants (*Lycopersicon esculentum* Mill., cv. House–Momotaro) were grown in nutrient solution with a dissolved O$_2$ concentration of either 1 mg · liter$^{-1}$ (hypoxia) or 6 mg · liter$^{-1}$ (normoxia) for 7 days starting at the fifth leaf stage. The dissolved O$_2$ levels in nutrient solution did not affect polyamine biosynthetic enzyme activities and free polyamine contents in the roots. Plants in hypoxic nutrient solution grew poorly and exhibited a significant increase in lactate dehydrogenase activity and lactate content in the roots compared with those grown in normoxic nutrient solution. However, preincubation of plants in 0.5 mM putrescine–containing normoxic nutrient solution for 6 hr promoted root growth and inhibited the rise of lactate fermentation in the roots after day 5 under hypoxia. These results indicate that putrescine interferes with hypoxically induced lactate fermentation in the roots; it alleviates hypoxia injury of roots and increases the root–zone hypoxia tolerance of tomato plants.

**Key Words:** lactate dehydrogenase, lactic acid, polyamine, root–zone hypoxia, tomato.

**Introduction**

Flooding the soil causes O$_2$ deprivation in the root–zone and severely limits growth and productivity of crop plants. Plants vary in their capacity to tolerate root–zone hypoxia. Some plants survive root–zone hypoxia by the adaptive development of aerenchyma in the root cortex, and others by metabolic adaptation to hypoxia including the regulation of glycolysis and ethanol and lactate fermentation in hypoxic roots (Subbiah and Sachs, 2003). However, the biochemical mechanisms that underly the metabolic adaptation to hypoxia are not well understood. Elucidation of such mechanisms may be helpful in devising effective measures against flooding damage to crop production.

Polyamines such as putrescine (Put), spermidine (Spd), and spermine (Spm) play important roles in plant defense to environmental stresses (Bouchereau et al., 1999). Lee et al. (1996) found that *Scirpus mucronatus* shoots exhibited a significant increase in arginine decarboxylase (ADC) activity and free Put content upon being submerged in water. The addition of an inhibitor of ADC to the medium inhibited shoot elongation under submergence, which was reversed by the concomitant addition of Put. Reggiani et al. (1989) also found that hypoxia–tolerant species have a larger capacity for enhancing Put biosynthesis in the shoot in an O$_2$–deprived environment than intolerant species have. These results indicate important roles of Put in shoot elongation under hypoxia, but there is little evidence available on the role of polyamines in this phenomenon.

Tomato (*Lycopersicon esculentum* Mill.) is intolerant to root–zone hypoxia. Its root growth was severely inhibited at a dissolved O$_2$ concentration (DO) of 1 mg · liter$^{-1}$ in nutrient solution (Guo et al., 1997). This poor root growth was associated with a marked increase in lactate dehydrogenase (LDH) activity and lactate content in the roots (Guo et al., 1999). Generally, the increase in LDH activity and the resultant lactate accumulation have been considered to be primarily responsible for the hypoxia and anoxia injury of the roots (Roberts et al., 1985). In this study, we examined the possibility that pretreatment of tomato roots with polyamines could alleviate growth inhibition of the roots in hypoxic nutrient solution through counteracting the hypoxia–induced activation of lactate fermentation in the roots.

**Materials and Methods**

Seedlings of tomato (*Lycopersicon esculentum* Mill.) cv. House Momotaro (Takii Seed) were grown in a half–strength Hoagland nutrient solution in a glasshouse. The nutrient solution was kept at 23°C with vigorous aeration to keep the DO level above 6 mg · liter$^{-1}$. At the third leaf stage, plants were moved to growth chambers kept at 28/22°C (day/night) in a light of 350 μmol · m$^{-2}$ · s$^{-1}$ over a 15–hr photoperiod under the same root–zone conditions as above. When plants had five expanded leaves, they were transferred to either normoxic (6 mg · liter$^{-1}$ DO) or hypoxic (1 mg · liter$^{-1}$ DO) nutrient solutions. One set of plants was preincubated in nor-
moxic nutrient solution containing 0.5 mM Put (sodium salt) for 6 hr prior to transfer to hypoxic nutrient solution. The DO level in nutrient solution was controlled as described by Guo et al. (1997).

During the course of the DO treatments, plants were periodically sampled in triplicate for fresh weight determinations. Root samples were stored at −80°C until enzyme and chemical analyses were conducted. Activities of ADC, ornithine decarboxylase (ODC), and S-adenosyl-L-methionine decarboxylase (SAMDC) and contents of acid-soluble free Put, Spd and Spm in the roots were determined as described by Song et al. (2001). LDH activity and lactic acid content in the roots were assayed by the methods described by Hoffman and Hanson (1986) and Rivoal et al. (1989), respectively.

Results and Discussion

The DO levels in nutrient solution did not affect polyamine biosynthetic enzyme activities in the roots (Fig. 1). ADC activity increased during the first 3 days of DO treatment and then declined, while ODC and SAMDC activities tended to decline during the DO treatment in both normoxic and hypoxic roots. The DO levels also did not free Put, Spd and Spm contents in the roots (data not shown) indicating that tomato roots are incapable of enhancing the polyamine biosynthesis in response to hypoxia.

