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Implication of Endogenous Cytokinins in the Growth Inhibition of Cucumber Plants by Supraoptimal Root-zone Temperature

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Summary

Seedlings of cucumber (*Cucumis sativus* L., cv. Sharp I) were grown in hydroponic culture at root-zone temperatures of 25, 35, and 38 °C for 10 days. Cytokinins in the leaves and roots were quantified at day 5 and 10. Zeatin and its riboside were the main cytokinins in the leaves, while the roots contained a considerable amount of isopentenyladenosine besides the above two cytokinins. High root-zone temperatures caused a marked decrease in cytokinin concentrations. The change was gradual at 35 °C but very rapid at 38°C, and fluctuated more in the roots than in the leaves. In plants grown at 38°C, cytokinin concentrations in the roots were very low already at day 5; at day 10 both the roots and leaves contained only negligible concentrations of cytokinins. Zeatin riboside was more sensitive to high root-zone temperatures than the other cytokinins in the leaves.

Cucumber plants were grown at 38°C for 10 days and treated with 0, 10^{-6}, 10^{-5}, and 10^{-4} M benzylaminopurine (BAP) to leaves in combination with 0, 10^{-6}, and 10^{-5} M abscisic acid (ABA) treatments to roots. Treatment of roots with 10^{-6} M ABA promoted slightly growth of BAP-untreated plants; but 10^{-5} M ABA was inhibitory, irresepective of BAP treatments. On the other hand, foliar sprays of BAP at 10^{-4} M caused a marked increase in the growth rate of ABA-untreated plants. Lower BAP concentrations had no significant effects. This growth-promoting effect of 10^{-4} M BAP sprays to leaves was not observed when the roots were supplied with ABA at either concentrations.

The results strongly suggest that inhibition of cytokinin synthesis in the roots and the resultant decrease in endogenous cytokinin concentrations in the leaves are the bases for growth inhibition of cucumber plants at supraoptimal root-zone temperature.

Introduction

Cucumber is a thermophilic plant whose optimal root-zone temperature lies around 25 °C in the mature growth stage (Sasaki and Itagi, 1989). Young cucumber plants can grow normally at 32 °C, but its growth rate is inhibited above 35°C; at 37 to 38 °C they develop very small, chlorotic leaves and poor roots with thick, bent tips (Du and Tachibana, 1994a; Wang and Tachibana, 1995).

We previously studied the physiological causes of growth inhibition in cucumber plants at supraoptimal root-zone temperatures, with respect to water relations, mineral nutrition, root respiration, photosynthesis, sugar metabolism in roots, and abscisic acid concentrations in leaves and roots (Du and Tachibana, 1994a, 1994b, 1995; Wang and Tachibana, 1995). However, changes in cytokinin concentrations in cucumber plants in response to elevated root-zone temperatures have not been elucidated.

Since cytokinins are mainly synthesized in roots (Skene, 1975), environmental stresses to roots may cause a decrease in cytokinin synthesis in the roots, which in turn may lead to a shortage of this hormone in the shoot. Root-zone environments such as drought (Itai and Vaadia, 1965), waterlog-

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ging (Burrows and Carr, 1969), salinity (Itai, 1978) and low temperature (Tachibana, 1988) are known to inhibit cytokinin production in the roots.

Information on cytokinin concentrations in plant tissues as influenced by supraoptimal root-zone temperature is limited. Itai et al. (1973) found that bean roots exposed to a short-term heat stress treatment had a dramatic decrease in cytokinin concentrations in the root xylem exudate. Kuroyanagi and Paulsen (1988) conjectured that wheat plants grown at 35 °C root-zone temperature contained a deficient level of cytokinin in the leaves, based on an increase in protease activity and a decrease in protein content. However, they did not determine the endogenous cytokinin concentrations in the plant tissues. Thus, the objective of our present study was to investigate the effect of supraoptimal root-zone temperature on endogenous cytokinin concentrations in cucumber plants and whether those changes are causally related to their growth inhibition.

Materials and Methods

1. *Effect of root-zone temperature on cytokinin concentrations in roots and leaves (Exp. 1)*

Seedlings of cucumber (*Cucumis sativus* L., cv. Sharp I) were raised in a glasshouse on gravel culture. Twenty plants each were then transplanted at the first true leaf stage to three hydroponic beds, containing 250 liters of 1/2 strength Hoagland solution. The solution was controlled at 25 °C, circulated continuously with a small pump, and aerated intermittently for 15 min every hour. After the plants were acclimated to the water culture conditions for 3 days, the solutions in two beds were raised to 35 and 38°C. Ten plants were sampled from each treatment 5 and 10 days after the onset of the treatments and 100-g portions of roots and leaf blades were immediately frozen separately in liquid nitrogen and stored at − 30 °C for cytokinin analysis.

2. *Effect of BAP and ABA treatments on plant growth at supraoptimal root-zone temperature (Exp. 2)*

Cucumber seedlings cv. Sharp I were raised as in Experiment 1. Six plants were planted in each of five hydroponic beds containing 250 liters of 1/2 strength Hoagland solution held at 25°C. After 3 days, the temperature of the solution in four beds was raised to 38°C, while that in one bed was left at 25°C. When the solution temperature reached 38°C, cis, trans- (+) ABA (natural ABA, a gift from Torey Ind. Inc.) was added to the nutrient solution at 0, 10^{-6}, and 10^{-5} M. Simultaneously, all expanded leaves in the 38 °C treatments were sprayed with an aqueous solution of BAP at 0, 10^{-6}, 10^{-5}, and 10^{-4} M, containing 0.01% Tween 20. This foliar spray was repeated again after 6 days. Plants grown at 25°C received no hormone treatments and served as the control. Plants were harvested after 10 days of root-zone temperature treatments, and their leaves, stem plus petioles, and roots weighed.

3. *Quantitation of cytokinins*

The method described by Horgan (1978) was followed for extraction and purification of cytokinins in roots and leaf blades. Briefly, 100-g of frozen tissues were immersed in Bielski solution (Bielski, 1964) and left at − 30 °C for 24 hr in the dark. The solution was then removed by filtration and the tissues were homogenized in acidic methanol (methanol: water: formic acid = 6: 4: 1, by volume). After standing at − 30 °C for 24 hr, the homogenates were filtered through filter paper under suction. The filtrates were combined with the previous Bielski extracts and evaporated to dryness at 40 °C in vacuo. The residues were dissolved in 30mM acetic acid and passed through a PVPP column to remove phenolics and pigments. The eluate was adjusted to pH 8.2 and partitioned four times against water-saturated n-butanol. Butanol extracts were then dried and redissolved in a minimum volume of 35% ethanol. Cytokinins in the 35% ethanol eluates were further purified and separated by gel filtration with a Sephadex LH-20 gels. Cytokinins in the eluates were quantified by soybean callus test using 6 callus pieces of cv. Acme and 25ml of Miller's media without replication (Tachibana, 1988).

Results

1. *Effect of root-zone temperature on cytokinin concentrations in roots and leaves (Exp.1)*

The effects of high root-zone temperatures on plant growth was essentially the same as those reported previously (Du and Tachibana, 1994a).

Shoot and root growths were significantly inhibited at 38°C at day 5; the upper leaves became chlorotic at days 6 to 7. At 35°C, plant growth was slightly inhibited at day 5, and remained small until day 10, as compared to those grown at 25 °C, particularly the roots. Leaf chlorosis did not occur at 35°C.