Plants in the hypoxic nutrient solution grew very poorly compared with those in the normoxic nutrient solution (Fig. 2). Plants, preincubated in Put-containing nutrient solution, grew better than did Put-unreared control plants after day 5 in hypoxic nutrient solution. This effect of Put pretreatment was particularly obvious in root growth; the root fresh weight in control plants at day 7 was only 33% of that in plants grown in normoxic nutrient solution; it increased to 68% in Put-pretreated plants. Preliminary experiments showed that Spd and Spm were ineffective regardless of their concentrations. In addition, Put pretreatment exerted little influence on plant growth under normoxia. Thus, it can be argued that high levels of Put in the roots could ameliorate hypoxic injury in the roots, promote root growth and shoot growth under root-zone hypoxia.

Fig. 1. Effect of dissolved O₂ concentrations (DO) in nutrient solution on activities of polyamine biosynthetic enzymes (arginine decarboxylase, ADC; ornithine decarboxylase, ODC and S-adenosylmethionine decarboxylase, SAMDC) in tomato roots. □: 6 mg · liter⁻¹ DO; ●: 1 mg · liter⁻¹ DO. Bars indicate SE (n = 3). NS means no significant difference between treatments at P ≤ 0.05.

Fig. 2. Time-course changes in fresh weights of shoot and roots of tomato plants during growth in nutrient solutions with a dissolved O₂ concentration (DO) of 6 (□ and A) or 1 (● and B) mg · liter⁻¹ without putrescine pretreatment or 1 mg · liter⁻¹ after putrescine pretreatment (△ and C). The photograph was taken at day 7. Bars indicate SE (n=3). Means with different letters are significantly different between treatments at P ≤ 0.05. NS means no significant difference between treatments.

Consistent with previous results (Guo et al., 1999), tomato plants exhibited a remarkable increase in LDH activity and lactate content in the roots upon transfer to hypoxic nutrient solution (Fig. 3), a characteristic feature of hypoxia-sensitive plants (Rivoal and Hanson, 1994). However, in Put-pretreated plants, this increase in lactate fermentation did not proceed after day 3 of hypoxia treatment. After day 5, Put-pretreated hypoxic roots showed a decrease in lactate content to the level in
Fig. 3. Time-course changes in lactate dehydrogenase (LDH) activity and lactic acid content in tomato roots during growth in nutrient solutions with a dissolved O₂ concentration (DO) of 6 (□) or 1 (●) mg \cdot liter⁻¹ without putrescine pretreatment or 1 mg \cdot liter⁻¹ after putrescine pretreatment (▲). Bars indicate SE (n=3). Means with different letters are significantly different between treatments at P≤0.05. NS means no significant difference between treatments.

Under hypoxia, with little changes in LDH activity indicating an increased efflux of lactate from the roots to the medium. However, coincident with this decrease in lactate content, roots under hypoxia grew more rapidly in Put-pretreated plants than did control plants (Fig. 2). This response indicates that the decrease in lactate in the roots may account for a major part of growth promotion in Put-pretreated roots over control roots under root-zone hypoxia.

The above results suggest that Put interferes with hypoxically induced lactate fermentation in tomato roots and results in alleviating lactate-mediated injury in the roots and enhancing root growth under hypoxia. Thus, genetic and cultural improvement of the root capacity for enhancing Put biosynthesis in response to hypoxia could be a useful measure against flooding stress damage to crop production. The mode of Put functions in suppressing the hypoxically-induced lactate fermentation remains to be clarified.

Literature Cited


Solvent-free cultivation of tomato plants (Solanum lycopersicum) is essential for the normal germination and tube growth in tomato (Lycopersicon esculentum Mill.) pollen. Plant Sci. 161: 507–515.


Literature Cited


溶存酸素濃度の低い培養液での
トマト根の生育と乳酸代謝に及ぼす
根へのプトレシ前処理の影響

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要旨

根域低酸素耐性の小さいトマト植物（品種. ハウス桃太郎）を、第5葉軸時に溶存酸素濃度（DO）の低い培養液（1 mg \cdot liter⁻¹）と高い培養液（6 mg \cdot liter⁻¹）に区別して、7日間水耕栽培した。根のポリアミン合成酵素活性および遊離態ポリアミン含量は培養液のDOレベルの影響を受けなかった。低DO培養液では生育が抑制され、根の乳酸脱水酸酵素活性および乳酸含量が著しく高まった。しかし、低DO培養液に移す前の6時間ごとに5 mMプトレシ含有培養液で前培養した植物では、5日目以降に根の生長が良好になり、乳酸脱水酸酵の活性化が抑制された。以上の結果は、根のプトレシ濃度が高いと、酸酵酸素不足による乳酸発酵が抑制され、その結果、根の障害が軽減されてトマトの根域低酸素耐性が高まることを示唆する。