Cytokinin concentrations in roots of cucumber plants grown at 25, 35 and 38°C for 5 and 10 days are shown in Fig. 1. Cucumber roots contained a considerable amount of isopentenyladenosine (2iPA) besides zeatin (Z) and zeatin riboside (ZR). Cytokinin concentrations in roots grown at 35 °C were almost the same as those grown at 25°C at day 5, but ZR and Z at the higher temperature decreased to less than a half at day 10, whereas 2iPA changed little. However, the roots grown at 38 °C had much lower concentrations of all three cytokinins at day 5, and only negligible concentrations existed at day 10.

Fig. 2 shows the cytokinin concentrations in leaves of plants grown at different root-zone temperatures for 5 and 10 days. Z and ZR were the main cytokinins in the leaves of plants grown at 25°C; 2iPA was barely detectable. The effects of high root-zone temperature on cytokinin concentrations in leaves were relatively small as compared to those in roots. Plants grown at 35 °C showed a slight decrease in both ZR and Z concentrations in leaves at day 5. Concentrations of these two cytokinins particularly ZR were further decreased after 10 days at 35°C. On the contrary, 2iPA concentrations in leaves were much higher in plants grown at 35 °C than were those grown at 25°C. Plants grown at 38 °C showed a marked decrease in ZR in leaves at day 5, but the decrease in Z was relatively small. At day 10, however, the concentrations of both ZR and Z decreased to very low levels; 2iPA was not detectable.

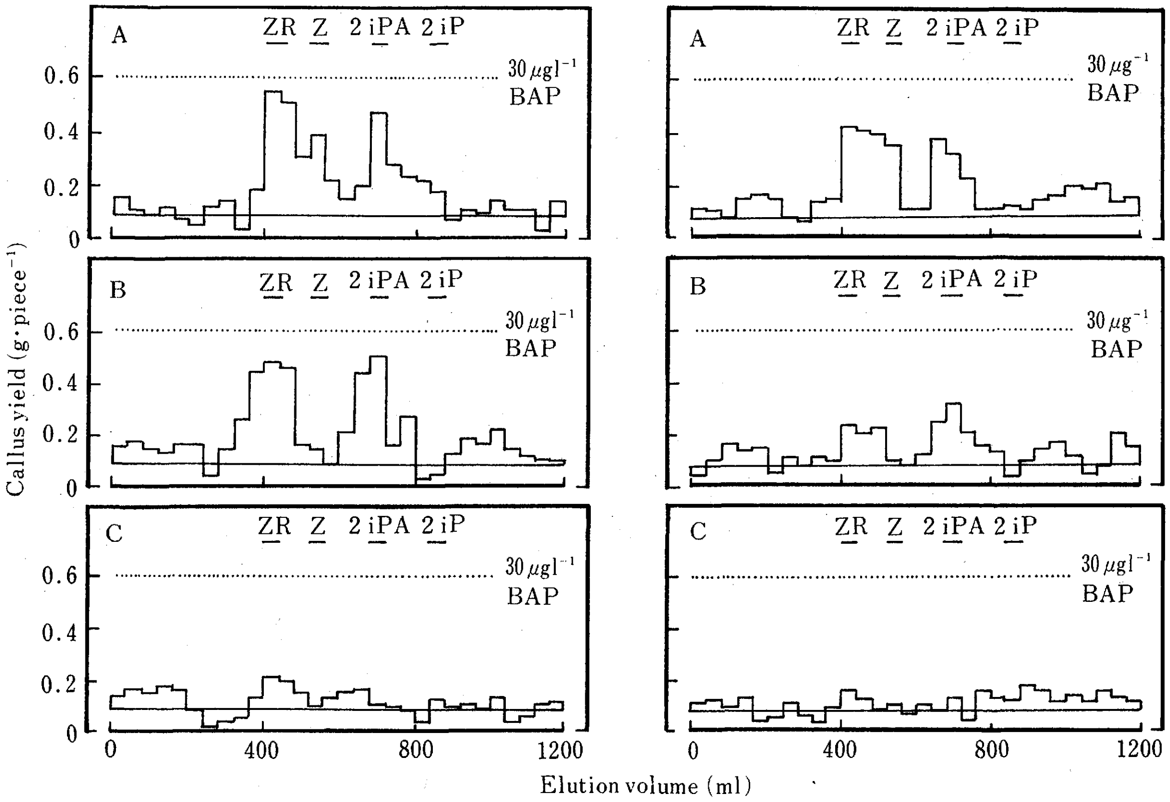

Fig. 1. Histograms of cytokinin concentrations in the roots of cucumber plants grown at 25°C (A), 35°C (B) and 38 °C (C) root-zone temperatures for 5 days (left) and 10 days (right). Cytokinins were extracted from 100g of fresh roots, fractionated on Sephadex LH-20 with 35% ethanol as eluting solvent, and assayed for cytokinin activity by soybean callus test. Abbreviations: ZR; zeatin riboside, Z; zeatin, 2iPA; isopentenyladenosine, 2iP; isopentenyladenine.

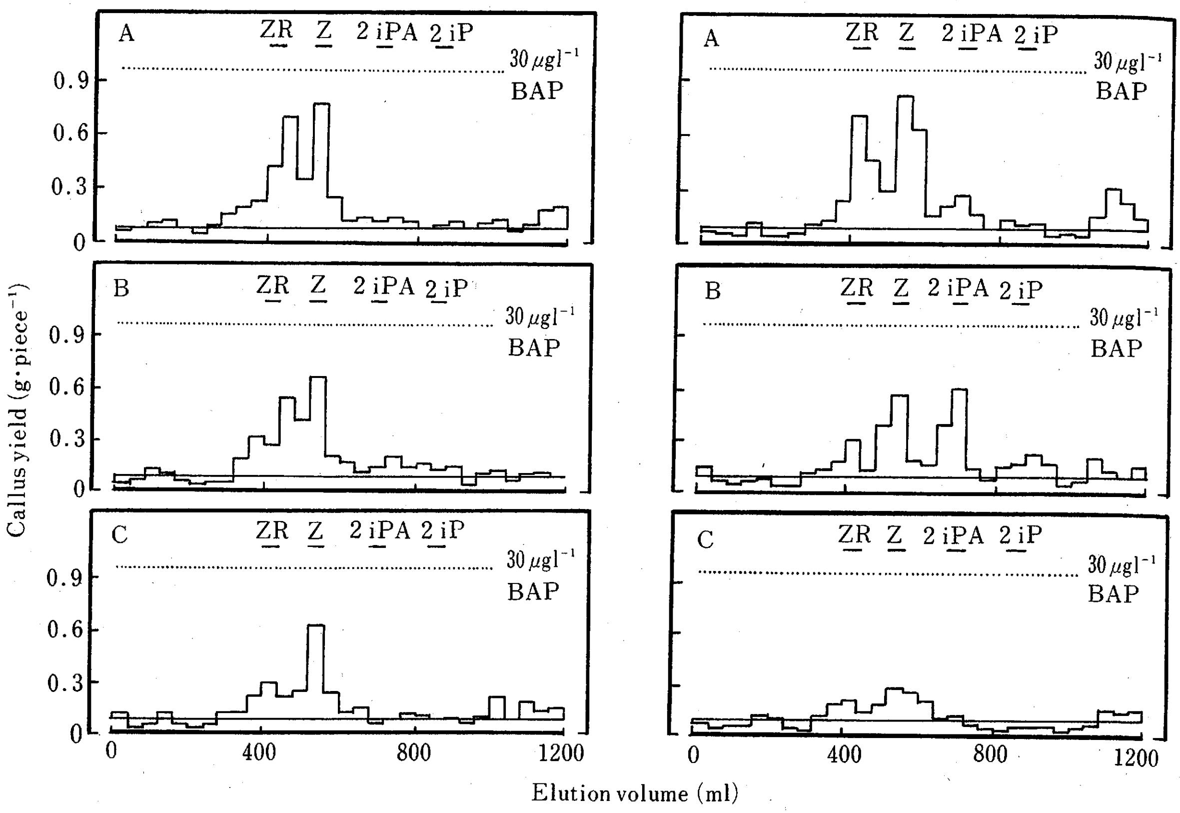

Fig. 2. Histograms of cytokinin concentrations in the leaves of cucumber plants grown at 25 °C (A), 35 °C (B) and 38 °C (C) root-zone temperatures for 5 days (left) and 10 days (right). Cytokinins were extracted from 100 g fresh leaves. For the abbreviations see the legend of Fig. 1.

2. *Effect of BAP and ABA treatments on plant growth at supraoptimal root-zone temperature (Exp. 2)*

Figs. 3 and 4 show the effect of BAP and ABA treatments on the dry weight of the whole plants, and leaves and roots of cucumber plants grown at 38°C for 10 days, respectively. The corresponding dry weights of the control plants grown at 25°C without hormone treatments are given in the figure legends. Plants grown at 38°C without any hormone treatment accumulated less dry matter, compared to the control plants grown at 25°C: specifically 56% (whole plant), 43% (leaves) and 34% (roots).

When ABA was not supplied to roots, plant growth at 38 °C was promoted by foliar sprays of BAP. The growth-promoting effect of BAP was greater as its concentrations were increased; at 10^{-4} M, the whole plant dry weight increased to 83% of the control plants grown at 25 °C without any hormone treatments. The growth-promoting

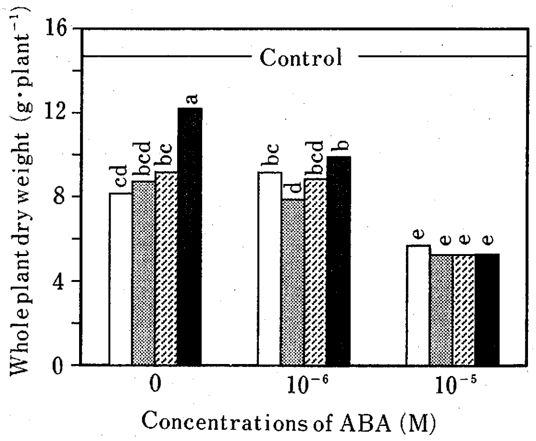

Fig. 3. Bar graphs depicting the effects of foliar sprays of benzylaminopurine (BAP) in combination with root treatment with abscisic acid (ABA) on the dry weight of whole cucumber plants grown at 38 °C root-zone temperature for 10 days. The whole plant dry weight of plants grown at 25°C without hormone treatments (Control) was 14.56g. BAP concentrations: □; 0 M, ░; 10^{-6} M, ▨; 10^{-5} M, ■; 10^{-4} M. Bars with the same letter are not significantly different at $P = 0.05$.

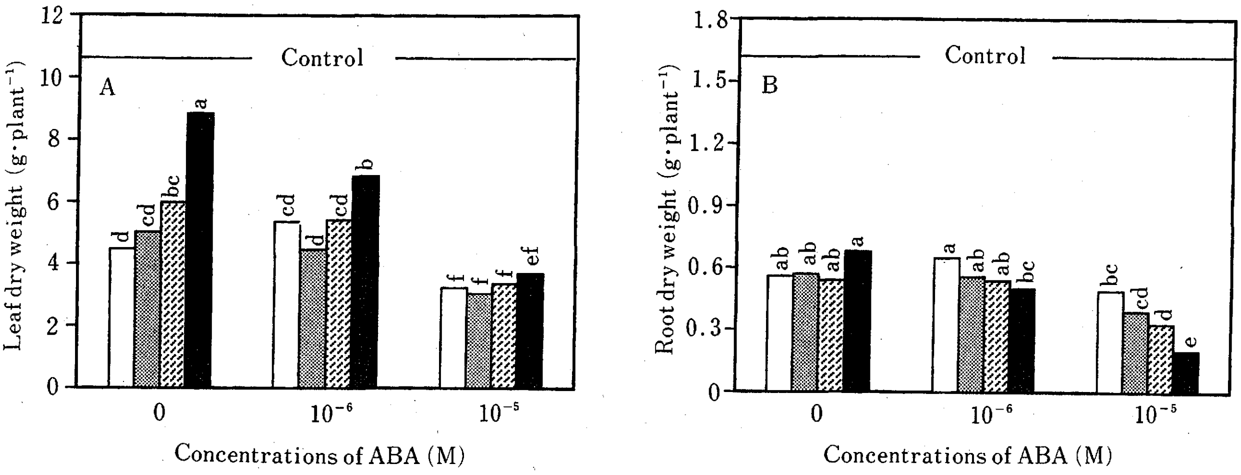

Fig. 4. Bar graphs showing the effects of foliar sprays of benzylaminopurine (BAP) in combination with root treatment with abscisic acid (ABA) on the dry weight of leaves (A) and the roots (B).The plants were grown at 38 °C root-zone temperature for 10 days. The leaf and root dry weights of plants grown at 25 °C without hormone treatments (Control) were 10.57 g and 1.62 g, respectively. BAP concentrations: □; 0 M, ▩; 10^{-6} M, ▨; 10^{-5} M, ■; 10^{-4} M. Bars with the same letter are not significantly different at$P=0.05$.

effect of BAP was achieved mainly by the increase in leaf areas but not in leaf number. Upper leaves of plants grown at 38°C without hormone treatments became chlorotic but this condition was reversed by BAP application.

When plants were not sprayed with BAP, ABA treatment to roots at 10^{-6} M caused slight, insignificant increases in root and leaf dry weight accumulations. However, ABA at 10^{-5} M was inhibitory to growth, particularly to leaf growth. Furthermore, the growth-promoting effect of BAP was completely negrected by simultaneous application with 10^{-6} M ABA to roots. At 10^{-5} M ABA, root growth was inhibited more as the concentration of BAP was increased. In this case, water content of leaves was 77 and 79% at 10^{-4} and 10^{-5} M BAP, respectively, in contrast to 82% at 10^{-6} and 0 M BAP. Leaf chlorosis in plants grown at 38 °C without any hormone treatment was partially alleviated by ABA treatments.

Discussion

Itai et al. (1973) found a rapid decrease in cytokinin concentrations in the xylem exudates of bean roots exposed to 47.5°C for only 2 min. Cheikh and Jones (1994) reported that exposure of in vitro-grown maize kernels to 35°C for 4 or 8 days resulted in great suppression of dry weight accumulation and increased kernel abortion, accompanied by a marked decrease in cytokinin concentrations.

Since roots are believed to be a main cite of cytokinin synthesis in plants, our results in Exp. 1 suggest that supraoptimal root-zone temperature inhibited cytokinin production in cucumber roots and consequently reduced the amount of cytokinins translocated to the shoot. Very low cytokinin concentrations in the roots grown at 38°C for 5 days suggest that the response of cytokinin synthesis in the roots to high temperature is very rapid.

In Exp. 2, BAP was sprayed on cucumber grown at 38 °C root-zone temperature to examine whether the decrease in cytokinin concentrations in leaves causes growth inhibition of cucumber plants. Concurrently, ABA treatment to the roots was examined, because in our earlier study endogenous ABA was markedly decreased in cucumber roots grown at 38 °C root-zone temperature (Du and Tachibana, 1995).

In roots untreated with ABA, foliar sprays of BAP at 38 °C ameliorated growth inhibition significantly. This growth-promoting effect of BAP was greater as the concentration was increased. Cheikh and Jones (1994) also observed that the inhibition of in vitro growth of maize kernels by heat treatment at 35 °C was significantly reversed by 10^{-5} M BAP. We found that foliar sprays of 10^{-5} and 10^{-4} M BAP to cucumber plants grown at 25°C root-zone temperature did not promote plant growth. Therefore, BAP spray to leaves promoted plant growth probably by blocking a drastic decrease in cytokinin activities which may have occurred in cucumber plants grown at 38°C root-zone temperature. Thus, we conclude that the decrease in cytokinin concentrations in the leaves is implicated in growth inhibition of cucumber plants at supraoptimal root-zone temperatures.

Cytokinins are known to stimulate cell division, cell enlargement, chloroplast formation, synthesis of chlorophylls and proteins (Skene, 1975), and inhibit *de novo* synthesis of protease (Martin and Thimann, 1972). Thus, it seems that these cytokinin-related processes were impaired in the leaves of cucumber plants grown at supraoptimal root-zone temperatures. Kuroyanagi and Paulsen (1988) observed a marked increase in protease activity and a decrease in protein content in leaves of wheat grown at 35°C root-zone temperature. We also found a significant increase in protease activity in leaves of cucumber plants grown at 38°C for 10 days over those grown at 25°C (Tachibana and Du, unpublished). Likewise, leaf chlorosis and small leaf areas in plants grown at 38 °C may well be attributable to the deficiency of cytokinins.

Blokhin (1986) observed that inclusion of 4.4×10^{-8} M BAP to the nutrient solution caused an increase in thickening as well as dry matter accumulation in corn and cucumber roots grown at normal root-zone temperature. We found that root growth of cucumber at 38°C was also promoted, although insignificantly, by treating the leaves with 10^{-4} M BAP. However, this promotion is attributable to the increase in shoot growth and a resultant increase in the supply of photosynthates to the roots, since, in our preliminary experiments, addition of 10^{-7} and 10^{-6} M BAP to nutrient solution resulted in further growth inhibition of roots grown at 38°C.

Previously, we found that treating cucumber roots kept at 38 °C with $3\text{x}10^{-7}$ M ABA significantly increased plant growth, whereas it was ineffective at 25°C (Du and Tachibana, 1995). In this study, a single treatment of 10^{-6} M ABA to roots did not significantly alleviate growth inhibition by high root-zone temperature. Furthermore, the simultaneous treatment of ABA to roots and BAP to leaves resulted in disappearance of growth promotion by BAP. So it seems that by treating roots with 10^{-5} M ABA and supplying BAP to leaves impeded root growth. These interactive effects of BAP and ABA are difficult to explain without information on ABA and cytokinin activities in the plants. It is known that ABA in leaves of cucumber plants with roots exposed to 38 °C increases to excessively high concentrations after 10 days (Du and Tachibana, 1995). Therefore, it seems possible that BAP treatment to leaves could have increased endogenous ABA to an excessive level in the 38°C-grown plants, probably through decreasing the water content in the leaves.

Literature Cited

Bielski, R. L. 1964. The problem of halting enzyme action when extracting plant tissue. Anal. Biochem. 9: 431-442.

Blokhin, V. G. 1986. Concentration dependence of the effect of 6-benzylaminopurine on root growth in plants of different species. Soviet Plant physiol. 33: 828-833.

Burrows, W. J. and D. J. Carr. 1969. Effects of flooding the root system of sunflower plants on the cytokinin content in the xylem sap. Physiol. Plant. 22: 1105-1121.

Cheikh, N. and R. J. Jones. 1994. Distribution of maize kernel growth and development by heat stress. Role of cytokinin/abscisic acid balance. Plant Physiol. 106: 45-51.

Du, Y. C. and S. Tachibana. 1994a. Effect of supraoptimal root temperature on the growth, root respiration and sugar content of cucumber plants. Scientia Hort. 58: 289-301.

Du, Y. C. and S. Tachibana. 1994b. Photosynthesis, Photosynthate translocation and its metabolism in roots of cucumber plants at supraoptimal root temperature. J. Japan. Soc. Hort. Sci. 63: 401-408.

Du, Y. C. and S. Tachibana. 1995. Effect of supraoptimal root temperature on ABA levels in cucumber plants and its control by ABA applied to roots. Acta Hort. 394: 227-234.

Horgan, R. 1978. Analytical procedures for cytokinins. p. 97-114. In: J. R. Hillman (ed.). Isolation of plant growth substance. Cambridge Univ. Press, Cambridge.

Itai, C. A. 1978. Response of *Eucalyptus occidentalis* to water stress induced by NaCl. Physiol. Plant. 43: 377-379.

Itai, C. A. and Y. Vaadia. 1965. Kinetin-like activity in root exudate of water stressed sunflower plants. Physiol. Plant. 18: 941-944.

Iati, C. A., A. Ben-Zioni and L. Ordin. 1973. Correlative changes in endogenous hormone levels and shoot growth induced by short heat treatment to the roots. Physiol. Plant. 29: 355-360.

Kuroyanagi, T. and G. M. Paulsen. 1988. Mediation of high-temperature injury by roots and shoot during reproductive growth of wheat. Plant Cell Environ. 11: 517-523.

Martin, C. and K. V. Thimann. 1972. The role of protein synthesis in the senescence of leaves. I. The formation of protease. Plant Physiol. 29: 64-71.

Sasaki, K and T. Itagi. 1989. Effect of root cooling treatment of nutrient solution on growth and yield of tomato, cucumber and melon. Environ. Control in Biol. 27: 89-95.

Skene, K. G. M. 1975. Cytokinin production by roots as a factor in the control of plant growth. p. 365-396. In: J. G. Torrey and D. T. Clarkson (eds.). The development and function of roots. Acad. Press, London.

Tachibana, S. 1988. Cytokinin concentrations in roots and root xylem exudate of cucumber and figleaf gourd as affected by root temperature. J. Japan. Soc. Hort. Sci. 56: 417-425.

Wang, Y. H. and S. Tachibana. 1995. Growth and mineral nutrition of cucumber seedlings as affected by elevated air and root-zone temperature. J. Japan. Soc. Hort. Sci. 845-852. (In Japanese with English summary).

根域の高温によるキュウリの生長抑制における内生サイトカイニンの関与

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摘　要

根域の高温による生育抑制に植物体のサイトカイニン濃度が関係しているかどうかを明らかにするため，キュウリ‘シャープⅠ’の幼植物を液温25，35，38°Cの培養液で10日間水耕栽培し，5日目と10日目に根と葉のサイトカイニン濃度を測定した．根のサイトカイニン濃度は，35°C区では5日目には25°C区のそれと大差がなかったが，10日目には顕著に低下した．38°C区の根では5日目にすでに著しく低い濃度になっており，10日目にはサイトカイニン活性はほとんど検出されなかった．一方，葉のサイトカイニン濃度に対する根域の高温の影響は根ほどには大きくなかったが，38°C区では5日目にゼアチンリボシドが減少し，10日目にはゼアチンリボシドがさらに減少するとともに，ゼアチン濃度も非常に低くなった．

次に，‘シャープⅠ’を液温25°Cと38°Cで10日間水耕栽培し，温度処理の開始時に38°C区の植物だけに，0，10^{-6}，10^{-5}，10^{-4} Mベンジルアミノプリン(BAP)の葉面散布と0，10^{-6}，10^{-5} Mアブシジン酸(ABA)の培養液添加を組み合わせて処理した．BAP処理は6日後にもう一度行った．ABA無処理の場合には，38°C区のキュウリの生長はBAPの葉面散布によって促進され，10^{-4} Mでは葉の乾物重が25°C区のそれに近くなったが，ABAを処理した場合にはBAPの生長促進作用がみられなくなった．

以上の結果から，根域の高温によるキュウリの生長抑制には，根でのサイトカイニン合成の抑制とそれによる葉のサイトカイニン濃度の低下が直接関与していると推察される．

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